nomenon in recent years (*9*), the ability of phospholipids to combine with monosaccharides to form lipid-soluble complexes was well recognized in the late 19th century, when considerable debate raged (*10*) as to whether such "jecorins" were true compounds preexisting in various tissues or were physical aggregations assembled by the extraction procedures. For the formation of the complexes studied here, drying at 50° to 60°C from mixtures containing ethanol or higher alcohols appeared to be the most effective procedure, but neither heat nor alcohol is absolutely essential to the phenomenon. In fact, dry sugars will dissolve directly into highly nonpolar solvents containing phospholipids already in solution. In our experimental system, the solubilization of glucose into a hexane vehicle proceeded very slowly at room temperatures even with rather rapid agitation, so that, with an excess of available sugar, a nearly steady rate of its appearance in the filtered solution was maintained for many hours. This rate was approximately proportional to the total amount of the dissolved phospholipid (rather than to its concentration), and the final quantity of sugar that went into solution at the steady state (requiring about 4 hours at 50° to 60°C) closely approached a 1 : 1 molecular ratio to the phospholipid present (as given by analysis for total P). With the ethanol-drying procedure, however, at least twice this amount of sugar was often carried into hexane by various phospholipids.

Doubt regarding the relevance of these observations to the red cell's sugar transport system naturally arises from the facts that (i) the extent of this sugar-complexing potentiality in the material extractable from the membrane vastly exceeds the density of the functional transport site, and (ii) the phospholipids have failed, in our experimental systems, to move perceptible amounts of sugar out of an aqueous phase into a nonaqueous phase. However, several properties of the intact cell transport system are paralleled in the phenomenon of lipid-sugar complex formation. Thus, during incubation of the erythrocytes with the "protein reagent," 1-fluoro-2,4-dinitrobenzene (DNFB), the glucose-transport capacity of the cells progressively and irreversibly deteriorates (*11*), so that after varying exposure to the agent, washing, and resuspension in ordinary medium, cells of varying residual transport capacity are obtainable. Concomitantly, there is a marked diminution in the degree to which the phospholipids extracted from the ghosts of these cells will form the hexane-soluble complex with glucose (Table 2), although the P content of the extracts is not appreciably altered. Also stilbestrol, which blocks the sugar-transport system of the intact cell in a fully reversible manner (*12*), appears to displace an equimolecular quantity of glucose from the lipid complex when it is presented during drying of the mixtures from ethanolic solution. Finally, there are small differences among the common monosaccharides with respect to the extent of their recoverability in hexane after drying with given proportions of phospholipid, which parallel substantially their larger differences with respect to affinity for the transport system in the red cells (*13*). However, this relation breaks down entirely in application to mirror-image specificity: while the transport system notably distinguishes between sugar enantiomorphic pairs, this type of specificity seems to be totally lacking in the phenomena of complex formation with extracted phospholipids.

PAUL G. LEFEVRE, KATHRYN I. HABICH
HELEN S. HESS, MELINDA R. HUDSON
Department of Pharmacology, University of Louisville School of Medicine, Louisville, Kentucky

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Detection and Quantitation of Fallout Particles in a Human Lung

Abstract. *Portions of an adult human lung were studied by autoradiography in order to detect the presence of fallout particles. The radioactivity in the remainder of the tissue was determined with a gamma-ray spectrometer. Four particles were found and their activities were determined. From the measurement for total-fission-product activity in the lung tissue it was calculated that there were approximately 264 particles in the right lung at the time of death.*

Radioactive particles formed during the atmospheric testing of nuclear weapons have become dispersed as worldwide fallout. The presence of these particles in the body is a potentially serious source of radiation exposure in the human population. We have found nothing in the literature to show that anyone has analyzed tissue for the presence of these discrete particles in order to assess the hazard they present. However, two authors have reported the detection of fission products in the lung. In 1959, Zr^{95} and Nb^{95} were quantitatively measured, and Ru^{103} was detected in ashed human lung tissue (*1*). In April 1962, Cs^{137} and Zr^{95} were measured in human lungs by external counting (*2*).

In the summer of 1962 we began work at the University of Michigan's School of Public Health to determine if it was feasible to detect individual radioactive particles in human lung tissue by autoradiographic techniques. After preliminary work had indicated that this was possible, an entire right human lung was obtained for counting and auto-

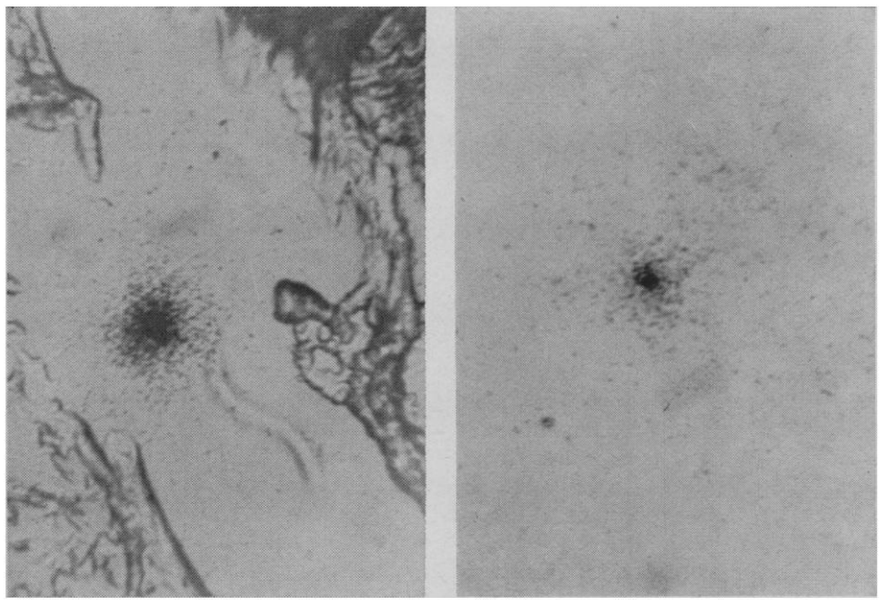

Fig. 1. Photomicrographs of radioactive fallout particles detected in tissue of human lung by autoradiography. (Left) Activity of 1.8 $\mu\mu$c; (right) activity of 1.5 $\mu\mu$c.

radiography. The results of this study are reported here.

The right lung was obtained from a diabetic, but otherwise normal, 78-year-old white male, on 26 March 1963 (*3*). Immediately after excision the hilar lymph nodes were dissected away and the lung was divided into five segments, designated in Table 1. Twelve small cubes of tissue were removed from areas throughout the lung for sectioning. The remainder of the tissue was ashed at 550°C. A count was made of the ash with a gamma-ray spectrometer in order to identify and measure gamma-ray-emitting radionuclides.

The small cubes removed for sectioning were freeze-dried and embedded in paraffin by a vacuum process. Twenty micron sections were cut, placed on glass slides (coated with gelatin acetate) by means of a dry-mounting technique, deparaffinized, and dipped in Kodak NTB-3 nuclear track emulsion. The slides were exposed in the presence of a desiccant at 4°C for 40 days. After developing and mounting, ten slides from each cube were scanned, at magnification of 100, to determine whether discrete radioactive particles were present. Four hilar lymph nodes were handled in like manner; however, the slides from these were scanned at magnification of 430. At least three control slides, without tissue, were prepared, along with the slides from each cube of tissue, to determine the background radiation of the film. The area of film around each tissue slice was also considered to be a control area.

Table 1. Results of determinations of radioactivity in lung-tissue ash, made with a gamma-ray spectrometer.

Segment No.	Anatomical region	Wet weight (g)	Total activity in each segment (10^{-5} μc)*				Particles found	Number of slides scanned
			Ce^{141}, Ce^{144}, + Pr^{144}	Ru^{103}, Ru^{106}, + Rh^{106}	Zr^{95} + Nb^{95}	K^{40}		
1	Inferior lobe: superior segment medial basal posterior	132	4.37 ±25 %	1.20 ±85 %	5.76 ±11 %	10.16 ±58 %	2	40
2	Inferior lobe: anterior basal lateral basal	63.9	3.99 ±28 %	1.47 ±70 %	3.33 ±18 %	11.70 ±51 %	2	20
3	Middle lobe: lateral medial	63.0	2.87 ±38 %	6.50 ±17 %	1.82 ±31 %	8.08 ±73 %	0	20
4	Superior lobe: anterior	72.7	1.84 ±57 %	<1.00	3.09 ±19 %	15.0 ±40 %	0	20
5	Superior lobe: apical posterior	55.1	2.58 ±41 %	1.28 ±80 %	3.33 ±18 %	8.70 ±67 %	0	20
Totals in right lung		387	15.65	10.57	17.33	53.64	4	

* 95 % Error = 196.0 $\{[c/m\ (uncor) + c/m\ (bkg)]/t\}^{1/2} \div c/m\ (cor)$, where c/m (uncor) = counting rate before subtraction of scattering components; c/m (cor) = counting rate after subtraction of scattering components; t = counting time (100 minutes); and c/m (bkg) = background counting rate.

The results of the determinations of gamma-ray activity for the tissue ash are shown in Table 1. The fission products detected were Ce^{141}, Ce^{144}, + Pr^{144}; Ru^{103}, Ru^{106}, + Rh^{106}; and Zr^{95} + Nb^{95}. The total activity detected in the lung from these three combinations of radioisotopes was 43.6×10^{-5} μc. Four radioactive particles were found on the 160 slides scanned. These were all found in tissue of the inferior lobe. No particles were found in the lymph nodes, on any of the control slides, or on areas of the tissue slides around the tissue section. Figure 1 shows two of the particles detected in the lung tissue by autoradiography.

Preliminary work was carried out to obtain a film calibration procedure. Filter papers from local monitors of radioactivity in the atmosphere were examined by autoradiography to detect any radioactive particles (Kodak "no-screen" x-ray film was used). When a particle was found, a small area of filter paper around it was punched out, a count was made with a beta-ray counter, and the filter paper was placed on a subbed (coated) microscope slide and separated from the particle by dissolution, in preparation for microscopic autoradiography. The slide was dipped in a 1-percent solution of collodion to fix the particle to the slide and to simulate a tissue slice. Some of the particles were found to be agglomerations, which were broken up by this process. The slides were then dipped in NTB-3 photographic emulsion and handled in the manner described for the tissue slides. Two groups of ten particles were studied by autoradiography; however, only six particles which did not split apart during processing were used for calibration. The diameter of the area totally blackened by exposure to the radiation was measured, and the diameter of the blackened area was plotted against the total number of β-ray disintegrations. From the curve the approximate activity of the particles found in the lung tissue could be obtained. Since the resolution of the film, and therefore the diameter of the spot, is a function of β-energy (*4*), the calibration curve derived by using the radioactive particles

in the air is applicable to the particles found in the lung only if the isotope compositions of these two media are the same. (We show later on that the isotope compositions were roughly equal.) The activities thus determined for the four particles or particle combinations found in the tissue were 1.6, 1.8, 1.5, and 1.7 $\mu\mu$c, respectively. By taking the average for these values and comparing it with the value for total-fission-product activity detected in the lung, assuming this activity is all in particulate form, one can calculate that the lung of our study contained approximately 264 particles at the time of death. The isotope composition of the radioactive particles was not determined because of their very low activities. However, a comparison has been made between the isotope content of the lung and of air, and the results agree fairly well. During the 4-week period just before the lung was received in this laboratory the relative proportions, in the filter samples, of the γ-emitting fission-product combinations of Table 1, cols. 4–6, in the order of the table columns, were as follows: 1.0 to 0.8 to 1.3. For the lung tissue, the averages for the same activities [weighted for the amount of tissue (by weight) in which they occurred] are 1.0 to 0.6 to 1.2. While there may be some differences between the relative proportions in air and in the lung, we do not think these differences would appreciably affect the applicability of the calibration procedure to the lung particles, in view of the preliminary nature of the results.

Although an actual dosage of radiation to the lungs is difficult to calculate because of the many unknown physical and biological parameters, one can calculate by Loevingers' equations (*5*) that a particle of Zr^{95} in equilibrium with Nb^{95}, having an activity equal to the average for the four particles found, with a 120-day half-life in the lung, would deliver a total of 2×10^3 rad at a distance of 10 μ. A sphere of this radius could contain as many as 16 cells. At a distance of 1 mm from the particle the dose would fall to 0.2 rad.

It is felt from these preliminary findings that more work is needed in locating, measuring, and tracing these particles in human tissue, so that the ultimate disposition and radiation dose can be established.

AUDREY V. WEGST
CHARLES A. PELLETIER
G. HOYT WHIPPLE
School of Public Health,
University of Michigan, Ann Arbor

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Intercellular Diffusion

Abstract. *Fluorescein-sodium, a fluorescent tracer whose molecular weight is 376, diffuses rather freely from the interior of one cell to another in a gland epithelium* (Drosophila) *but does not diffuse along the intercellular space to the exterior. The permeability of the junctional surfaces of the cell membranes appears to be high, in contrast to the nonjunctional surfaces and intercellular spaces which represent strong diffusion barriers.*

A recent study on permeability properties of an epithelium (*Drosophila* salivary gland cells) revealed that small ions, such as K^+, Na^+ and Cl^-, move rather freely from one epithelial cell to another. The permeability of the epithelial cell membrane was found to be so modified at the junctional surfaces between cells that, in contrast to the rest of the cell surface, the junctional surfaces offer no substantial restriction to ion flow (*1*, *2*). We have now extended this study to ions of larger size with the aid of a fluorescent tracer, fluorescein-sodium.

Fluorescein-sodium (molecular weight 376) diffuses readily through cytoplasm, where it is detectable at very low concentrations ($10^{-7}M$, above *p*H 7). We have injected fluorescein-sodium into single cells with micropipettes and have followed its diffusion through a chain of cells in a beam of ultraviolet light. The salivary gland cells of *Drosophila* are arranged in a single layer. The cells are large (about 100 μ in diameter) and quite transparent to visible and ultraviolet light. Approximately 5×10^{-9} ml of fluorescein-sodium (10mM fluorescein in saline solution) were injected into a given cell. This represents about 1/70 of the (single) cell volume, a change in osmolarity of less than 0.1 percent for the single cell, and a change of less than 0.0005 percent after final dilution into the whole epithelium. Such injections appeared to have no damaging effects; the cell volume, the cell transparency, and the cell membrane potential remained unaltered.

The injected fluorescein-sodium is visible initially as a fluorescent bleb of a few microns in diameter around the micropipette. From there it spreads through the cytoplasms of the injected cell and adjacent ones (Fig. 1). Within 3 to 20 minutes all cells, except a few near the gland duct, become fluorescent.

The diffusion of the injected fluorescein appears to be strictly from cell to cell. There were no detectable leaks of the substance to the exterior. To observe possible leakage through the cell surface membranes or intercellular spaces, the epithelium was moved from time to time to a new position in the bathing solution, and the solution was scanned for fluorescence. This is a fairly sensitive method. When cells were punctured experimentally, leaks to the exterior were detectable through single holes of 1 to 3 μ diameter. In intact cells, fluorescein-sodium never appeared in the exterior, neither at the basal nor at the luminal sides of the cells.

Thus, while there is no substantial barrier to diffusion across the surface of the cell membrane at the junction between cells, there appears to be a strong barrier to diffusion across the

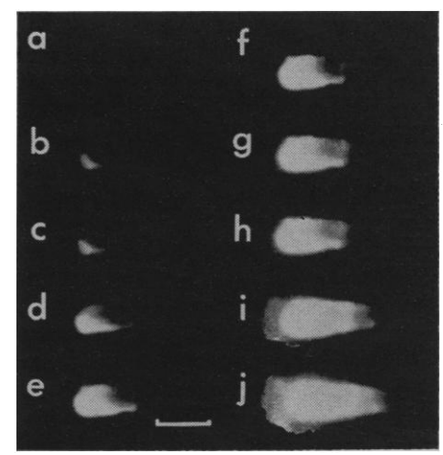

Fig. 1. Intercellular diffusion of fluorescein-sodium. The gland is isolated, placed in a physiological solution, and viewed under a compound microscope in an ultraviolet darkfield. Photomicrographs: *a*, at moment of injection of about 5×10^{-9} ml of fluorescein-sodium into one of the 200 cells of the gland; *b*, 2 minutes; *c*, 4 minutes; *d*, 6 minutes; *e*, 8 minutes; *f*, 10 minutes; *g*, 12 minutes; *h*, 14 minutes; *i*, 16 minutes; *j*, 18 minutes after injection. Note the absence of fluorescence in the solution bathing the gland. Calibration, 300 μ.