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.

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References and Notes

1. T. Schonfeld *et al.*, *Nature* **185**, 192 (1960).
2. J. Rondo *et al.*, *ibid.* **195**, 851 (1962).
3. The lung was obtained with the permission of Dr. A. James French, chief of the Department of Pathology, University of Michigan Medical Center.
4. G. A. Boyd, *Autoradiography in Biology and Medicine* (Academic Press, New York, 1955), chap. 6.
5. G. S. Hine and G. L. Brownell, Eds., *Radiation Dosimetry* (Academic Press, New York, 1956), chap. 16.

4 December 1963

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

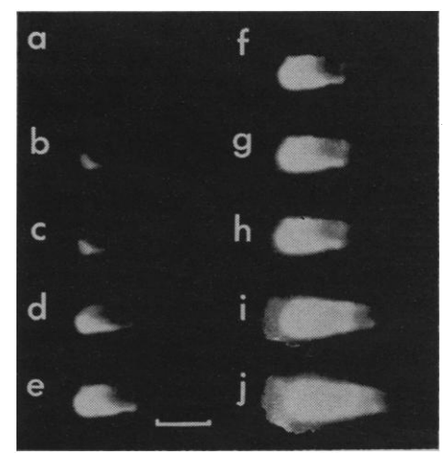

Fig. 1. Intercellular diffusion of fluorescein-sodium. The gland is isolated, placed in a physiological solution, and viewed under a compound microscope in an ultraviolet darkfield. Photomicrographs: *a*, at moment of injection of about 5×10^{-9} ml of fluorescein-sodium into one of the 200 cells of 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.

surface in contact with the cell exterior, and to diffusion in the direction of the intercellular space to the exterior. For small ions the latter barrier amounts to a resistance of, at least, 10^4 ohm cm^2 (*2, 3*).

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References and Notes

1. Y. Kanno and W. R. Loewenstein, *Nature* **201**, 194 (1964).
2. W. R. Loewenstein and Y. Kanno, *J. Cell Biol.*, in press.
3. For a comparison with other epitheliums, see M. G. Farquhar and G. E. Palade, *J. Cell Biol.* **17**, 375 (1963). For a morphological description of the present epithelium, see J. Wiener, D. Spiro, and W. R. Loewenstein, *J. Cell Biol.*, in press.
4. We are indebted to Drs. D. D. Potter and J. Nichols for suggesting fluorescein as a convenient tracer, and to the National Science Foundation and National Institutes of Health for financial support.

23 December 1963

Thermal Denaturation of Collagen in the Dispersed and Solid State

Abstract. *Thermal denaturation temperature of newly reconstituted collagen fibrils from rat tail tendons is 52°C compared with 42°C for neutral solutions. This suggests that the increase in concentration of collagen within the fibril increases the stability of the individual molecules. The absence of firm intermolecular bonds in these fibrils rules out crosslinking as an explanation for increased stability. "Aging" at 37°C up to 1 year raises the shrinkage temperature of reconstituted fibrous gels by 4° to 6°C and greatly increases resistance to dissolution at high temperature. The newly formed fibrils dissolve without shrinking, whereas older gels exhibit shrinkage before dissolution. Since nearly all extractable collagen is in the form of fibrillar aggregates in tissue, it is unlikely that thermal denaturation occurs at body temperature; therefore it could not be involved as a necessary stage in collagen resorption.*

The relationship between denaturation of collagen in the solid and dissolved state has physiologic significance, since it has been proposed that denaturation of extractable collagen in vivo at physiologic temperature is a necessary stage in the catabolism of collagen, preparing it for proteolytic digestion (*1*).

It is unlikely that any appreciable amount of collagen in vivo is in molecular, or even small aggregate, dispersion in the tissues since negligible amounts of collagen are extractable at physiologic temperature and *p*H. Neutral extractable collagen is, in all probability, in the form of fibrils which dissolve in the cold, much like the newly reconstituted thermal gels (*2*).

Denaturation of collagen, as now generally visualized, is a phase transition or "melting" of the highly ordered molecule to a random state. Shrinkage of collagen in the solid state and the denaturation of dispersed molecules are considered to be different manifestations of the same phenomenon. Flory and Garrett (*3*) showed that the temperature of denaturation is concentration-dependent above a certain minimum level, and that it exhibits a smooth transition from the dispersed to the solid phase.

In an effort to shed further light on the relationship between melting in the dispersed and solid state, the denaturation temperature of dissolved collagen at neutral *p*H was compared with the temperatures of shrinkage and dissolution of reconstituted fibrils of increasing age and diminishing extractability, and also with those of the native tissue.

Freshly removed tendons from young adult rats were dissolved in 0.1*M* acetic acid in the cold; the solution so obtained was clarified by sedimentation at 100,000*g* for 1 hour and then filtered. Clear solutions of collagen (approximately 0.2 g/100 ml) were prepared by reconstitution of the lyophilized material in phosphate buffer, *p*H 7.6, Γ/20.4, at 5°C, as previously described (*4*). Opaque rigid gels composed of striated fibrils were formed from the cold neutral solutions by warming to 37°C for 10 minutes (*4*). Some gels were formed in 0.2*M* and 1*M* NaCl at *p*H 6 to 7. Collagen gels, 40 × 3 mm, prepared in this manner in hematocrit tubes under sterile conditions, were incubated at 37°C for periods ranging from 15 minutes to 1 year before use.

The temperature of shrinkage was measured by suspending the tubes in duplicate or triplicate in a water bath in which the temperature was raised at a rate of 1°C per minute. Shrinkage of gels was readily apparent and could be measured on the scale of the tube. Final denaturation (dissolution) was manifested as a loss in opacity and the rising of bubbles through the clear solution.

Samples of whole intact tendons from the same animals were freely suspended in capillary tubes. After incubation under the same conditions as the gels, measurements of the shrinkage temperature were made in the same manner.

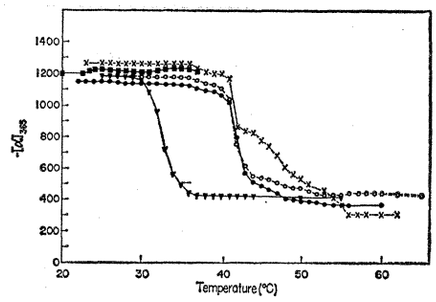

Fig. 1. Denaturation of collagen in solution measured by optical rotation. Solid triangles, acetate ionic strength, 0.15; *p*H 4.2. All other curves represent collagen in phosphate ionic strength, 0.4; *p*H 7.6 with varying concentrations of arginine. Closed circles, 0.4*M* arginine; open circles, 0.2*M* arginine; ×-×, 0.1*M* arginine; solid squares, no arginine.

Denaturation of collagen dissolved in phosphate and acetate buffers was measured by the change in optical rotation at 365 mμ again with a continuous temperature increment of 1°C per minute. Collagen concentrations, about 0.15 percent, were the same as those in the solutions from which thermally reconstituted gels were prepared. Thermal precipitation was prevented by the addition of 0.1 to 0.4*M* arginine (*5*). By comparing denaturation temperature over this range of arginine concentrations it could be shown that this agent did not greatly influence the results, nor did it affect the shrinkage and denaturation of the gels.

Reconstituted collagen gels incubated at 37°C for 1 hour or less dissolved completely on cooling to 5°C, indicating the absence of firm crosslinking between molecules; the newly formed

Table 1. Temperature of shrinkage and denaturation in solid state, in degrees Celsius.

Time of incubation	Reconstituted gels		Intact tendons	
	Shrinkage*	Denaturation†	Shrinkage*	Denaturation†
15 min	None	52	54–58	59
6 hr	45–52	52	56–58	58
22 hr	49–54	53–55	56–58	58
48 hr	50–53.5	54	57–59	59
10 days	49–53	58		
28 days	49–55	57		
375 days	53–56	None		

* Temperature at beginning and end of shrinkage. † Temperature of complete dissolution of gel or tendon.