Enteric Entry of Microcrystals.* (30347)

Hobart A. Reimann, Joseph E. Imbriglia and Thelma Ducanes
Departments of Medicine and Pathology, Hahnemann Medical College and Hospital, Philadelphia, Pa.

Crystals of tricalcium phosphate, the component of a dentrifrice, were in the enteric mucosa (Fig. 1), abdominal lymph nodes, lungs, myocardium and elsewhere in a patient who inadvertently had inhaled and ingested the substance for years and died of carcinomatosis(1). Questions arose as to whether particles in the intestinal lumen had entered the mucosa directly or had been deposited hematogenously after inhalation and passage through the lungs, and whether casual or continual exposure for years to small amounts of various microcrystalline substances contained in many household, medical diagnostic and therapeutic, and commercially used products is hazardous.

Experimental. Tricalcium phosphate contained in a popular denture cleanser was fed to one group of 6 white rats. To see whether other commonly used finely pulverized substances act similarly, silica flour (powdered sand, alpha quartz), the chief component of powdery kitchen cleansers was fed to a second group of rats. A third group ingested a barium sulfate preparation used for diagnostic skiagraphy. The size of the highly insoluble crystals of the substances ranged from 44 μ to less than 0.1 μ. There is evidence that particles smaller than 1 μ are the most dangerous(2). To prevent dust, inhalation and pulmonic entry, 50 g of the substances were mixed with egg albumin as a binder and mixed with 1000 g of macerated standard food pellets with 30 ml glycerin added to delay drying. The mashes were remolded into pellets and fed.

Rats ingested from 60 g to 100 g of the respective substances during 6 to 8 weeks without evident harm. Crystal-free food was fed for 4 to 10 days before they were killed. At necropsy, the enteric lumen was flushed with water to rid crystals from the mucosal surface. Sections of ileum were fixed and stained in the routine manner. They were examined with ordinary light and with phase-polarized light microscopy and a gamma quartz compensator. A 12.5$\times$ eye piece and a 10$\times$ objective were used for scanning; 40$\times$ and 100$\times$ objectives to ascertain that crystals were in the tissue, not on it, and to distinguish them from extraneous material. Electronmicrographs of specially fixed tissue were made at the Delaware Scientific Laboratories, Inc., Wilmington, Del.

Results. In rats that had ingested large amounts of the 3 substances, single and clumped fragmented crystals had entered the intestinal mucosa probably by phagocytosis. A cluster of sand particles is shown in a villus in an area of mild inflammation (Fig. 2). In Fig. 3, large and small crystals are impacted in a pouch surrounded by leukocytes, epithelioid cells and fibroconnective tissue. Fig. 4 is the same area enlarged and with polarized light. Large and small crystals are visible. Electron micrographs (Fig. 5 and 6) show what appear to be phagocytosed sand particles smaller than 0.01 μ in the microvilli and cytoplasm of an epithelial cell. Identification by means of X-ray diffraction analysis was obscured by changes incidental to fixation and staining. Particles of barium sulfate also entered the gastric, ileal, and colonic mucosa.

After penetrating the villi, crystals entered

FIG. 1. Human enteric mucosa; birefringent tricalcium phosphate crystal in a macrophage (1,250 $\times$). Polarized light and a first-order red-plate compensator by Dr. D. J. McCarty(1).

FIG. 2. Rat ileum. Villus with a cluster of 13 or 14 sand crystals the size of cell nuclei and smaller. Diffuse lymphocytic infiltration of the tunica propria. Areas of focal inflammation extended into the submucosa and muscularis. Crystal-free villi not inflamed. Polarized light.

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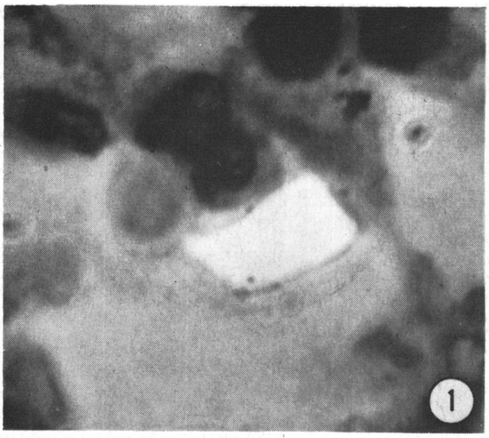

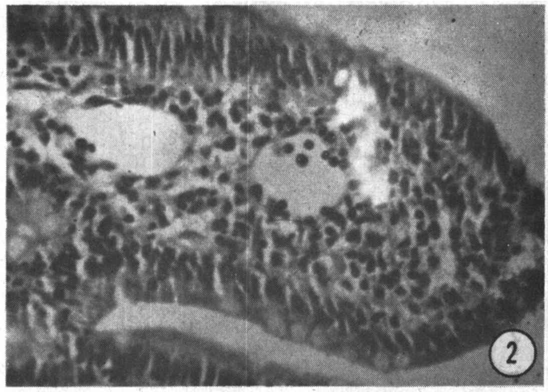

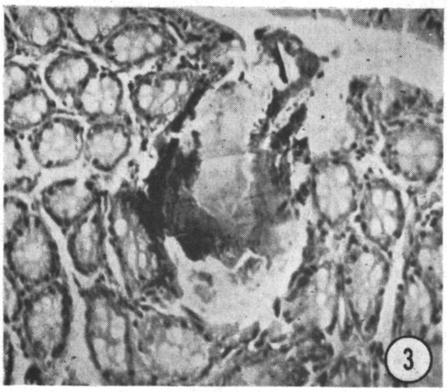

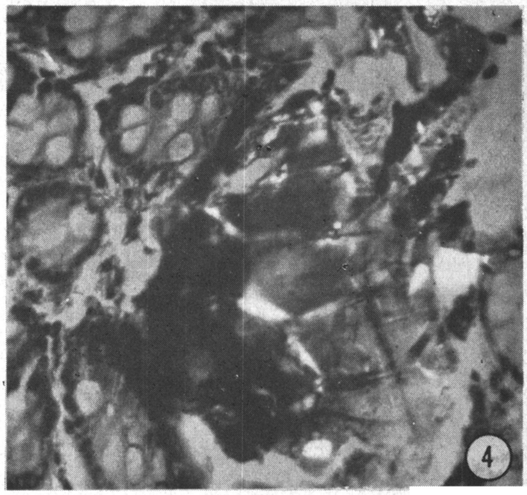

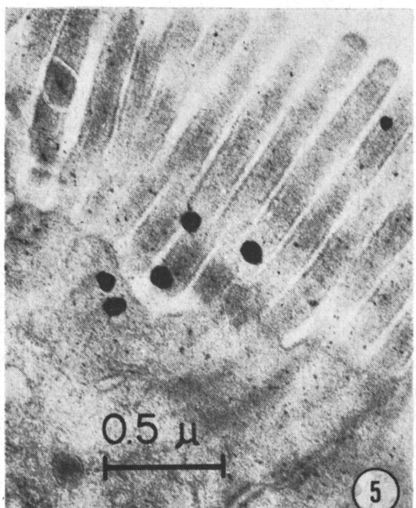

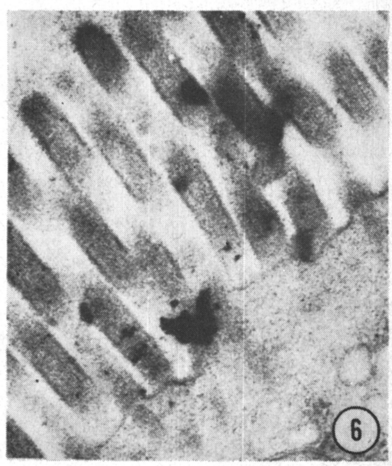

FIG. 3. Rat ileum. Sand impacted in a mucosal crypt. Mild granulomatous reaction surrounded by fibroconnective tissue and epithelioid cells.

FIG. 4. Area of Fig. 3. Polarized light shows large and small crystals.

FIGS. 5 & 6. Rat ileum. Particles of sand in microvilli. The particles resemble those of hydroxylapatite in tissue in Fig. 4 of Giacomelli, Spiro and Wiener, *Electronmicroscopy*, Fifth Internat. Congress, 1962: V 2; 6, and of phagocytosed carmine particles in vaginal epithelial cells(4).

the blood directly or by way of the lymphatic channels and thoracic duct or by both routes. Transit of particles through the intestinal wall probably was rapid and caused only mild inflammation. Crystals then were dispersed systemically and deposited in viscera including the lungs, myocardium, and brain. Free or phagocytosed crystals in normal tissue probably were of recent deposit, others excepting barium sulfate had provoked an inflammatory reaction or older granulomas (3).

Similar instances may occur in man more often than is suspected after long exposure to one or to several artificially pulverized substances. Four or five different substances may accumulate locally or systemically and cause lesions. For example, a person may use a dentifrice, talc powder, a kitchen cleanser, daily for many years and may take kaolin, an aluminum hydroxide-magnesium silicate or a bismuth preparation for medication, and have repeated diagnostic skiagraphic barium-meal studies made. These products are composed of micron-sized, insoluble, supposedly inert particles which are able to enter the body by the phagocytic action of enteric epithelial cells or through the lungs.

By electron microscopy, microbes also were shown in the sections of ileum. They had entered the epithelial cells and were phagocytosed in the cytoplasm(5).

Summary. Micron- and submicron-sized crystals of tricalcium phosphate, of sand and of barium sulfate ingested by rats entered the intestinal epithelium. They then were distributed and deposited in various organs where tricalcium phosphate and sand incited inflammation and granulomas. Crystals of tricalcium phosphate had caused similar changes in a patient. Particles of these and of other supposedly inert, insoluble substances contained in widely used products enter the body by way of the enteric and respiratory tracts after inadvertent exposure to them or by their deliberate use.

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Preservation of Living Cells at −79°C with Dimethyl Sulfoxide.* (30348)

BERTHA A. BOURONCLE (Introduced by Charles A. Doan)
Department of Medicine, Ohio State University, Columbus, Ohio

Dimethyl Sulfoxide (DMSO) has been known since 1867, its use being primarily as a solvent in the wood and pulp industry. More recently Dimethyl Sulfoxide has been shown to have protective action against freezing damage to a variety of living cells and organisms(1-7).

The present report is a study of the toxicity of DMSO, as well as the different factors influencing the effectiveness of this compound in the preservation of HeLa and Fl-amnion cells at −79°C. This is of importance due to the extensive use of these cells in many aspects of research. These observations may also be applied to the preservation of a broad variety of primary explants or lines of tissue culture cells, as well as of other tissues and organs.

Materials and methods. Each culture bottle, containing a 5-day culture of HeLa or Fl-amnion cells, is trypsinized, then centrifuged and the supernatant discarded. Ten ml of tissue culture planting media is added to the

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