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)

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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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TABLE I. Viability of Frozen HeLa and Amnion Cells as Determined by Supravital Stain (Neutral Red-Janus Green) and *in vitro* Growth After Replantation.

Method of storage at −79°C	Time of storage	Percentage of viable cells by S–V stain		Growth after 48 hr replantation		Growth after successive passages	
		HeLa	Amnion	HeLa	Amnion	HeLa	Amnion
None	Fresh cells	93	94	++++	++++	++++	++++
No DMSO	2–6 days	8	10	0	0	0	0
10% DMSO	2–6 "	92	93	++++	++++	++++	++++
Idem	13–18 "	93	91	++++	++++	++++	++++
"	1 mo	88	93	++++	++++	++++	++++
"	2 "	92	90	++++	++++	++++	++++
"	3 "	88		++++		++++	
"	4–6 "	70	82	+++	+++	++++	++++
"	10 "	61		+++		++++	
"	12 "	65	61	+++	+++	++++	++++
"	15 "	50		++		++++	

cells and mixed with a pipette.

The cells are counted in a hemocytometer. The cell suspension is again centrifuged for 10 minutes at 1000 rpm and the supernatant is discarded. Per each 1,000,000 cells per ml counted, 1 cc of the following "freezing solution" is added: Tissue culture media, 75%; human serum, 15%; DMSO, 10%.

One ml of the above cell suspension containing 1,000,000 cells per ml is placed into a 3 ml constricted neck "Neutroglas" ampule which is immediately sealed in a glass flame and placed directly into a Harris Freezer at −79°C.

In further experiments the procedure of freezing was the same but the proportion of cells or of DMSO was altered.

Cell viability studies. 1) Techniques of thawing and planting cells. The ampules are thawed rapidly with gentle agitation in a 37°C water bath. The cell suspension is centrifuged at 1000 rpm for 10 minutes and the supernatant is removed. The cells for each vial are resuspended in 10 cc of tissue culture media and placed into a tissue culture bottle in the incubator at 37°C. The degree of growth is microscopically observed at 24 hours and 48 hours and expressed as 0 to 4+.

2) Supravital staining technique with neutral red and janus green. We employed the technique described by Doan and Ralph(8), which has proved to be the most reliable and simple technique for viability studies of HeLa and amnion cells. The living cells show a normal morphology with the granules stained with neutral red. Early signs of degeneration are expressed by overstaining of the granules and mitochondria. Dead cells do not pick up the stain.

3) Technique for autoradiography. We used a modification of the stripping film technique described by Doniach and Pelc(9). The slides are stained with Giemsa. The number of cells incorporating H-3 thymidine is determined by counting 3,000 cells.

Results. Following the technique described, we have been successful in preserving HeLa and Fl-amnion cells at −79°C in 10% DMSO, 15% human serum and 75% tissue culture media for as long as 15 months (Table I).

Up to 3 months the microscopic growth of the cells on replantation after 48 hours was 4+ and the supravital stain showed from 88% to 93% of viable cells. From 4 to 12 months the microscopic growth of the cells on replantation after 48 hours was 3+ and the supravital stain showed from 61% to 82% viable cells. After 15 months the microscopic growth of the cells on replantation after 48 hours was 2+ and the supravital stain showed 50% viable cells. Both lines of cells, Fl-amnion and HeLa, preserved equally under identical conditions.

Autoradiography of amnion cells from the fresh culture bottle after 5 days growth, as compared to thawed frozen cells after 6 months storage, showed the incorporation of tritiated thymidine to be from 37% to 41%, thus showing that after months of storage the viability of frozen cells is similar to fresh aliquots.

TABLE II. Effect of Different Concentrations of DMSO in Preservation of HeLa and Amnion Cells at -80°C.

Conc of DMSO in storage at -79°C for 17 to 28 days (%)	% of viable cells by S-V stain		Growth after 48 hr replantation		Growth after successive passages	
	HeLa	Amnion	HeLa	Amnion	HeLa	Amnion
None	8	9	0	0	0	0
2	40	57	+	++	++	++++
5	84	90	++++	++++	++++	++++
10	86	92	++++	++++	++++	++++
15	50	53	++	+++	++++	++++
20	19	17	±	+	0	0
30	0	0	0	0	0	0

To determine whether the concentration of cells has any influence on the survival of cells preserved in 10% DMSO at —79°C, Fl-amnion cells in concentrations of 1,000,000 per ml to 10,000,000 per ml were frozen for 15 to 54 days. Regardless of cell concentration, microscopic growth of the Fl-amnion cells, 48 hours after replantation, was 4+ and the supravital stain showed from 90 to 94% of viable cells. The practical application of this experiment is that large concentrations of cells can be stored in one vial, saving valuable storage space in the freezer. On replantation, the number of tissue culture bottles to be replanted from each vial depend on the cell concentration in the vial. If the frozen vial contains 10,000,000 cells per ml, a total of 10 bottles are replanted after thawing.

In all our experiments we kept the human serum at a constant concentration of 15% during freezing. To determine what concentration of DMSO was most satisfactory in the preservation of HeLa and Fl-amnion cells, varying concentrations of DMSO were used, ranging from 0 to 30%. The results (Table II) show that optimum concentrations of DMSO were from 5 to 10%. The microscopic growth after 48 hours was 4+ and the supravital stain showed from 84 to 92% viable cells. Concentrations of 2% and 15% gave less satisfactory results.

In an attempt to determine the toxic level of DMSO to tissue culture cells growing at 37°C, various concentrations of DMSO (from 0 to 30%) were added to the culture media of each suspension of 1,000,000 cells per ml. The cells were then planted in tissue culture bottles and incubated at 37°C. The microscopic growth was evaluated after 24 and 48 hours' incubation. Inhibitory effect was found when the cells were incubated with DMSO in concentrations of 1.5%. Concentrations of DMSO of 5% or over produced complete inhibition of cell growth after 24 hours exposure.

In our next experiment, the Fl-amnion cells were exposed to different concentrations of DMSO for 1 and 2 hours at 4°C and 37°C. Exposure of the cells for 1 or 2 hours, to DMSO concentrations of 20% or over, showed a definite inhibitory effect in the growth of Fl-amnion cells.

Discussion. We have developed a technique for preservation of HeLa and Fl-amnion cells at —79°C with DMSO, which is simple and may be used in any research laboratory.

The optimum concentration of DMSO is from 5 to 10%. Prolonged exposure of the tissue culture cells to DMSO at 1.5 to 5%, or shorter exposure to higher concentrations at either 37°C or 4°C, are toxic to the cells.

We have found that the slow cooling rate essential in the preservation of other living cells or organisms, does not appear to be critical in preservation of HeLa and amnion cells. Under the conditions of our experiment, by placing the cell suspension in "freezing solution" directly into the —79°C Harris freezer, we were able to preserve HeLa and amnion cells for 3 months with 90% recovery; and for as long as 15 months with 50% recovery.

The viability of the preserved cells was tested by the microscopic observation of the growth of the cells in tissue culture bottles after replantation; by incorporation of tritiated thymidine; and by the neutral red-janus

green supravital technique. We have found that this latter technique is very reliable in predicting viability of cells and correlates closely with the microscopic growth after replantation. In contrast, the eosin exclusion technique proved to be unreliable.

It is possible that this simple technique can be applied for preservation of a broad variety of primary explants or lines of tissue culture cells, and for the preservation of other tissues and organs.

Summary. A simple and reliable technique for preservation of HeLa and Fl-amnion cells at −79°C with DMSO has been developed in our laboratory. The optimum concentration of DMSO is from 5% to 10%; using this concentration we have been able to preserve HeLa and Fl-amnion cells at −79°C for as long as 15 months. Large concentrations of cells as high as 10,000,000 per ml can be stored per vial. Studies of the toxicity of DMSO on tissue culture cells demonstrated that either prolonged exposure to low concentrations or shorter exposure to high concentrations of DMSO, at 37°C or 4°C, are toxic to the cells.

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Myosin Synthesis in Chronic Heart Failure.* (30349)

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Studies on the mechanism of chronic congestive heart failure have centered primarily on the identification of abnormalities in the energy production of the failing heart and on changes in the physicochemical properties of the contractile proteins(10). Recent work has failed to confirm alterations in molecular weight of myosin of failing hearts(9), but evidence has been presented suggesting alterations in energy metabolism of the diseased heart(6). The involvement of protein metabolism in congestive heart failure was suggested from experiments on animals with experimental left ventricular failure(8,5).

Presently an abnormality of the contractile proteins is still considered a possible factor in the pathogenesis of myocardial insufficiency, and an impairment in myocardial energy production could lead to defective protein synthesis and thus affect myosin. For these reasons investigation of myosin synthesis in the failing heart appeared to be of interest.

Material and methods. Left heart failure was produced in male rabbits of approximately the same age by constriction of the ascending aorta above the sinus of Valsalva

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