fresh growth medium. The suspended cells were then centrifuged for about 10 minutes at approximately 1000 rev/min.

This process was repeated twice; after the second washing the cells were suspended in 25 ml of growth medium in a 100-ml serum bottle and the viability was determined. Cell suspensions were then placed on the shaker, the cells were counted daily, and the medium was changed at various intervals until a maximum cell population was reached.

L cells, continuously cultured in a serum-free system and frozen and stored as described, gave the highest recovery of viable cells (90, 88, and 86 percent) in medium containing 4 percent DMSO (Fig. 1*a*). Furthermore, at 3-, 5-, and 6-percent concentrations of DMSO, 80, 81, and 84 percent, respectively, of the cells were viable after storage for 1 month.

Cells frozen in 1 percent DMSO gave evidence of survival but failed to grow after incubation in fresh medium. The trypan-blue dye-exclusion method indicated that all cells were dead after incubation for 3 days. Cells frozen without DMSO appeared to be dead immediately after thawing.

Eight percent DMSO provided the most consistent viability values for HeLa cells (87, 81, and 86 percent) after storage (Fig. 1*b*). With cat kidney cells, 4 pecent DMSO showed the best viability (74, 81, and 86 percent) throughout the storage period (Fig. 1*c*).

The peak growth yields of L, HeLa, and cat kidney cells after storage in 4, 8, and 4 percent DMSO, respectively, for 1 month were 51.0, 47.0, and 41.0 $\times$ 10^5 cells per milliliter, respectively (Fig. 2). These growth curves compare favorably with those obtained with unfrozen controls.

Thus L and cat kidney cells frozen in 4 percent DMSO and HeLa cells frozen in 8 percent DMSO gave higher recovery than was observed with the other concentrations of DMSO, but they also grew in the presence of DMSO near these optimum concentrations. The epithelial-like HeLa cells required higher concentrations of DMSO for optimum recovery than did the fibroblast-like L and cat kidney cells.

We have shown that it is possible to grow and preserve mammalian cells suspended in a simple chemically

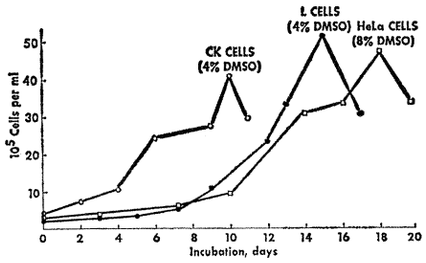

Fig. 2. Growth of L, HeLa, and cat kidney (*CK*) cells after storage in liquid nitrogen for 1 month at optimum concentration of dimethylsulfoxide (DMSO).

defined medium with DMSO as an additive in the complete absence of serum or serum products. The typical growth curves obtained from stored samples indicate that the conditions employed have not altered this important characteristic. These techniques might be applicable to the preservation of other materials (tissues, whole organs, and so forth) where protein-containing additives such as serum would be undesirable.

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References and Notes

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3. Kahlenberg-Globe Equipment Co., Sarasota, Fla.
4. BF-3, Biological Freezer, Union Carbide Corporation, Linde Division, 270 Park Ave., New York 10017.
5. Frozen Semen Products, Division of Cryo-Therm, Inc., R.D. #1, Breinigsville, Pa.
6. We adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

23 June 1965

Sulfur Dioxide in City Atmospheres

In "Air pollution affects pattern of photosynthesis in *Parmelia sulcata*, a corticolous lichen" [*Science* **148**, 1600 (1965)], L. Pearson and E. Skye report data (from a Warburg apparatus) which indicate that lichen disks in atmospheres contaminated by sulfur dioxide show "morphologic and photosynthetic abnormalities similar to those of lichens from an industrial center in Sweden." They suggest that "some kinds of lichens may be absent from city environments because of atmospheric pollutants which destroy chlorophyll." Although the authors are careful not to state unequivocally that the responsible atmospheric pollutant is sulfur dioxide, this is in fact the only pollutant studied in the report, and the inference is present that sulfur dioxide is in fact *the* pollutant. The concentrations of sulfur dioxide used in the experiments were 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 percent by volume (uncorrected for solubility in the water present). Sulfur is present in polluted atmospheres primarily as sulfur dioxide, sulfur trioxide, and sulfate. In a study of ten cities during 1953–54 (Ann Arbor, Michigan; Akron, Ohio; Charleston, West Virginia; Cincinnati, Ohio; Detroit, Michigan; Elizabeth, New Jersey; St. Louis, Missouri; Washington, D.C.; and Whiting, Indiana), sulfur dioxide ranged from less than 0.01 to 0.38 parts per million by volume, with an average of 0.06; corresponding total sulfate values were less than 0.01 to 1.22, with an average of 0.10 [J. Cholak, L. J. Schafer, W. J. Younker, D. Yeager, *A.M.A. Arch. Ind. Health* **11**, 280 (1955)]. These concentrations appear to be in the same range as similar data from other cities throughout the world. Effluent flue gases from coal-burning plants typically have sulfur dioxide concentrations of 0.1 to 0.3 percent by volume. Pearson and Skye's experimental conditions represent sulfur dioxide concentrations of 100 to 100,000 parts per million, values which range from about 260 to 260,000 times larger than the maximum concentrations actually found in urban atmospheres. The data shown in Pearson and Skye's Fig. 1 are for the highest sulfur dioxide concentration (100,000 parts per million), which leads one to suspect that their highest concentrations are required for significant effects. Atmospheric pollution is a complex problem of growing, worldwide importance. Because of the economic and political problems connected with it, scientists should be especially careful to present their results so as to avoid unwarranted conclusions. This could have been done in Pearson and Skye's report by including a comparison of the sulfur dioxide concentrations used in their experiments with typical concentrations found in polluted atmospheres.

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