the Periodic Table, the "inert gases," because of their presumed nonreactivity, have occupied an isolated position, apart from the other groups of chemical elements. Discovery that at least two of these elements readily form stable compounds opens the door to investigations which should more closely integrate this group with the rest of the Periodic Table.

C. L. CHERNICK, H. H. CLAASSEN
P. R. FIELDS, H. H. HYMAN
J. G. MALM, W. M. MANNING
M. S. MATHESON, L. A. QUARTERMAN
F. SCHREINER, H. H. SELIG
I. SHEFT, S. SIEGEL, E. N. SLOTH
L. STEIN, M. H. STUDIER
J. L. WEEKS, M. H. ZIRIN
Argonne National Laboratory, Argonne, Illinois

References and Notes

1. See for example, Mellor's *Modern Inorganic Chemistry*, G. D. Parkes, Ed. (Longmans, Green, London, 1961).
2. Based on work performed under the auspices of the U.S. Atomic Energy Commission.
3. A. Van Antropoff, K. Weil, H. Fraüenhof, [*Naturwissenschaften* **20**, 688 (1932)] claimed the synthesis of a krypton chlorine compound with the aid of an electric discharge. O. Ruff and W. Menzel [*Z. Anorg. Allgem. Chem.* **213**, 206 (1933)] attempted to make the fluorine compound by an analogous technique and found no evidence of reaction. D. Yost and A. L. Kaye [*J. Am. Chem. Soc.* **55**, 3890 (1933)] found no reaction between krypton and chlorine under ultraviolet irradiation and between xenon and fluorine in an electric discharge. Yost and Kaye pointed out that Pauling and others had suggested the possibility of such reactions and that their results were only tentative. However, Antropoff [A. Van Antropoff, H. Frauenhof, K. H. Krüger, *Naturwissenschaften* **21**, 315 (1933)] conceded a partial misinterpretation of his earlier results, and there has been little subsequent research.
4. C. D. Cooper, G. C. Cobb, E. L. Tolnas, *J. Mol. Spectry.* **7**, 223 (1961).
5. N. Bartlett, *Proc. Chem. Soc.* **1962**, 218 (1962).
6. At Argonne National Laboratory the reaction with platinum hexafluoride was duplicated and xenon was shown to react at room temperature with ruthenium hexafluoride, but not with uranium, neptunium, or iridium hexafluorides. When the ruthenium hexafluoride system was studied quantitatively, a larger than equimolar consumption of the hexafluoride was observed, and some reduction of the ruthenium seemed to occur. This suggested the role of a hexafluoride as a fluorine carrier and led to the studies with xenon and fluorine. Xenon also reacts with plutonium hexafluoride. The course of this reaction, however, has not yet been elucidated (M. Steindler and J. Fischer, Argonne Chem. Eng. Div., private communication).
7. H. H. Claassen, H. Selig, J. G. Malm, *J. Am. Chem. Soc.* **84**, 3593 (1962).
8. M. H. Studier and E. N. Sloth, *J. Phys. Chem.*, in press.
9. Other species found (for example, Xe, O_2, H_2F_2) can be explained without reference to xenon compounds.
10. Xenon which had been irradiated with slow neutrons in a nuclear reactor was used to prepare the xenon tetrafluoride used in the hydrolysis experiment described. The behavior of the xenon was followed by observing the gamma activity associated with the resulting mixture of radioactive xenon isotopes.
11. P. R. Fields, L. Stein, M. H. Zirin, submitted for publication.

2 October 1962

Azeotropic Freeze-Drying

Abstract. Spheres of comminuted frozen meat were freeze-dried by boiling in toluene under reduced pressure. The dehydration was performed at 30°C and apparently took place without melting the ice. The dried product rehydrated readily and showed all the characteristics of freeze-dried defatted meat.

Freeze-drying is possible without vacuum, so long as the water vapor pressure of the medium surrounding a frozen specimen is below the vapor pressure of ice at that temperature. This process was demonstrated in the dry air freeze-drying experiments of Meryman (*1*) and Lewin and Mateles (*2*).

Azeotropic mixtures are composed of two or more compounds that distill together without decomposition or reaction. The boiling point of an azeotropic mixture is constant, and is lower than the boiling point of its constituents. The composition of the azeotropic vapor depends upon the molecular weight and vapor pressures of the mixture components at a given temperature. The purpose of our work was to test the feasibility of azeotropic freeze-drying.

At 0°C water and toluene form an azeotrope with a vapor pressure of 12.1 mm-Hg. Of this pressure 4.6 mm represents the vapor pressure of ice and 7.5 mm the vapor pressure of toluene. The composition of the azeotrope is 10.7 percent water and 89.3 percent toluene. If the temperature is increased, but the water is still frozen, the composition of the azeotrope changes. The vapor pressure of the solvent increases, while the vapor pressure of water depends on the temperature of ice. The azeotropic mixture becomes unsaturated. The degree of saturation depends on the rate of heat supply to sublimate the ice, and on the rate of vapor removal from the boiling flask. The vapor pressure at the solvent-ice contact is greater than the vapor pressure of solvent in other parts of the flask. Thus, violent boiling occurs around a frozen specimen. Due to the temperature difference between the condensor and the boiling flask, the azeotrope is rapidly removed. Heat of 620 cal is necessary to sublimate 1 g of ice at 0°C, and since the vapors are rapidly removed, it is not likely that the ice will melt. These postulates were tested experimentally.

Approximately 300 ml of toluene were placed in a 700-ml round bottom flask and heated to 30°C. The flask was fitted with an insulated Bidwell-Sterling receiver arm, a condensor, a manometer and a vacuum line. A 7-g sphere of frozen (−15°C) comminuted meat, about 1.5 cm in diameter, was placed in the flask and a vacuum was drawn immediately. When the pressure reached 33 mm-Hg (in approximately 60 seconds) the contents of the flask surrounding the meat sample came to a vigorous boil. Vapors rosè to the condensor, where they separated into water and toluene. Water settled in the Bidwell-Sterling receiver while toluene returned to the flask. At the end of 90 minutes, 4.8 ml of water had accumulated in the receiver, and the boiling subsided. At that time air was allowed to enter the system. The sample was withdrawn and placed for 45 minutes in an air oven at 101°C to dry off the solvent.

After drying in the oven, the sample had the typical porous structure of freeze-dried meat. The weight of the dry material was 1.5 g and its dimensions appeared unchanged. Upon immersion in water, the material promptly rehydrated to the consistency and appearance of very lean, raw, ground meat. Its taste was that of uncooked beef.

HUGO E. WISTREICH
JEREMY A. BLAKE
Research and Development Laboratory, Preservaline Manufacturing Company, Flemington, New Jersey

References

1. H. T. Meryman, *Science* **130**, 628 (1959).
2. L. M. Lewin and R. I. Mateles, *Food Technol.* **16**, 94 (1962).

26 April 1962

Simple Apparatus for Ultrafiltration

Abstract. A rapid and highly efficient ultrafiltration apparatus has been devised from readily available laboratory materials costing less than $5. Employing a fritted glass tube to support an evacuated dialysis bag, it accommodates as little as 1 milliliter and is ideally suited to dialysis of small volumes and concentration of macromolecules for chromatography and electrophoresis.

In our laboratory it is routinely necessary to concentrate protein solutions for electrophoretic analysis and to remove protein from an amino acid solution for chromatographic analysis. These two operations are carried out simultaneously with the apparatus shown in Fig. 1. The grommet (Walsco Electronics, No. 7034-F; inside diameter, ¼ in.; mounting hole, ⅜ in.) is

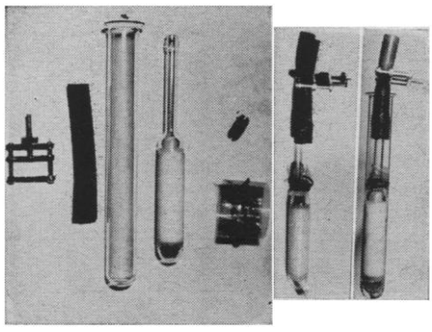

Fig. 1 (left). Materials for the ultrafiltration apparatus. (Left to right) Clamp, vacuum tubing, test tube, fritted immersion filter, grommet, dialysis tubing, and rubber band. Fig. 2 (right). Ultrafiltration apparatus (left) assembled, showing how the rubber band is used to seal the dialysis bag to the grommet, and (right) complete with close-fitting test tube; 2 ml fills the tube to the level of the grommet.

positioned as close as possible to the fritted portion of the immersion filter (Thomas, No. 5151-C; fritted length, 2 in.). The moistened dialysis tubing (diameter when inflated, 5/8 in.) is formed into a bag by tying a knot in one end and is gently pulled over the moistened filter and grommet. A rubber band is wrapped around the bag two or three times, forcing the dialysis tubing into the groove of the grommet to provide a vacuum-tight seal (see Fig. 2, left).

The assembly is then evacuated with a water aspirator and sealed with the clamp. Wetting the entire bag during evacuation helps to obtain maximum vacuum. The material to be concentrated or dialyzed is placed in a test tube of a size determined by the volume of liquid. A thin-wall test tube (Thomas, No. 9444; 150 × 18 mm) of 18-mm outside diameter is suitable for 1 or 2 ml of liquid. If proteins are to be concentrated, the completed assembly (Fig. 2, right) is placed in the refrigerator. In about 2 hours 75 percent of the liquid will be inside the bag. It can be removed by inserting an 8-inch length of thin plastic tubing (Adams, intramedic No. PE 205) and withdrawing the liquid into a syringe (fitted with a 16-gauge needle for use with the designated tubing). The assembly can be re-used if it is stored in water to prevent drying out of the bags.

DONALD A. BURNS
JOYCE N. BUDNA
JOAN M. CHAMBERLIN
Dover Medical Research Center, Dover, Delaware
8 March 1962

Defect in Small Millipore Filters Disclosed by New Technique for Isolating Oral Treponemes

Abstract. Three Millipore filters (100, 50, and 10 mμ) can effectively and easily be used to isolate oral spirochetes from a mixed oral inoculum. The spirochetes appear pure in the underlying agar, owing to their ability to grow through the filter. Their passage through the 10-mμ filters may be attributed to the instability of this pore size on storage.

Strains of spirochetes will filter or grow through porcelain, paper, and collodion filters into underlying agar or broth media (*1*). The pore size of these filters was sufficiently large to permit passage of the slender, motile spirochetes through the filter (*2*). Membrane filters with more uniform pore sizes (Millipore Filter Corporation, Bedford, Mass.) have been used by us in experiments intended to separate oral treponemes from a mixed oral inoculum, and to determine the smallest pore size that would permit passage of the spirochetes (*3*).

The 0.45- and 0.3-μ filters were sterilized by autoclaving at 15 pounds for 15 minutes; the 100-, 50- and 10-mμ filters were sterilized by high voltage irradiation (MF types HA, PH, VC, VM, VF-white-plain-47 mm) before purchase. The filters were placed on the surface of PPLO medium (Baltimore Biological Laboratories) containing 1.2 percent agar, with sterile forceps, touching only one peripheral area of the filter. The inoculated filter plates were incubated in the upright position, anaerobically, in Brewer jars, in an atmosphere of 95 percent H_2 and 5 percent CO_2 at 37°C for a 5- to 8-day period. A drying agent, $CaCl_2$, was placed in each jar to remove excess water.

For primary isolation of spirochetes, freshly obtained debris from the gingival crevice of man was used. This mixed inoculum was collected in tubes containing 2 ml of PPLO broth or phosphate buffer (*p*H 7.0) and dispersed on a Vortex mixer. It was inoculated with a Pasteur pipette as approximately 0.05-ml drops on the filter surface within 30 minutes after collection. Oral bacteria, as well as spirochetes, were found beneath 0.3- and 0.45-μ filters, demonstrating the inadequacy of these pore sizes in the mechanical separation of treponemes from a mixed oral inoculum. When filters measuring 100, 50 and 10 mμ were used, spirochetes appeared as multiple foci of dense growth beneath most inoculation sites (Table 1). Occasionally, a Gram-positive coccus or rod was recovered from beneath the 100-mμ filter. These contaminants on subculture were capable of growing into the agar medium. When the oral inoculum contained swarming organisms, they would cover the entire surface of the filter and adjacent agar but would not migrate under the filter from the periphery.

A laboratory strain of *Treponema microdentium* with a diameter consistently greater than 100 mμ as determined by electron photomicrographs was employed to study this grow-through phenomenon in greater depth. This particular strain grows on most commercially available media when in association with an anaerobic diphtheroid (strain JB3B) and a fusiform (strain JF_5) (*4*).

This combination was prepared for inoculation by removing agar, containing spirochetes and supporting organisms, from 5-to-10-day-old well plates with a sterile scalpel. This material was introduced into a glass tissue grinder and homogenized with 3 ml of PPLO broth. The resulting mixture was placed as single drops from a Pasteur pipette at five or more sites on each filter surface. The spirochetes appeared beneath the 100-, 50-, and 10-mμ filters at most inoculation sites, with results varying with shipments of filters (Table 1).

Experiments were designed to determine whether the spirochetes were growing through the filter or migrating laterally to by-pass the filter. No migration of bacteria or spirochetes beyond the edge of the filter and back under it to the site of spirochetal proliferation was ever observed. The hazes continued to appear when the filter borders were sealed with sterile paraffin, or when the filter edge extended beyond the support of the underlying agar. The pattern of the haze below the filter corresponded to the outline of the inoculum on the filter surface. When spirochetes and sup-

Table 1. Passage of spirochetes through millipore filters. Data are given in terms of haze/inoculation site.

100-mμ filter (VC)	50-mμ filter (VM)	10-mμ filter (VF)
	Oral debris	
12/34*	2/10	8/12
15/15*		
	Treponema microdentium†	
4/21*	55/55*	106/112*
117/117*	1/45*	17/140*
90/90*	0/20*	

* Results obtained with different shipments of filters. † In conjunction with supporting organisms, JB3B and JF5.