cigarettes a day or more—about 0.205 percent per year (*20*)—would, on this basis, be about 1300 rem over a 25-year period. A dose of this magnitude from polonium is probable only in localized areas of the bronchial tree, but the causes of lung cancer may not be identical in the two cases (particularly in the Schneeberg miners whose deaths occurred before cigarette smoking was widespread) because of the presence of strong cocarcinogens in cigarette smoke (*21*). Because of the well-known synergistic action of ionizing radiation and cigarette-smoke extracts, or other chemical agents, in experimental cancer production (*22*), the presence of these chemical promoters might lead to cancer from radiation doses at least an order of magnitude less than the figure of 1300 rem. A dose of 100 to 200 rem to the bronchial epithelium may be highly significant, therefore, and even doses at the lower estimate of 36 rem may not be negligible if the dose-response curve for cancer induction is linear for alpha-emitting substances. This general comparison is independent of the relative biological effectiveness chosen for alpha particles.

We support the view that other chemical factors, particularly cocarcinogens, as well as physiological effects, such as alterations of ciliary activity by cigarette smoke, probably play an important part in the genesis of bronchial cancer in smokers. Our present conclusion is that Po^{210} inhaled in cigarette smoke may act as an important initiator in the production of bronchogenic carcinoma.

EDWARD P. RADFORD, JR.
VILMA R. HUNT
Department of Physiology, Kresge Center for Environmental Health, Harvard School of Public Health, Boston, Massachusetts

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23 December 1963

Novel Filter for Biological Materials

Abstract. *Thin plastic sieves with precisely controlled hole size and density can be made by irradiating plastic films with fission fragments and etching out the material traversed by the fragments. These filters may be used for the nondestructive separation of cells of closely similar sizes.*

Studies of radiation-damage tracks in certain solids (*1*) have recently led to a method of drilling fine holes of adjustable size and number in thin sheets of these solids (*2*). The method has now been extended to a number of plastics which are commercially obtainable in sheet form. From plastic film it is now possible to produce fairly large numbers of filters which have important advantages over conventional filters in certain research fields such as cytology and bacteriology.

Briefly, the technique is to bombard a plastic sheet at near normal incidence with fission fragments, which produce continuous trails of radiation-damaged material on the sheet. Fine holes are formed by preferentially dissolving the damage trails in a suitable reagent, which then continues to enlarge the holes at a uniform rate until the desired size is reached. The number of holes is equal to the number of fission particles traversing the plastic film (*2*).

Only heavy, high-energy particles produce continuous trails of chemically altered material. Alpha particles from a Po^{210} source, although not suitable for most plastics, so alter the local structure of cellulose nitrate that holes form (Fig. 1). Lighter particles such as protons, electrons, x-rays, and γ-rays do not produce this effect.

Filters with hole sizes ranging from about 1 μ or less up to about 10 μ and with about 2 percent open area have been made from 0.013 mm (1/2 mil) films of polycarbonate resins and polyester resins. Filters have also been made from a number of experimental plastic films which are not yet commercially available. Extremely uniform holes are formed in polycarbonate film after irradiation and etching (see cover photograph). Figure 2 is a graph of hole diameter plotted against etching time in NaOH solution for an 0.013 mm film. The rate of hole enlargement increases with stirring rate, reagent concentration, and temperature.

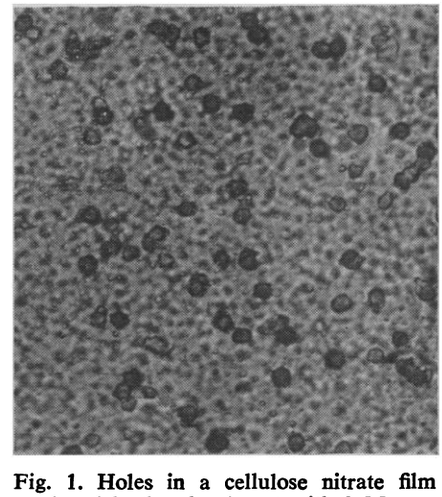

Fig. 1. Holes in a cellulose nitrate film produced by bombardment with 3 Mev α-particles followed by a 3-minute etch in 6*N* NaOH aqueous solution at 75°C.

These filters must be thin enough ($\lesssim$ 0.018 mm) to be penetrated by the bombarding particles, but filters which are several centimeters in diameter and only 0.006 mm thick are quite tough and can support a large weight of liquid without the necessity for a supporting framework. Because of the random positions of the holes, the fraction of the filter surface which is open cannot exceed about 2 percent without some overlap of holes. With increasing porosity the efficiency of a filter in separating particles of closely similar size is impaired.

Various plastics which are suitable for filters resist attack by strong acids, weak bases, and many organic solvents such as acetone and xylene. Some of them can also be used at temperatures up to approximately 175°C and, therefore, can be conveniently sterilized.

Irradiated and etched plastic filters have a number of advantages over conventional filters of fiber and metal mesh. The number and diameter of holes can be easily adjusted by varying the irradiation time and etching time. The holes are circular cylinders which have exactly the same diameter within the resolution of an optical microscope. For 6-μ holes the variation in diameter is less than 0.5 μ. Because of the ideal geometry of the holes, the filter does not become clogged and delicate particles such as blood cells can be filtered without destruction by gravitational action rather than by application of a differential pressure. Because of the transparency and chemical resistance of these sieves, materials such as cells and substrates can be collected, stained, and viewed *in situ*. Specifically, the study of the structure of blood cells is facilitated because cells are flattened onto the top surface of the filter prior to fixation and staining.

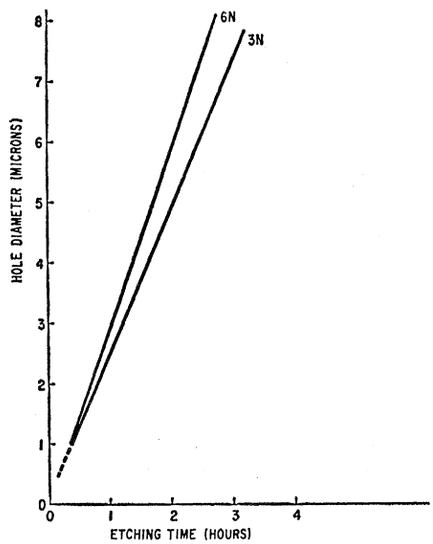

Fig. 2. Etching characteristics of polycarbonate film in unstirred aqueous solutions of 6*N* and 3*N* NaOH at 75°C. The film thickness decreases as the hole size increases, so that a film containing 6-μ holes has been reduced in thickness by about 6 μ.

Recently, with H. M. Rozendaal, we have used 6-μ plastic sieves to separate HeLa cells (diameter about 10 μ) which had been added to human blood. Experiments now in progress by S. H. Seal (Sloan-Kettering Institute) have as their goal the nondestructive filtration of free-floating cancer cells from the blood of patients harboring malignant disease (*3*). Because of the precise uniformity of hole sizes in irradiated and etched filters, it should be possible in cytological studies to separate large numbers of cells of uniform sizes to facilitate the study of cell growth and multiplication.

R. L. FLEISCHER
P. B. PRICE
E. M. SYMES
General Electric Research Laboratory, Schenectady, New York

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Precipitins in the Rabbit Produced by Protein Polysaccharide from Bovine Nasal Cartilage

Abstract. *Precipitins have been produced in the rabbit by injecting protein polysaccharide from bovine nasal cartilage in Freund's adjuvant. These antibodies did not precipitate either protein polysaccharides extracted from other species, or the separated protein and carbohydrate moieties of the antigen. It is postulated that the antibodies are directed toward determinants consisting of carbohydrate and amino acid residues linked in three-dimensional arrangements specific for different protein polysaccharides.*

In the past decade, several unsuccessful attempts have been made to demonstrate the antigenicity of acid mucopolysaccharides (*1*). The failure of hyaluronic acid and chondroitin sulfate (either injected alone or absorbed on different bacteria) to induce production of specific antibodies in the rabbit is not surprising, in view of the fact that preparations of these mucopolysaccharides obtained from different species have proved to be chemically identical.

Recent studies have demonstrated that chondroitin sulfate exists in cartilage firmly bound to a complex protein structure of which keratosulfate, neutral sugars, and sialic acid also seem to be part (*2*), and that hyaluronic acid, isolated from human synovial fluid, contains a small but firmly bound protein moiety (*3*).

On the basis of these findings, chondrotin sulfate and hyaluronic acid may be considered very large prosthetic groups of conjugated proteins whose tissue or species specificity may be determined by the structure of the protein moiety or by the secondary and tertiary structure of the complex as a whole. This report shows the production of antibodies against such complexes.

Protein polysaccharide was extracted from nasal septa of steers 11 to 14 months old. Isolation, purification, and fractionation into "light" and "heavy" fractions were achieved according to the techniques described by Schubert and co-workers (*4*).

Sixty milligrams of unfractionated complex, such as potassium salt (Table 1), were dissolved in 5 ml of saline solution, then emulsified with 15 ml of Freund's adjuvant containing 25 mg of *Mycobacterium phlei* to which merthiolate was added to insure a 1:10,000 final concentration. Intramuscular injections of portions corresponding to 1 mg of protein polysaccharide were given at weekly intervals to 2.5-kg male white albino rabbits. The total amount of antigen administered was 4 mg. Four weeks after the last injection, the rabbits were bled and the serum obtained (containing merthiolate to 1:10,000 final concentration) was absorbed with fresh calf serum in order to remove possible antibodies to fractions of bovine serum protein. It was then assayed for preci-