lower in light and moderate smokers of cigarettes, a somewhat younger group on the whole, than in the other groups. It is clear that cigarette smoking does not tend to promote development of disorders that may contribute to the development of arteriosclerosis.

The mean and median total-blood cholesterol levels (determined by the Zak method), that were known for the various groups studied are listed in Table 4. These determinations were made for the most part during hospital admissions for various illnesses and therefore do not represent usual blood levels of cholesterol during periods of good health. Cholesterol levels for patients with severe liver disease or biliary obstruction were excluded from the tabulations. It may be seen that there are no striking differences in the mean and median values for any of the groups, although both are higher by about 20 to 30 mg per 100 ml in heavy smokers of cigarettes or of pipes and cigars than in nonsmokers. None of the values is above the accepted upper limits of normal. It is possible, nevertheless, that the higher incidence of severe sclerosis of the aorta and the slightly higher incidence of myocardial infarction in cigarette smokers may be related to these slight differences in blood cholesterol concentrations.

The results of this analysis based on necropsy statistics indicate that if an association exists between smoking practices and the development of arteriosclerosis or lesions resulting therefrom, it is at best tenuous and inconclusive. A sizable minority of heavy smokers of cigarettes seem to develop sclerotic changes in their aortas at a faster rate than nonsmokers and tend to have slightly higher blood cholesterol levels. The incidence of myocardial infarction is only very slightly higher in heavy smokers of cigarettes than in nonsmokers, and there is no consistent rise in the incidence of such lesions with degree of cigarette smoking. The incidence of other types of lesions related to arteriosclerosis is not affected by smoking habits.

The findings do not preclude the possibility that heart attacks due to myocardial infarction may be more severe clinically, and more often fatal during their acute phase, in heavy smokers of cigarettes than in nonsmokers (*7*).

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8 October 1962

Synthetic Acrylic Gel: A New Medium for the Study of Immune Precipitates

Abstract. The physicochemical properties of photocatalyzed Cyanogum gel offer practical advantages in the analysis of specific immunological precipitation techniques: use of any buffer at any *p*H; most probable neutrality for stains or reagents; remarkable resolution of the immunological precipitates, visible microscopically; possibility of direct dark-background examination of the precipitates, so that their kinetics can be studied and they can be photographed before dyeing and staining. This gel seems particularly suitable for immunoanalysis of complex and unstable antigenic products, such as tissue homogenates.

The electrophoretic study of hemoglobin by means of a synthetic gel, Cyanogum, was reported in 1959 by Raymond (*1*). Interesting results were also reported (*2*) with the use of Cyanogum gel for the electrophoretic separation of human serum. However, this gel has not yet been utilized for immunological studies. This report describes its use as a medium for the double diffusion of antigen and antibody to form immune precipitates, and includes a description of a method for photopolymerization of the gel which avoids the use of the β-dimethylaminopropionitrile catalyst (*3*).

The uncatalyzed Cyanogum 41 (*4*) is a mixture of two organic monomers: acrylamide and N,N′-methylenebisacrylamide. The catalysis of this mixture results in linear polymers of acrylamide, joined by three-dimensional "methyl" bridges. Two essential physicochemical properties of Cyanogum, "gel" consistency and hydrophilia, result from its "spongy" structure and the exclusive presence of amide groups along the chains (*5*).

A constant thickness of the gel and an adequate horizontal position of the glass plate, which supports it, are easily achieved by allowing the whole to rest on a layer of mercury (Fig. 1). The adhesiveness of Cyanogum gel to the glass plate is usually excellent when the suggested concentrations are used, and if the glass is not strongly scoured. No preliminary coating of the glass plate is needed.

Because of its elasticity, polymerized Cyanogum is cut with difficulty. The troughs and reservoirs needed for immunological studies are formed by means of molds or templates, preferably made of Plexiglas (methacrylate), which are placed in the unpolymerized solution. These molds are easily removed from the gel, especially when they have been coated with a thin layer of siliconated oil (*6*).

The Cyanogum gel concentrations that permit adequate diffusion of proteins are between 3 and 10 percent with an optimal range between 4 and 5 percent for most common procedures. At higher concentrations, the

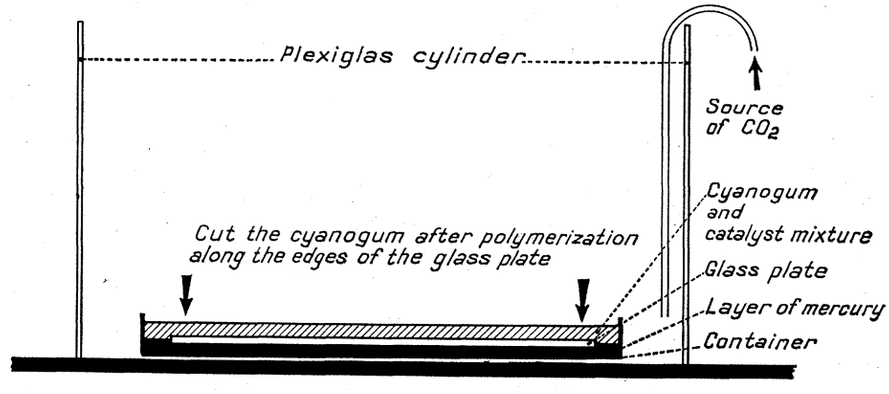

Fig. 1. Vertical section of the apparatus designed for the photopolymerization of regularly thin layers of Cyanogum gel in CO_2 atmosphere.

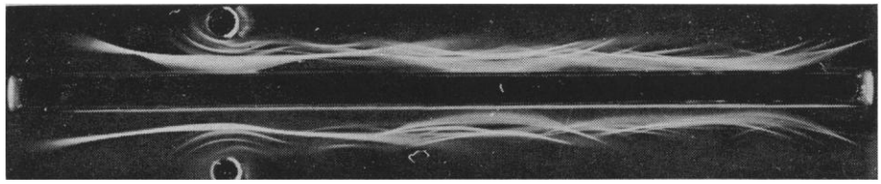

Fig. 2. Photograph of immunoelectrophoresis in 4.25-percent Cyanogum gel (140 × 90 × 1 mm), polymerized by photocatalysis, with the use of borate buffer of *p*H 7.5. Electrophoretic separation of the antigen: 320 volts for 60 minutes (40 ma), anode in the right. Antigens: 6 μl of normal human serum concentrated 2 times by means of lyophilization (upper reservoir); 6 μl of normal human serum in normal concentration (lower reservoir). Immune serum: 0.2 ml of duck specific antihuman serum.

rate of diffusion of proteins is not only decreased generally, but that of certain protein categories is completely arrested.

The catalyst system (photocatalysis by riboflavin), for 100 ml of 4.25-percent Cyanogum, consisted of a freshly prepared mixture of the following: stock solution of 30-percent Cyanogum, 13.98 ml; 0.64-percent solution of $Na_2S_2O_8$, 0.74 ml; 0.005-percent solution of riboflavin (*7*), 11.68 ml; water or buffer, 73.6 ml.

Since polymerization is initiated by light, the gel is exposed to a 6-minute period of lighting with two 500-watt photoflood lamps, well centered and placed at 0.40 m above the Cyanogum gel. The photocatalysis of thin layers of Cyanogum is correctly achieved only in the presence of a CO_2 atmosphere. However, the role of CO_2 is not only to suppress the air-oxygen inhibitory action, since its replacement by an N_2 atmosphere does not result in identical photocatalysis. The specific effect of CO_2 is probably due to the transitory decrease in *p*H, which returns to the initial level a few moments after the gel is reexposed to atmospheric air.

Slight qualitative and quantitative differences in immune precipitates are frequently observed between identical gel plates, depending upon whether they have been exposed to daylight or kept in a dark chamber (*8*). Darkness also prevents the nonspecific precipitates around unstable products, a frequent observation after many days of immune reaction. The photocatalytic action of riboflavin does not seem to alter the immune precipitates once they are formed.

After elimination of the excess proteins by a 48-hour immersion of the gel strips in a water bath, enhancement of the precipitated bands is obtained either by overprecipitation with dilute ethanol or ebullition, or by use of the standard staining procedures of ordinary agar gel. Washed and dyed gel strips can be kept in the hydrated form for a long time or they can be plasticized according to the following method. The gel strips are immersed for 2 hours in an aqueous solution of 2-percent glycerin. Then they are covered with a sheet of cellophane previously dipped in the glycerin solution, avoiding the trapping of any air bubbles. Finally, they are dried at 40°C for a few hours. The resulting films are smooth and translucid, and can be kept indefinitely.

Immunological study of small quantities of normal human serum by means of a specific horse antiserum (*9*), when the reservoirs are separated by a distance of 4 mm, results in the formation of 11 different precipitates. The immunoelectrophoretic study of 6 μl of normal human serum by means of a specific duck antiserum shows 25 different bands of precipitation (Fig. 2). Excellent immunological precipitates are observed with 1-mm-thick strips of Cyanogum gels, with or without preliminary electrophoresis at 4.25 to 4.5 percent concentration, and with the use of borate buffer (*p*H 7.5; 0.14*M*). When the reservoirs of the antigens and the immune sera are separated by a distance of 4 mm, the first immune precipitates appear after 3 hours. They correspond to the most rapidly diffusible and the most concentrated antigens. After 72 hours, all the immune reactions are completed and definitive. This prolonged period calls for the maintenance of the gels in a humid atmosphere.

Because of their particular physical and chemical properties, Cyanogum gels may have numerous practical advantages for the study of immunological precipitations, with or without preliminary electrophoretic fractionation. These include: the possible utilization of any buffer solutions of any *p*H; the lack of affinity toward dyes or reagents; the high capacity for resolution of even microscopic immune precipitates. For these reasons, Cyanogum gels seem especially well adapted for the immunological study of unstable biological materials with complex antigenicity, such as tissue homogenates (*10*).

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1 October 1962

Visual Observations of Nightglow from Manned Spacecraft

Abstract. The luminous band around the horizon noted by J. Glenn in the first U.S. manned orbital flight is attributable to airglow. Dip-of-the-horizon measurements on the star γ Ursae Majoris showed that the band is centered at an elevation of 91 kilometers or somewhat higher. The edge-on brightness of the airglow layer was 6×10^{-7} candles per square centimeter.

The luminosity of the night sky in places remote from artificial illumination was discovered a long time ago and has been studied by many researchers in many countries. The sources of the light are radiations and scattered sunlight from interplanetary space, radiation and starlight from intersteller space, and atomic and molecular radiation from the earth's atmosphere, generally from above the ozonosphere. These atmospheric sources of light are either the aurorae or the night airglow. The physics of these phenomena has recently been comprehensively reviewed by J. W. Chamberlain (*1*). One