

estimated distance to the Crab Nebula of 2 kparsec (6). If the sources are at this distance, then the average electron density in the interstellar medium is ≤ 0.028 electron cm^{-3} (7). If most of the electrons are close to the sources, then the interstellar electron density is even less.

The time variations of the sources are also unusual. The pulses tend to be isolated, although in NP 0532 they have been observed as close together as 0.21 second; NP 0527 is further distinguished by the brevity of its active periods, generally lasting no more than a few minutes during the observing period (15 minutes).

If the sources each have a unique period, then these periods must be less than 0.25 and 0.13 second for NP 0527 and NP 0532, respectively. These limits were determined by checking whether the time intervals between adjacent pulses were consistent with any single period. The upper limits on the source periods were constrained by the time resolution of the observations. If observations with greater time resolution

fail to yield unique periods for these sources, they then constitute a new class of radio sources distinct from the highly periodic pulsars. In Fig. 3 are shown those pulses that were observed on 21 October 1968. No pattern is evident.

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8. We are grateful for the assistance of W. D. Brundage and K. A. Braly and for the support of the National Radio Astronomy Observatory.

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27 November 1968

Surface Structure of Polymers: Glancing-Angle Electron Diffraction Study of Polyethylene

Abstract. *The surface regions of polyethylene nucleated and crystallized in contact with both a high-energy surface (gold) and a low-energy surface (fluorinated ethylene-propylene copolymer Teflon) have been examined by means of glancing-angle electron-diffraction techniques. Examination of these surfaces has been confined to a maximum depth of 120 angstroms. The surface region of the polyethylene generated in contact with the gold is considerably more crystalline than the surface generated in contact with the fluorinated ethylene-propylene copolymer Teflon. These results tend to corroborate recent wettability and infrared studies. Apparently, the surface structure of polyethylene is highly dependent upon the method of preparation.*

Wettability (1) and infrared (2) examination [attenuated total internal reflection (ATR)] of polymer surfaces, particularly polyethylene, have indicated that when a polymer is nucleated and crystallized in contact with a low-surface-energy phase [for example, vapor, fluorinated ethylene-propylene copolymer (FEP) Teflon, and so forth] an amorphous surface layer is generated, and when a high-surface-energy phase (for example, gold, metal oxide, and so forth) is used, a crystalline interfacial region is produced in the polymer when the melted polymer solidifies. In the above experiments, it was important to (i) provide for ex-

tensive wetting of the substrate by the melted polymer, (ii) maximize the area of the solid-liquid interface, and (iii) minimize the contribution of the liquid-vapor interface.

Since wettability determinations are confined to the outermost surface layer, the change in wettability when nucleation is initiated by a high-energy surface is attributed to a variation in surface density. Although an increase in the density of the surface layer may be interpreted as an increase in the extent of crystallinity, no direct evidence for crystallinity can be obtained from a wettability measurement. The evidence obtained from a detailed analysis of the

infrared reflection of the absorption bands at 720 and 730 cm^{-1} offers firm proof that crystallinity in the surface region of polyethylene is enhanced when nucleated in contact with a high-surface-energy phase. However, since the depth of penetration of the infrared reflection experiments (2) is about 2 μ (3), considerably thicker than the surface layer (about 10 to 100 Å), we explored the possibility of using other nondestructive techniques which are confined to the surface layer (< 100 Å) to corroborate the wettability results. With glancing-angle electron diffraction, we examined the surface layer structure (about 60 to 300 Å in depth) to ascertain the extent of crystallinity.

A detailed account of the experimental variables and an analysis of the glancing-angle electron-diffraction technique is found elsewhere (4). An RCA-EMD-2 electron-diffraction unit operated at 50 kv was used. During glancing-angle electron diffraction, the beam is incident at angles of 0.02 to 0.1 radian. In order to eliminate prolonged exposure of the polymer film to the electron beam, we determined a proper angle of tilt on trial samples (the beam being stationary while the holder has 3 degrees of freedom). This enabled a diffraction pattern to be obtained in 2 (maximum 3) seconds.

The structure of the surface region of a high density (0.95 g cm^{-3}) polyethylene compound (Marlex 5003, Phillips Petroleum Co.) was examined. Two distinct types of surfaces were prepared. One specimen (treated) was nucleated and crystallized in contact with a high-energy surface (gold), whereas the other specimen (untreated) was nucleated and crystallized in contact with a low-energy surface (FEP Teflon). Both polymer films were prepared by being placed in contact with these surfaces for ½ hour at 200°C in nitrogen, and then being cooled slowly (1°C min^{-1}). It is important that the substrates be removed from the polyethylene by nonmechanical means so as not to damage the surface region which was generated by contact with the substrate. The gold was effectively removed by amalgamation with mercury.

The effectiveness of this method of removal is shown by the complete absence of any diffraction pattern due to gold. The FEP Teflon-polyethylene composite fell apart when cooled to room temperature. Both ATR and wet-

tability indicate that no FEP Teflon remained on the polyethylene. If a vapor phase had been used instead of the FEP Teflon to generate the polyethylene surface, similar results would have been obtained except that the extent of surface roughness would have precluded examination by glancing-angle electron diffraction. Both wettability and infrared analysis experiments have indicated that the surface region of the polymer that crystallized when in contact with the gold was crystalline, whereas the specimen that solidified in contact with FEP Teflon remained noncrystalline.

Diffraction patterns were taken of the treated as well as untreated surfaces. In all cases the diffraction patterns obtained from the treated surfaces show several dense bands. These bands indicate definite changes in order-development of structure other than that of the bulk polymer. The diffraction patterns from untreated surfaces show no structure at all.

The calculated interplanar spacings, considering the broadness of the rings, at 4.20, 3.06, 2.5, and 2.26 Å, correspond quite closely to Bunn's (5) data for polyethylene, at 4.15, 3.72, 2.98, and 2.48 Å. From the diffraction data, the crystallite size is estimated to be 20 to 30 Å in diameter.

Inhibition of Cell Growth in vitro by Adenosine 3',5'-Monophosphate

Abstract. Adenosine 3',5'-monophosphate, at a concentration of 40 micrograms per milliliter, inhibits the growth of HeLa and strain L cells in culture. The inhibition becomes progressively greater during the incubation of the cells. Adenosine 5'-monophosphate and adenosine, metabolites of adenosine 3',5'-monophosphate, do not affect the growth of either cell culture. This suggests that adenosine 3',5'-monophosphate enters the cell without alteration. Dibutyl-adenosine 3',5'-monophosphate, reported to have a greater activity than adenosine 3',5'-monophosphate on several tissues, inhibited the growth of the cells much less.

The action of steroids on cell cultures has not been extensively studied; however, they generally inhibit the growth of cells in culture (1). Hydrocortisone inhibits the growth of several human cell lines (2) and also inhibits the growth of chick heart fibroblasts and L cells (3). Arpels, Babcock, and Southam examined the effects of eight steroids on 12 human cell cultures, and cytotoxic effects were observed after incubation of the cell cultures for several days (4).

Adenosine 3',5'-monophosphate (3',

Glancing-angle electron diffraction confirms the earlier results obtained with wettability and infrared techniques. The nature of the interfacial region, with respect to crystallinity, is related to the substrate used to generate the polymer surface.

The glancing-angle electron diffraction not only furnished clear evidence of structural changes in the treated polyethylene film, but also proved that the substrates used in the formation of the film were removed completely. Thus, the increase in wettability of these films (1) is not due to traces of remaining substrate but is due solely to morphological changes in the surface structure of the polymer.

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26 September 1968; revised 19 November 1968 ■

HeLa cells (line 229) were maintained in Eagle's medium (9). Both Eagle's and Waymouth's mediums were supplemented with 5 mg of neomycin sulfate, 0.2 mg of amphotericin B, and 10 ml of fetal calf serum per 100 ml of solution. The pH of the medium was adjusted to 7.6 at the beginning of all experiments.

L cells, obtained from 4-day-old stock cultures, were detached by scraping, and the HeLa cells were detached by treatment of the 4-day-old cultures with 0.1 percent trypsin (1:300, Baltimore Biological Laboratories). The cells were suspended by agitation, centrifuged, resuspended in fresh medium, and counted in a Coulter counter. Two milliliters of the medium containing 50×10^3 cells were pipetted into Leighton tubes.

Adenosine 3',5'-monophosphate (Calbiochem, lots 73435 and 72982) and N⁶-2'-O-dibutyl-adenosine 3',5'-monophosphate (Boehringer-Mannheim, lots 6068310 and 06477308) were dissolved directly in prepared culture medium and filtered through a prewashed Millipore filter.

The addition of 3',5'-AMP (0.1 mg/ml) to the tissue culture medium inhibits growth of L (Fig. 1) and HeLa cells. This inhibition becomes progressively more severe with time; the L cell count in the 3',5'-AMP is 58 percent of the control at 2 days and 16 percent of the control at 4 days. The HeLa cell count is 55 percent of the control at 2 days and 27 percent of the control at 4 days. The addition of 5'-AMP to the cell cultures does not significantly inhibit growth. Adenosine was also added at the same molar concentration and it had no effect on the growth of the cell culture. The dose response to 3',5'-AMP was investigated (Table 1), and at a concentration between 0.04 to 0.01 mg/ml the inhibition disappears.

To determine whether the growth retardation is the result of permanent cell damage or is reversible, we grew L cells in a Blake bottle in medium containing 3',5'-AMP (0.1 mg/ml). After 4 days of incubation, the control cells and those in 3',5'-AMP were counted. The cell count in the 3',5'-AMP flask was only 14 percent of that in the control. The 3',5'-AMP-treated cells were washed, suspended in fresh culture medium, and placed in Leighton tubes; they were then counted after incubation for 2 days and 4 days. Cell counts were compared to those for control cells not in 3',5'-AMP. The 3',5'-

5'-AMP) has been proposed as an intermediary or second messenger in the action of estrogens (5), epinephrine, glucagon, adrenocorticotrophin, luteotrophic hormone, and serotonin (6). Because the steroids inhibit growth of cell cultures, we determined whether 3',5'-AMP would mimic this effect.

Strain L cells (NCTC clone 929) were maintained in Waymouth's medium MB752/1 (7), modified by replacing the salt solution with the tris (hydroxymethyl) amino methane-citrate salt solution described by Paul (8).