length should diminish with prolonged incubation, but also demonstrates that this technique can be used to prepare tri-, tetra- and pentanucleotides of defined sequence.

After studying reaction conditions and chromatography techniques we developed the following procedure for routine preparations of large amounts (about 100 μg) of oligonucleotides. In a 0.1-ml reaction mixture were 10 to 30 mM NDP; 10 mM magnesium acetate, 0.2M glycine buffer at pH 9.3, 0.1 mM $CuSO_4$, 0.40M NaCl, 7.5 mM dinucleotide primer, and 0.12 mg of enzyme per milliliter (*9*). The reaction mixture was incubated at 33°C for 14 to 24 hours, the longer time being necessary for the polymerization of purine nucleoside diphosphates. The course of a typical reaction is shown in Fig. 3, where ApU is used to prime the polymerization of ADP. Samples were removed from the reaction at the indicated times and applied to chromatography paper. The chromatograms were developed overnight with a solvent composed of equal parts of 95 percent ethanol and 1M ammonium acetate. The appearance of the dried chromatograms under a source of ultraviolet light is reproduced in Fig. 3. At zero time the only visible components are ADP, ApU and a trace of 5′-AMP which usually contaminates commercial preparations of ADP. After 2 hours of incubation the polymerization of ADP has leveled off at about 50-percent incorporation, the commonly misnamed "equilibrium" state. A subsequent 22 hours of incubation results in a redistribution of chain lengths, and during this process the true equilibrium state is approached. In order to remove excess ADP, which partially overlaps the ApUpA region, the reaction mixture is heated for 1 minute at 100°C to denature polynucleotide phosphorylase, and it is then treated with 20 μg of purified alkaline phosphatase for 2½ hours at 37°C. In large-scale preparations of oligonucleotides, the entire reaction mixture may now be applied to paper for chromatography in the usual way (*1*). Dried chromatograms are soaked for 1 hour in absolute ethanol in order to remove ammonium acetate, and the soaking is followed by a 10-minute wash in ether. The various oligonucleotide components are then cut out, eluted with H_2O and lyophilized.

We tested purity of oligonucleotide fractions by hydrolyzing a portion in alkali and determining the reaction products. In one experiment, alkaline hydrolysis of ApApU gave only AMP-2′, 3′ and uridine in a ratio of 2.05:1.00; in a second experiment, hydrolysis of ApApUpU gave only AMP-2′, 3′; UMP-2′, 3′; and uridine in a ratio of 2.16 : 1.05 : 1.00. These results not only show the high degree of purity of the oligonucleotide fractions but also demonstrate the reliability of the determination of chain length on the basis of chromatographic mobility.

This technique is exceedingly useful for the preparation of large (0.1 to 1 mg) amounts of many tri-, tetra- and pentanucleotides of defined base sequence. Of the NDP's that we have studied in this reaction (ADP, UDP, CDP, IDP, XDP), all give excellent results. Poor yields obtained with GDP are due to the very slow phosphorolysis of the polyguanylic acid chains originally synthesized, and this in turn results in low yields of the shorter chains.

Oligonucleotides synthesized in this fashion have been valuable in studies of coding specificity, in both the specific binding of aminoacyl-sRNA to ribosomes (*10*) and in polypeptide synthesis (*11*).

R. E. THACH
PAUL DOTY

Department of Chemistry, Harvard University, Cambridge, Massachusetts

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6. Only the polymerization kinetics of purine nucleoside diphosphate have been studied in detail. Preliminary studies on the kinetics of pyrimidine nucleoside diphosphate polymerizations have indicated similar behavior, but specific exceptions to the general rule may occur (*8*). In any case, the methods developed here for the synthesis of tri-, tetra-, and pentanucleotides apply equally well to both purines and pyrimidines.
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9. NaCl is included specifically for the purpose of depressing the rate of the polymerization reaction which proceeds in the absence of primer (*1, 4*). The extent to which this concentration of salt inhibits the formation of long polymer chains (*4*) under the conditions employed is not significant.
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12. This work, as well as that previously reported (*1*), was supported by the National Institutes of Health grant HD-01229.

23 March 1965

Glossopteris Discovered in West Antarctica

Abstract. *Leaf impressions from the Polarstar Formation in the northern Ellsworth Mountains are the first* Glossopteris *and the oldest identifiable plant fossils reported from West Antarctica. Their occurrence in a thick, probably marine, geosynclinal sequence in close association with coals and probable glacial deposits increases the similarity between the late Paleozoic history of Antarctica and the other southern continents.*

Three geologic expeditions, sponsored by the University of Minnesota, to the Ellsworth Mountains have made discoveries in the Polarstar Formation which now demonstrate the existence of *Glossopteris* in West Antarctica. In 1961 to 1962 the party collected several argillite specimens with bedding plane markings within the lower 15 m of the formation on the east side of Mt. Wyatt Earp (Fig. 1) in the northern Sentinel Range. The markings are mainly smooth, structureless, linear impressions or low ridges 1 to 2 mm wide and up to 9 cm long. They might be interpreted as animal trails, ice crystal impressions, or plant stems, but their existence indicated the possible presence of fossil plants in the formation. The following season definite plant stems with cell structure and carbonaceous matter were found in slates and argillites higher in the formation on Mt. Weems and on the ridge to the north. Continued searching in the area with helicopter support during 1963 to 1964 culminated in the discovery of abundant *Glossopteris* leaves in the upper part of the exposed Polarstar Formation at 77°33′15″S, 85°54′W, on a ridge 2.4 km northeast of Polarstar Peak. This *Glossopteris* site lies near Mt. Ulmer, considered the highest peak in the Sentinel Range by Ellsworth when he first viewed the mountains from the north during his 1935 flight across West Antarctica.

The general geology of the Ellsworth Mountains has been described (*1*); most exposed bedrock consists of intensely folded metasedimentary rocks. Metamorphism is low grade, but fair cleavage is present locally and quartz-chlorite-epidote veins are common. In the southernmost Sentinel Range and to the south at a few localities in the Heritage Range igneous rocks occur as lava flows and tabular mafic intrusives. The metasedimentary rocks total a minimum of 12,000 m in thickness and are considered mainly Paleozoic in age (*2*). A fossiliferous Upper Cambrian limestone near the middle of this sequence crops out in the Heritage Range, but mainly younger strata are exposed in the Sentinel Range. These younger beds have been divided into, in ascending order, the Crashsite Quartzite, the Whiteout Conglomerate, and the Polarstar Formation.

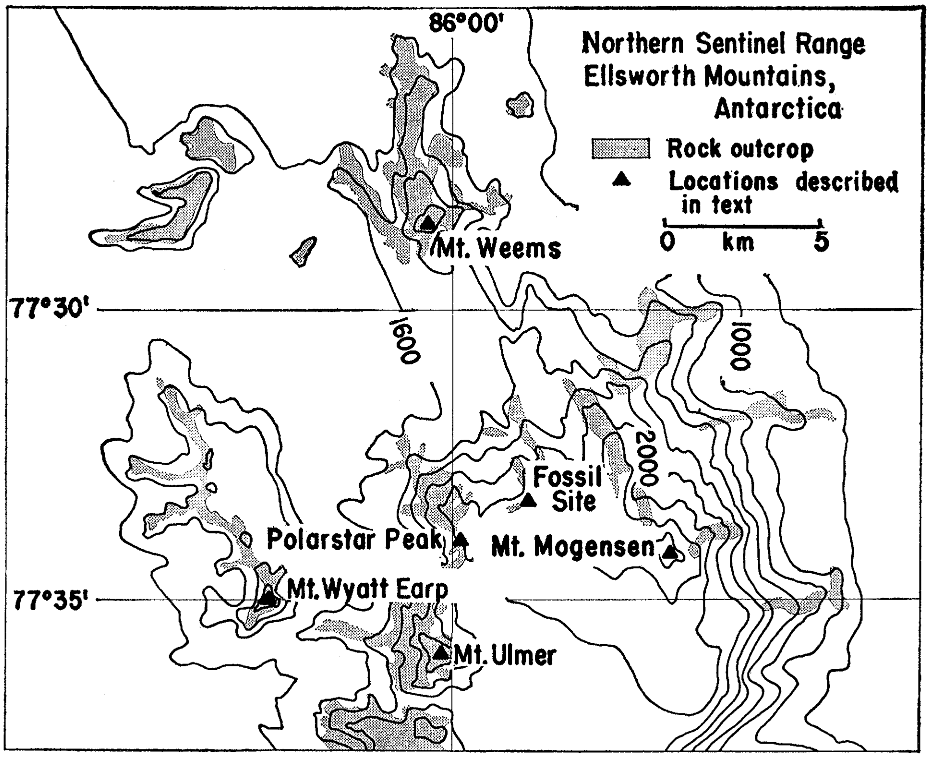

Fig. 1. Locality map of northern Sentinel Range, Ellsworth Mountains, West Antarctica. Contour interval, 200 m. [Map after U.S. Geological Survey Newcomer Glacier Sheet, 1960]

The Whiteout Conglomerate crops out in a discontinuous belt along the east side of the Ellsworth Mountains extending from the north end of the Sentinel Range to the central Heritage Range, a distance of 307 km. This formation's most distinctive characteristics are its great thickness, its massiveness, and its lack of sorting. The total thickness is at least 760 m, and no systematic variation has been detected along the strike. Careful study of well-exposed contacts suggests the Whiteout Conglomerate is conformable with both the underlying and overlying formations. Except for a few shaly or silty lenses up to 15 m thick the formation is massive and lithologically homogeneous. Many large outcrop areas are devoid of bedding, and only a rare weathered surface reveals faint lamination in the conglomerate. The detrital fraction of the formation displays a size range from clay-sized particles to a 5.4-m boulder; in a typical locality the fragments larger than 5 mm comprise about 10 percent of the rock. These boulders and large fragments consist mainly of quartzite, slate, phyllite, schist, marble, granite, quartz, and feldspar; some boulders are striated. Most boulders are rounded or subrounded, but the smaller fragments are rounded to angular, and mainly angular. The finer matrix of the rock is approximately half angular fragments of the same composition and half cementing aggregate of sericite, calcite, chlorite, quartz, and clay minerals. The Whiteout Conglomerate is actually a thick conglomeratic graywacke that probably was formed in quiet water from materials rafted by glacial ice, possibly under conditions similar to those on the Antarctic shelf today. The age of the formation is considered middle or late Paleozoic on the basis of the Cambrian fauna much lower in the section and the tentative Permian age assigned to the overlying formation.

The Polarstar Formation crops out only in the Sentinel Range east of the Whiteout Conglomerate belt. Its minimum exposed thickness is estimated at 1500 m from three incomplete measured sections on Mt. Ulmer, Polarstar Peak, and Mt. Weems. This formation consists of a clastic sequence of interbedded dark gray silty argillites and arenaceous rocks, which are mainly graywacke. Bedding is distinct and ranges from lamination to several feet in thickness; graded bedding is common in the coarser units, and some cross-beds, current ripplemarks, and slump structures also occur. The argillites consist of a very fine-grained dark matrix in which rest a few coarser grains of quartz, mica, or rock fragments. Pyrite and resinous matter are common, and carbonaceous fragments are abundant; two argillite specimens gave definite positive tests for carbohydrate-like material based on a phenol-sulfuric acid test (*3*). The graywackes are composed of fragments of quartz, plagioclase, mica, volcanic rocks, and metamorphic rocks in a fine matrix of sericite, chlorite, clay minerals, and opaque (carbonaceous?) material. Coal beds up to 30 cm thick are common in the upper quarter of the formation. The Polarstar Formation probably accumulated under nearshore marine conditions, perhaps in a prodeltaic environment. It is provisionally assigned a Permian age.

The original *Glossopteris* leaf discovery on the ridge northeast of Polarstar Peak was in beds near the top of the exposed Polarstar Formation. A 381-m section was measured along the ridge, with the highest beds at the southern end near the discovery site; an estimated 60 m of younger strata in the syncline to the west constitutes the highest known unit in the formation and in the mountains. While the sections were measured glossopterid leaves were collected from six units. The positions of these units, in meters above the base of this section, and their fossil contents are:

1) 67 to 71.5 m, argillite, abundant bronze-colored leaf impressions up to 11.3 cm long on one bedding surface.

2) 128 to 131 m, argillite with thin coal beds, common carbonaceous plant-stem fragments up to 7.5 cm long and 7.5 cm wide, rare incomplete leaf impressions up to 7.5 cm long on argillite bedding surfaces.

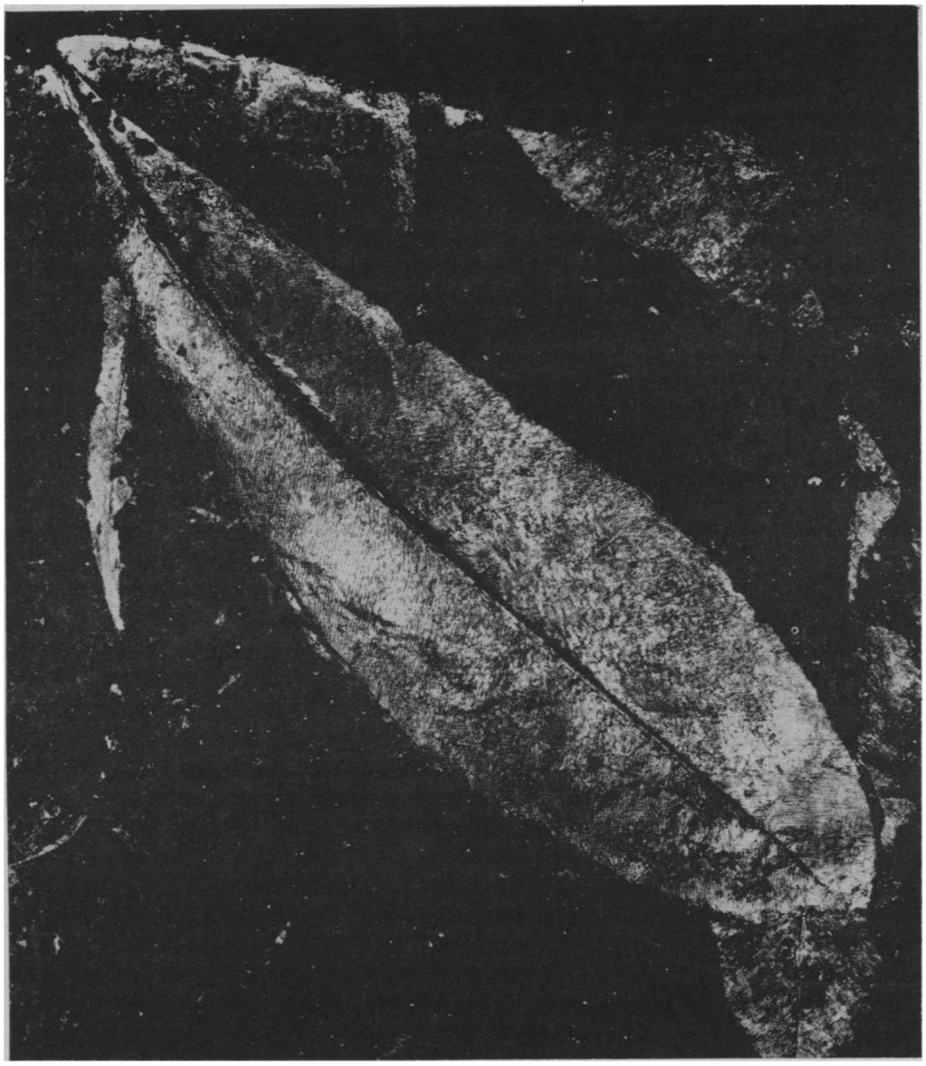

Fig. 2. *Glossopteris* leaf impression in argillite of the Polarstar Formation, Sentinel Range, Ellsworth Mountains, West Antarctica. The leaf is 16.9 cm long and has a bronze-colored organic coating emphasized in this high-contrast photograph. [Thomas Bastien]

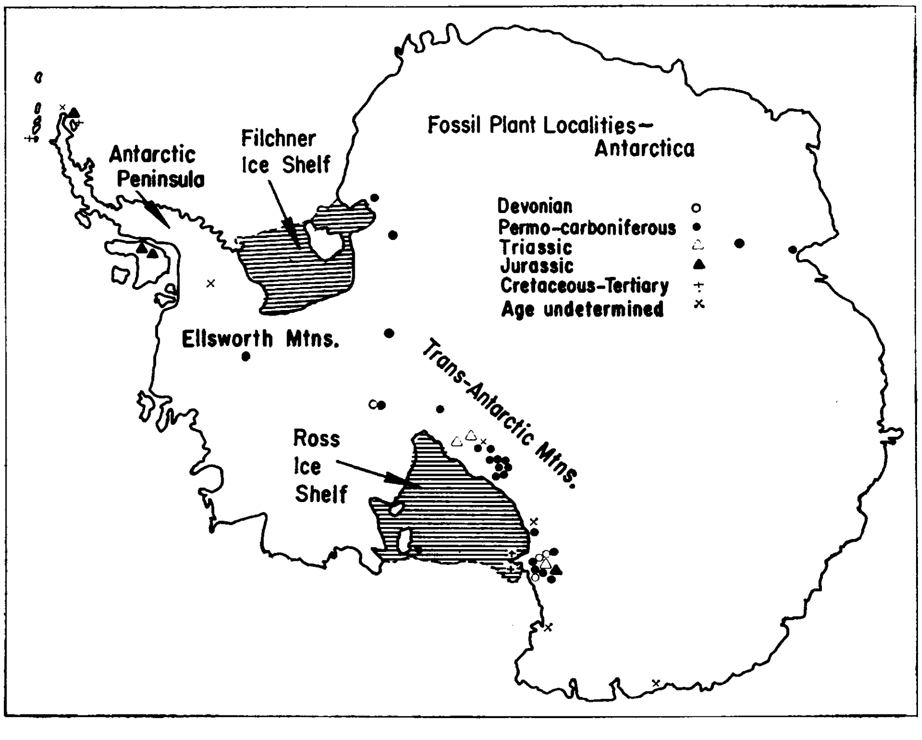

Fig. 3. Fossil plant localities in Antarctica. [After Plumstead (*11*)]

3) 180 to 187.5 m, argillite with thin coal beds, abundant leaf impressions up to 15.6 cm long on most argillite bedding surfaces.

4) 206 to 209 m, argillite with thin coal beds, incomplete leaf impressions up to 10 cm long on argillite bedding surfaces within the uppermost 30 cm.

5) 227 to 236 m, argillite with coal beds up to 30 cm thick, poorly preserved leaf and stem impressions in argillite.

6) 261.5 to 352 m, three argillite units separated by massive graywackes, abundant and complete leaves up to 20 cm long in argillite, mainly in the lower 30 m.

Unit 6 includes the original leaf discovery site and yielded the bulk of the material collected, including the specimen in Fig. 2. The entire collection is now under study by J. M. Schopf, who reports after preliminary examination of part of the material: "The most common fossils are leaves of the genus *Glossopteris*. Evidently several species are represented, among which I would suggest preliminary comparison with *G. damudica* Feistmantel, *G. ampla* Dana, *G. indica* Schimper, and possibly *G. angustifolia* Brongniart. The taeniopteroid form which resembles *G. damudica* seems to be most common and, therefore, this assemblage has a little different aspect from others I have previously studied. Several small leaves similar to those which have been identified with *Gangamopteris cyclopteroids* Feistmantel are present and may have the greatest stratigraphic significance, since the absence of a midrib is a feature associated with the older stratigraphic range of these glossopterid plants. There is a possibility that one form which lacks a midrib may be specifically distinct. I have seen no elements which would mark this collection as younger than Paleozoic and my preliminary impression is that Permian fossils are represented" (*4*).

These fossil plants are significant in being (i) the first reported occurrence of *Glossopteris* in the geologic province of West Antarctica, (ii) the eighth fossil plant collection from West Antarctica and the first not obtained on the Antarctic Peninsula (Fig. 3), and (iii) the first identifiable pre-Jurassic plant remains from West Antarctica. The geographic source of the original leaves is unknown, but this is the first evidence for the extension of the *Glossopteris* floral province into the region on the Pacific side of the Transantarctic Mountains. In the Ellsworth Mountains *Glossopteris* occurs in a thick section which probably represents the folded, marine, geosynclinal equivalent of the thinner, flat-lying formations that yield a similar flora in the Transantarctic Mountains. On Polarstar Peak, *Glossopteris* is found in argillite interbedded with thin coals; the formation overlies a thick conglomeratic graywacke probably deposited under glaciomarine conditions. In South America (*5*), South Africa (*6*), and Australia (*7*), *Glossopteris* also occurs closely associated with coals and glacial deposits, and the thin, flat-lying cratonic *Glossopteris*-bearing beds pass oceanward into thick, deformed, partly marine, geosynclinal sequences. *Glossopteris* has also been found under similar geologic conditions in Madagascar (*8*) and the Falkland Islands (*9*) although coal beds seem to be absent in the latter area. Such similarities between the geologic history of the Ellsworth Mountains and these other regions of the southern hemisphere were predicted by du Toit (*10*), and they are compatible with his reconstruction of Gondwanaland and its bounding geosyncline.

CAMPBELL CRADDOCK
THOMAS W. BASTIEN
ROBERT H. RUTFORD
School of Earth Sciences,
University of Minnesota, Minneapolis
JOHN J. ANDERSON
Department of Geology,
University of Texas, Austin

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Pulse Radiolysis of Dioxane Solutions

Abstract. *Liquid dioxane gives transient absorptions owing to the production of free radicals and solvated electrons when subjected to a pulse of high-energy electrons (4 million electron volts) for a duration of 2 microseconds. Solutions of benzophenone give the ketyl radical and ketyl radical ions. Solutions of anthracene give anthracene in the triplet state and the anthracene anion. The formation of these species is largely prevented when hydrochloric acid is present.*

We have observed the transient absorptions produced when a 2-microsecond pulse of 4 million-electron-volt electrons is absorbed in liquid dioxane with an apparatus previously described (*1*). With pure dioxane, a 5000-rad pulse gives two species, one with a maximum at 290 mμ (half-life 300 μsec) and another (half-life 2 μsec) which absorbs increasingly from 500 mμ to 800 mμ, the limit of observation. At 650 mμ the absorption amounts to 1 percent.

It seems likely that the former is a free-radical product of the radiolysis, and the latter absorption, like the long wavelength absorptions produced in water and alcohols (*2, 3*), is due to solvated electrons.

With benzophenone present ($4 \times 10^{-2}M$) new absorptions (Fig. 1) are produced. That with a maximum at 545 mμ matches exactly the spectrum of the ketyl radical, $(C_6H_5)_2\dot{C}OH$, obtained by Bell and Linschitz (*4*), and their absorption coefficient gives the yield $G = 0.5$ (number of radicals per 100 electron volts). The half-life of the radical increases from 200 to 600 μsec when the pulse intensity is decreased ten times, an indication of partial removal by radical combination reactions. The small peak at 700 mμ matches the spectrum of ketyl ion $(C_6H_5)_2CO^-$ which is no doubt formed by electron attachment (*3*). It decays rapidly with a half-life of 7 μsec and has a yield of $G = 0.1$.

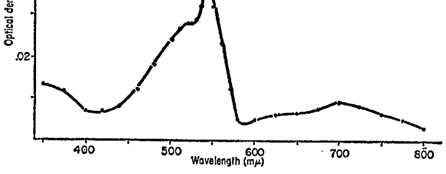

Fig. 1. Absorption spectrum of species produced immediately by a single pulse of 4 Mev electrons (5 kilorads) on 0.04*M* benzophenone in deaerated purified dioxane.

Anaerobic addition of a small amount of hydrochloric acid completely prevents the formation of these species, an effect which is paralleled in the aqueous system (*3*). In explanation of this, it was suggested that the ion is a precursor of the radical and that acid prevents ion formation since H_3O^+ captures the available electrons. This may also occur here, in which case all of the electrons, with a total $G = 0.6$, are captured by benzophenone. The alternative explanation, that the benzophenone radical is formed by the triplet states, as in the photochemical system (*4*)

$$(C_6H_5)_2CO^* + RH \rightarrow (C_6H_5)_2\dot{C}OH + R$$

(where RH is the solvent), would require quenching of the triplet state or its precursor (possibly an excited solvent molecule) by acid, which seems less likely than an electron-scavenging effect.

Similar experiments with anthracene solutions ($5 \times 10^{-3}M$) give transient spectra corresponding to the triplet state [peaks at 410 and 425 mμ (*5*)] and to the anthracene negative ion [peaks at 600 and 720 mμ (*6*)]. These species were also found in similar experiments when alcohols were used as solvents (*7*).

Acid (0.01*M*) removes the anion as expected, but it also removes 75 percent of the triplet state. This could arise again by the quenching processes mentioned above, but the alternative explanation that 75 percent of the triplet state is formed by neutralization of the anion

$$\text{Anthracene}^- + R^+ \rightarrow \text{Anthracene}^* + R$$

seems more likely.

These experiments indicate that secondary electrons produced by radiolysis of a medium of low dielectric constant such as dioxane may become solvated. Because of the greater probability of recapture of the parent ion in such media, however, the yield of solvated electrons is much smaller (not greater than $G = 0.6$) than in more polar media such as water and alcohols.

J. H. BAXENDALE
E. M. FIELDEN†
J. P. KEENE
University Chemistry Department and
Christie Hospital, Manchester, England