be demonstrated within 72 hours of antigenic stimulation, and was still detectable 10 days after stimulation, at which time the experiments were terminated. Both the number of successful experiments (22/54) and the inactivation titers obtained in our system appeared to be as high or higher than those obtained in other primary in vitro mammalian antibody systems in which bacteriophage has been the antigen (*2, 3*).

Preliminary experiments designed to show that the virus-neutralizing activity found in our concentrated culture fluids was due to an immunoglobulin and not to some nonspecific virus-neutralizing factor have been encouraging. The 7*S* and 19*S* gamma-globulin fractions were isolated from several of the concentrated culture fluids (6 days after antigen stimulation) with high inactivation titers by means of sucrose density gradient centrifugation. Titration of these and all other fractions of the density gradients showed the neutralizing activity to be concentrated only within the 7*S* and 19*S* peaks. Control samples showed less than 5 percent neutralization in all fractions. (Details of the above experiments as well as other experiments designed to characterize the nature of the neutralizing substance will be reported later.)

Assuming that the neutralizing activity directed against R17 demonstrated by the concentrated fluids was due to antibody, the possibility of a secondary response due to previous infection with this organism must be considered. Factors weighing against this possibility include both the early age of the animals at the time they were killed and the lack of any detectable neutralizing activity in their serums. However, since the R17 is a widely distributed coliphage, the possibility of primary in vivo exposure cannot be completely ruled out unless germ-free animals are used.

In order to detect the relatively small amounts of antibody produced in a system of this type, very sensitive assays are required. Two of the most sensitive systems include lysis of red blood cells as described by Jerne (*8*) and phage neutralization as described by Adams (*7*). Unfortunately, antigens found on red blood cells are as ubiquitous as are coliphages.

In the present experiments the presence of both spleen and thymus together in the cultures is required for production of the neutralization factor. The specific mechanism of spleen-thymus interaction in our system is yet to be defined. However, the lymphoid system of the mice used in these experiments is rapidly differentiating, and it has been widely reported that the thymus is required for the functional as well as morphological differentiation of lymphoid tissue (*9*). How the thymus functions in these respects is still obscure.

Especially significant in the above system is the absence of the requirement for any preliminary preparations of either antigen or tissues before primary stimulation with antigen.

GEORGE C. SAUNDERS
DONALD WEST KING
Department of Pathology, University of Colorado School of Medicine, Denver 80220

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Radiocarbon Samples: Chemical Removal of Plant Contaminants

Abstract. *Roots and similar plant matter can be efficiently removed from charcoal samples by nitration and acetone leaching after preliminary removal of humic acids and lignin by standard procedures.*

Some samples on which radiocarbon dating is desirable are mixtures of finely divided charcoal, plant matter (mainly root fragments), and soil organic matter. Physical separation of the contaminating plant matter is quite impractical because of the small particle size and the similar bulk densities of the charcoal and root fragments. Such samples commonly have to be discarded as unsatisfactory for radiocarbon dating, yet the geochronological significance frequently makes it highly desirable to salvage the charcoal for dating. In addition, the requirements for purity become more critical as the age of the samples increases (*1*) and when the samples are to be isotopically enriched (*2*).

Three chemical methods for removing plant contaminants from charcoal that have been tested by treating samples made up entirely of modern grass roots are (i) "wet combustion" with perchloric and nitric acids, (ii) bleaching with sodium hypochlorite followed by digestion in 72 percent sulfuric acid, and (iii) nitration of cellulose and leaching with acetone after bleaching. The last method is the easiest and most efficient and is described in detail.

Washed and dried grass roots were leached with hot sodium hydroxide solution to remove humic acids and lignin. Final delignification was obtained by bleaching the residue with sodium hypochlorite in hydrochloric acid solution (*3*). The remaining cellulose residues were removed by nitration and subsequent extraction of the nitrated cellulose with acetone. The test sample of grass roots was rendered completely soluble by this treatment, the only residue being inorganic matter (probably silica).

To test the method further, an artificial sample was prepared by mixing 8.18 g of charcoal that had C^{14} activity (sample A-482) (*4*) with 0.47 g of modern (August 1963) grass roots (sample A-487) with a fallout-produced radiocarbon activity equivalent to 173±2.4 percent of the modern activity (0.95 of standard oxalic acid).

The artificial sample consisting of 94.61 percent charcoal and 5.39 percent dried roots was pulverized, boiled in 15 percent sodium hydroxide solution for 1 hour, and washed and filtered on glass paper. The residue was delignified by slowly adding 150 ml of sodium hypochlorite (Clorox) to the sample in 200 ml of 6*N* hydrochloric acid with constant stirring. After boiling this mixture for 20 minutes and filtering it, the cellulose in the residue was nitrated for 10 minutes with 400 ml of a mixture of concentrated nitric

and sulfuric acids (1 : 1). The sample was washed and the nitrated cellulose leached out with nine 200-ml portions of acetone; four more than required to obtain a clear solution.

The carbon and minor inorganic matter remaining in the filter were aerated with an aspirator; it was washed with distilled water, and dried in an oven. The carbon was converted to carbon dioxide and analyzed for radiocarbon in the standard manner. The radiocarbon activity observed, less than 0.55 percent of modern (*4*) and equivalent to an apparent age of more than 40,000 years, clearly demonstrates that plant contaminants can be completely removed from charcoal samples by the described chemical treatment.

To test the unlikely possibility that insoluble organic compounds might form in the acetone-nitrocellulose reaction and be absorbed by the charcoal, 3.95 g of young charcoal which was dated as being 1280 ± 130 years old (A-601) was contaminated with 5.93 percent of grass roots (A-487) as shown in Fig. 1 and purified in the same way as before. Ages calculated in two separate counts were 1300 ± 300 and 1410 ± 260 years which were averaged to 1360 ± 200 years (A-718). Because the difference between this result and that of the uncontaminated sample is approximately one-third of the standard deviation of the difference, there is 87 percent probability that the two samples have the same C^{14} content.

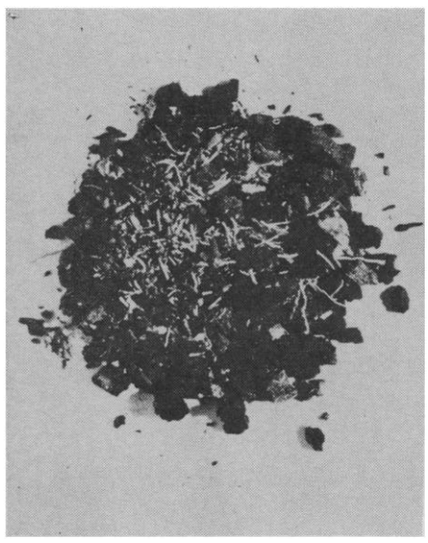

Fig. 1. Charcoal sample A-601 showing its appearance after contamination with 5.93 percent of modern grass roots (A-487). The pile is approximately 8 cm in diameter.

Therefore, the acetone has no significant effect on the C^{14} age of the sample.

This purification treatment, while suitable only for elemental carbon (*5*), is recommended for all charcoal samples suspected of being near the age limit of radiocarbon detection. Its application to very old samples requiring isotopic enrichment would appreciably improve the accuracy of the dating.

C. VANCE HAYNES, JR.
Geochronology Laboratories
University of Arizona, Tucson

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Internally Perfused Axons: Effects of Two Different Anions on Ionic Conductance

Abstract. *Voltage-clamped giant axons of squid, internally perfused with potassium chloride solutions, showed reduced initial transient membrane conductance to voltage and increased overall (leakage) conductance. Unclamped axons showed reduced action and resting potentials. Ionic conductances and membrane potentials were maintained or restored by perfusion with potassium fluoride solutions. As much as 90 percent of internal fluoride could be replaced with chloride without alteration of normal properties of membrane.*

The survival time of internally perfused giant axons of squid, in terms of resting potential, action-potential magnitude, and excitability, depends on, among other things, the type of anions contained in the perfusion solution (*1*). Our results now extend these findings by applying the voltage-clamp technique to study of the perfused giant axon.

The technique for simultaneous voltage-clamping and internally perfusing isolated, cleaned, giant axons of squid (*Loligo pealii*) has been described (*2*). Membrane potentials were measured with a glass microelectrode (3*M* KCl bridge connected to a calomel half cell) inserted just through the membrane for recording the potential with respect to an external glass-millipipette electrode (3*M* KCl-agar bridge connected to a calomel half cell). The electrodes were shorted together in 0.5*M* KCl between experiments; there was always less than 1-mv junction potential between them. No correction was made for additional junction potential after penetration of the membrane with the microtip electrode, because junction potentials of less than 1 mv were recorded between the two electrodes by the method of Baker, Hodgkin, and Shaw for various external- and internal-perfusion solutions (*3*).

Internal-perfusion flow rates were about 10^{-4} ml/sec. Internal solutions were changed by replacing one Sage electrolytically driven pump with another containing a different solution; the dead space of the cannula was exchanged within 2 minutes. The external-perfusion solution, artificial sea water, had the following ion concentrations (m*M*): Na^+, 430; K^+, 10; Ca^{++}, 10; Mg^{++}, 50; Cl^-, 560; tris buffer, 1. The first of three internal solutions had the following ion concentrations (m*M*): K^+, 456.6; F^-, 400; $HPO_4^=$, 26.6; $H_2PO_4^-$, 3.4. Potassium chloride replaced KF in the second solution, and the third contained nine parts of the KCl solution and one part of the KF solution. The *p*H of all solutions was between 7.2 and 7.4.

The presence of 26.6-m*M* $HPO_4^=$ in our solutions, although richer than the concentration used by Tasaki *et al.* (*1*), probably did not obscure the other anion effects studied; in most instances it was only 1/15 of the concentration of anions being tested.

After change of the internal solution from 400-m*M* KF to 400-m*M* KCl, the resting potential, E_{RP}, declined about 10 mv in 15 minutes while the action-potential peak voltage, E_{AP}, fell quite low. After 10 minutes of KCl perfusion the rate of action-potential decrease accelerated; rise in threshold to excitation