linol from a tartrate-buffered solution gave the trivalent chelate corresponding to the formula $Ce(C_9H_6NO)_3$. Identical results were obtained using the method of homogeneous precipitation (*3*). In both of these cases the cerium was present in the trivalent state in the chelate.

Since there is a difference in color of the two chelates—the trivalent chelate is yellow, whereas the tetravalent chelate is purple—it was thought that a study of the absorption spectra of the chelates might be useful as an analytic method. A method may be developed for the determination of cerium in a mixture with other rare-earth metal ions, since the other rare-earth chelates with 8-quinolinol are all yellow in color (*3*). Also, a study of the absorption spectra might be helpful in the elucidation of the structure of the tetravalent chelate.

The absorption spectra of $Ce(C_9H_6NO)_4$ in $CHCl_3$ and 1*N* HCl and $Ce(C_9H_6NO)_3$ in 1*N* HCl are shown in Fig. 1. The curves were determined with a Beckman, model DU, spectrophotometer, using 1.00-cm silica cells. The chelates were prepared by methods already described (*1, 2*). The absorption spectra of the two chelates in 1*N* HCl are identical, giving maxima at 312 and 360 mμ. 8-Quinolinol also gives identical maxima in 1*N* HCl, which indicates that the two chelates are completely dissociated in this solvent. This is what would be expected from the ready solubility of the chelates in strong acid solution.

The spectrum of the cerium (IV) chelate in $CHCl_3$ exhibits three maxima in the wavelength region studied. Maxima were observed at 307, 370, and 480 mμ. Obviously, the 307- and 370-mμ maxima are those of 8-quinolinol, but the 480-mμ maximum is caused by the chelate itself. This would indicate that the tetravalent chelate could not be an addition product having the composition $Ce(C_9H_6NO)_3 \cdot C_9H_6NOH$. The trivalent chelate was too insoluble to be studied in $CHCl_3$.

The maximum at 480 mμ could thus be used for the qualitative or quantitative determination of cerium, either alone or in the presence of other rare-earth metal ions. Further studies are being conducted on this determination.

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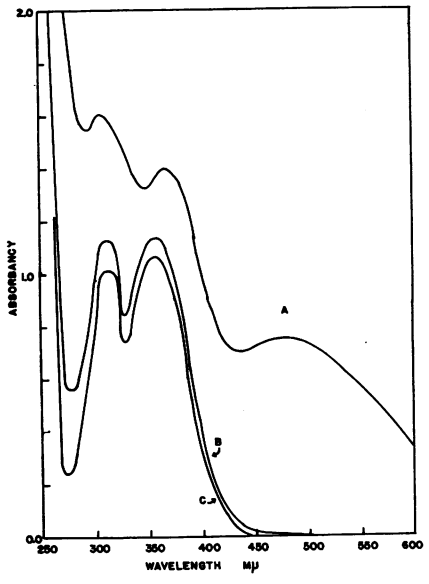

Fig. 1. The absorption spectra of the cerium 8-quinolinol chelates in various solvents. (*A*) $Ce(C_9H_6NO)_4$, $3.79 \times 10^{-4}M$ in $CHCl_3$; (*B*) $Ce(C_9H_6NO)_4$, $1.72 \times 10^{-4}M$ in 1*N* HCl; (C) $Ce(C_9H_6NO)_3$, $2.18 \times 10^{-4}M$ in 1*N* HCl.

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13 August 1956

X-ray Microscopy of Thin Tissue Sections

The methods of projection x-ray microscopy have now been developed to the point where applications can be sought in a wide variety of problems. In the field of biology one of these is the examination of sections of tissue. Thus, it will be instructive to view relatively thick slices stereoscopically and also to compare the appearance of thin sections under the optical and x-ray microscopes. For such comparisons the sections must be no more than a few microns thick, as indeed they must be for all x-ray microscopy at high magnifications if confusion owing to overlying detail is to be avoided. To obtain the necessary image contrast with thin biological preparations, very soft x-rays must be employed.

The experiments described here were made with a projection x-ray microscope, as described by Nixon and Cosslett (*1*). Absorption in the several centimeters of air through which the rays pass in this apparatus must be eliminated if soft x-rays are to be used. This was done successfully by circulating helium through the enclosed specimen and photographic chamber. In our early trials, gold or nickel foils about 1 μ thick served as target windows for the x-ray tube operated at 3 to 6 kv. Under these conditions some image contrast was produced by thin specimens, but it was not adequate in photographs of sections of soft tissue. Far better results were obtained using

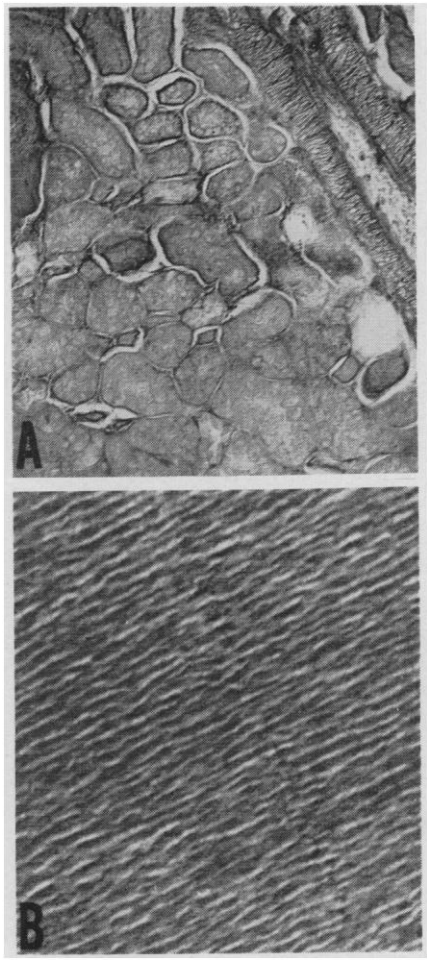

Fig. 1. (*A*) X-ray micrograph of a 10-μ section of frozen-dried mouse kidney. The paraffin-imbedded tissue was cut on a conventional rotary microtome. The section, mounted on a thin Formvar substrate, was deparaffinized before photography. (× 180) (*B*) X-ray micrograph of decalcified human dentin. A 5-μ section of the methacrylate-imbedded tissue was cut on a rotary microtome equipped with a glass knife, was mounted in Formvar, and the plastic was removed. (× 450).

aluminum as target window. Only a few percent of the K-radiation of aluminum, with a mean wavelength of 8 A, is absorbed in the helium, and very little white radiation of shorter wavelength is produced even when relatively high excitation voltages are applied to increase the efficiency of x-ray production. In this way very satisfactory photographs with exposure times of 5 to 10 minutes have been obtained by operating the tube at 10 to 15 kv, the target window being 7 μ of aluminum foil.

Figure 1 gives two examples of micrographs made under these conditions. The top picture (*A*) is of a 10-μ section through the cortex of frozen-dried mouse kidney at a magnification of about 180 times. The cells lining the convoluted tubules, which are the chief histological structures of this photograph, are discern-

ible, and much detail, including the musculature, is seen within the arteriole at the right. The bottom photograph (*B*) is of a 5-μ section through acid-decalcified dentin of a human tooth. The dentinal tubules that run lengthwise of the section are clearly visible at this magnification of 450 times, as are many small fibers associated with them.

There are still many evident improvements to be made in preparing sections for x-ray microscopy and in mounting them, which will certainly lead to corresponding improvements in the photographs obtained. Nevertheless, these examples demonstrate that microscopy with soft x-rays has already developed to the point where it can provide useful histological information.

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20 August 1956

Graft-Induced Transmission to Progeny of Cytoplasmic Male Sterility in Petunia

Many different manifestations have been revealed of hereditary properties of the cytoplasm. Thus, such different phenomena as maternal characters determined in the egg prior to fertilization, temporary environmentally induced, cytoplasmically determined characters, and interactions between nuclear genes and cytoplasmic factors have been demonstrated (*1, 2*). In a few instances (*3*), hereditary elements in the cytoplasm seem to be independent of nuclear control; one of these is the cytoplasmic male sterility in *Petunia*—the subject of this report (*4*).

The main avenue of attack on the nature of cytoplasmic inheritance has been to transfer by breeding different genomes into different cytoplasms. Despite the advantages of this method in demonstrating the matroclinous products, it neither proves that the cytoplasm as a whole by itself (a genuine "plasmon") determines hereditary traits in the same sense as nuclear genes do, nor can it prove that certain loci ("plasmagenes") are responsible for hereditary properties. Goldschmidt (*2*), while reviewing the body of evidence, tends to reject the plasmagene concept as suggestive, unnecessary, and misleading and chooses to find alternative interpretations to the facts. With certain assumptions Michaelis (*5*) formulated an interesting statistical model for hereditary units in the cytoplasm. Although his assumptions are reasonable, they are arbitrary; and the method is useful only for characteristics that express themselves in measurable gradations. Only in the case of the killer effect in *Paramecium* (*6*) has a self-reproducing hereditary particle of the cytoplasm ("kappa") been demonstrated. The behavior and chemical nature of this particle does not reveal whether it is an intimate part of the cytoplasm or a self-reproducing foreign inclusion producing deleterious effects in certain genotypes. Significant gaps thus exist in our knowledge about hereditary elements of the cytoplasm.

This preliminary report deals with an attempt to obtain evidence on the nature of cytoplasmic male sterility in *Petunia* by means of grafting. The following lines were used (*7*): (i) Northern Star, a fertile variety having a lavender corolla with a large white central star, and (ii) P-431-54 (ms), a completely male-sterile line having a dark garnet corolla and violet throat color. Anthers of the latter are greatly reduced in size and are devoid of functional pollen.

Self- and sib-pollinations of the Northern Star for two generations yielded only fertile progeny. By crossing the male-sterile line with Northern Star and three other unrelated varieties for two generations, exclusively male-sterile progeny were obtained (Fig. 1). These results, which conform to the much wider experience of other workers, suggest that this sterility factor is independent of nuclear genes.

Reciprocal grafts between fertile and male-sterile lines were made in two separate series of experiments in 1954 and 1955. No changes were noticed in the fertility or sterility of the graft components, but observations were limited by the short survival of the scions. All scions of 15 combinations of male-sterile and fertile died shortly after producing a few flowers; consequently, seeds could not be obtained from pollinations with fertile pollen. Scions of the fertile variety grafted on male-sterile stocks survived slightly longer than the reciprocal grafts. Seed was produced from two scions in each of the two series, from both sib- and self-crosses (the latter with pollen from the same flower as well as that from flowers of the donor plant). The germination rate of the seed in petri dishes was approximately 45 percent. All the work was conducted in a greenhouse in complete isolation from other lines of *Petunia*.

Progenies were grown from seeds produced by the scions and were subjected to additional test crosses to test the inheritance of male sterility. The results of these tests are summarized diagrammatically in Fig. 1. Progeny of the fertile scions grafted on male sterile stocks consistently included both fertile and sterile plants, whether from self- or sib-crosses. The number of mature plants obtained was small because the seeds germinated poorly in soil and many seedlings died. In the first series from selfing the scions with pollen of the same flower, three fertile and three male-sterile plants were obtained. Two of the male-sterile plants recovered some fertility after the third or fourth flower, and only one plant remained entirely sterile for the 12 months of its life. Seeds obtained from scion flowers pollinated with pollen from the donor plant produced eight fertile and two male-sterile plants. In the second series, 11 plants of a total of 38 remained completely male-sterile. All plants in both series showed the characteristic flower color of the Northern Star and no resemblance to the color of the male-sterile line or that of the F_1's.

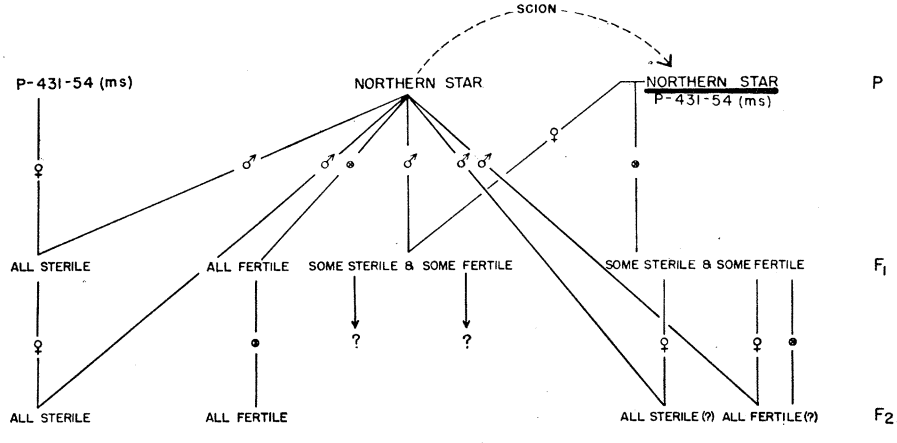

Fig. 1. Diagram of pedigrees. Graft combination indicated in upper right by fraction symbol, the scion being above, the stock below, the line.