The bottom pellet is discarded. In contrast to the nucleoprotein fraction, the protein fraction can be sterile-filtered through ultrafine filters and frozen without loss of biological activity.

Since lipid components had been demonstrated in the nucleoprotein fraction (*9*), it was of some interest to determine whether they were still present in the protein fraction. Upon analysis (*10*), no significant change in total lipid content (dry-weight basis) was found in the protein fraction (4 percent) as compared to the nucleoprotein fraction (3 percent). By careful low-temperature extraction with acetone, followed by extraction with 2 : 1 chloroform methanol, it was possible to remove 90 percent of the total lipid present in the protein fraction. No significant change in biological activity was noted in the delipidized protein fraction (Table 1).

Ascending electrophoretic patterns of the embryo protein fraction 0.1*N* $NaHCO_3$ buffer, *p*H 8.8, were obtained (*11*) and are reproduced in Fig. 1, along with a typical ascending pattern of embryonic nucleoprotein fraction. The leading peaks have disappeared or have become greatly diminished, while the major peak remains intact. Similar patterns have also been obtained with this buffer at other *p*H values and with other buffers. It is therefore evident that the protein fraction is more electrophoretically homogeneous than the nucleoprotein fraction.

In view of these results, it appears that the biological activity is independent of the presence of at least 90 percent of the nucleic acid or lipid in the nucleoprotein fraction. Since the biological activity of the embryo nucleoprotein fraction can be completely accounted for by the delipidized protein fraction, it is planned to examine the constituents of the latter for the source of biological activity. First, the very small trace residues of nucleic acids and lipids still bound to the remaining proteins should be separated, either individually or as nucleolipoproteins, and tested. In view of the inertness of the nucleic acid and lipid fractions heretofore extracted and tested in culture (*1, 9*), it is not very likely that the biological activity resides here. Second, we must consider the possibility that tightly adsorbed or complexed large or small molecules, chemically dissimilar from the majority of the proteins in the protein fraction, are the active factors. Separation and biological testing of these tightly bound complexed molecules appears to offer a more profitable approach. Third, the electrophoretic pattern indicates a possibility of polydispersity of chemically similar proteins in the main component. Further separation and testing of polymers that differ in molecular size from the main electrophoretic component of the protein fraction may reveal a unique biologically active polymer. Finally, if all polymers in the main component are found to be equally active, separation, identification, and testing of disintegration products of the protein fraction may reveal its true nature. The method of large-scale continuous-flow paper electrophoresis alone, or in conjunction with chemical pretreatment, lends itself to evaluation of all of these possibilities (*12*).

R. J. KUTSKY
Donner Laboratory of Medical Physics, University of California, Berkeley, and *Veterans Administration Hospital, San Francisco, California*

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Nucleic Acids and Survival of Excised Anthers in vitro

Abstract. Excised anthers of *Allium cepa* and *Rhoeo discolor* have been successfully cultured in modified White's medium supplemented with various concentrations of ribonucleic acid and deoxyribonucleic acid. Ribonucleic acid proved to be much more useful than deoxyribonucleic acid and reduced the time required for the completion of meiosis from 48 hours to 24 hours. The role of nucleic acids in the control of nuclear divisions has been indicated.

In an earlier communication (*1*) I had reported the successful culture of the excised anthers of *Allium cepa* with the help of kinetin and gibberellic acid. Since then I have tried to rear anthers in improved culture media with the help of nucleic acids. A correlation between the development of microspore mother cells and the synthesis of nucleic acids in the anther has been shown by the work of several investigators (*2, 3*). In most plants the meiotic synthesis of deoxyribonucleic acid (DNA) is completed by the diplotene stage and anthers excised at or after diplotene give little difficulty in culture. On the other hand, all attempts to culture preleptotene anthers have been completely unsuccessful. Obviously, the most important steps in the differentiation of the microspore mother cells occur during the premeiotic interphase and perhaps in the early leptotene.

The importance of the tapetum in the development of pollen has often been emphasized, and Cooper (*4*) showed that in *Lilium henryi* and *L. regale* DNA is transferred from the tapetal cells to the microspore mother cells during meiosis. These observations have been regarded by some as fixation artifacts (*3*). However, Linskens (*5*) has found that in *L. henryi* after the beginning of prophase, DNA secreted by the degenerating tapetal cells and nuclei appears in the form of nucleotides and other degradation components, and is taken up by the reduced microspore mother cells for synthetic processes.

It is well established that the DNA content of the nuclei in the anthers increases at three specific stages during pollen development: (i) preleptotene, (ii) shortly before the division of the microspore nucleus, and (iii) shortly after the microspore mitosis, in the generative nucleus (*3*).

In the light of these observations I have used ribonucleic acid (RNA) and DNA (concentrations, 10, 25, 50, and 100 mg/lit.) for the culture of excised anthers of *Allium cepa* and *Rhoeo discolor*. White's modified medium was used for the experiments; the technique was the same as that reported earlier (*1*). Whole anthers were excised from the bud and cultured in Pyrex test tubes on an agar medium. For each test at least two dozen cultures (each with five anthers obtained from a single bud) were made with suitable control. The tests were usually replicated after about a fortnight.

The anthers of *Allium cepa* normally do not show any development in the basic medium. When the basic medium was supplemented with different concentrations of RNA (*6, 7*), the best development occurred at a concentration of 50 mg/lit. (Fig. 1). Anthers excised at leptotene-zygotene formed 68 percent dyads and 10 percent tetrads after 1 day along with 12 percent of the mother cells at leptotene-zygotene and 10 percent at diplotene-diakinesis; 16 percent dyads and 84 percent tetrads after 2 days; and 98 percent tetrads with 2 percent degenerated cells after 4 days. When excision was done at diplotene-diakinesis, there were 2 percent mother cells, 22 percent dyads, and 76 percent tetrads after 1 day; 6 percent dyads, 5

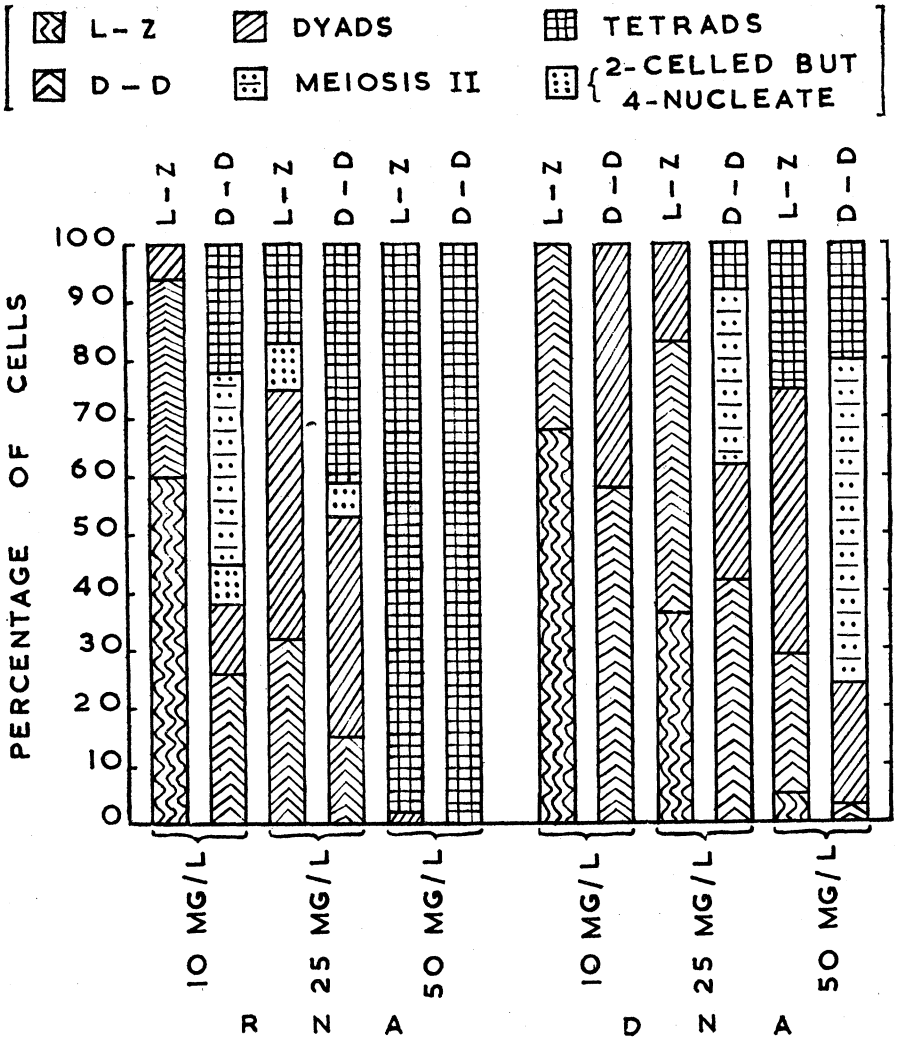

Fig. 1. Optimum development of anthers of *Allium cepa* excised at leptotene-zygotene (L-Z) and diplotene-diakinesis (D-D), in modified White's medium with different concentrations of RNA and DNA at 20°C.

percent mother cells in telophase II and 89 percent tetrads after 2 days; and 100 percent tetrads after 4 days (this is the best development obtained in *Allium cepa* anthers so far).

With DNA (*6, 7*) also, best results were obtained in *Allium cepa* at 50 mg/lit. (Fig. 1). Anthers excised at leptotene-zygotene showed 7 percent unchanged mother cells, 32 percent in diplotene-diakinesis, 39 percent at the dyad stage, and 22 percent at anaphase-telophase II after 1 day; 5 percent mother cells at leptotene-zygotene, 24 percent at diplotene-diakinesis, 46 percent dyads, and 25 percent tetrads after 2 days; thereafter there was no further development. When the anthers were excised at diplotene-diakinesis there were 3 percent mother cells at diplotene-diakinesis, 29 percent dyads, 58 percent at metaphase-anaphase-telophase of meiosis II, and 10 percent tetrads after 1 day; 3 percent unchanged mother cells, 21 percent dyads, 56 percent at metaphase-anaphase-telophase of meiosis II, and 20 percent tetrads after 2 days. No further development occurred.

In the basic medium the anthers of *Rhoeo discolor* showed best development only when they were excised at diplotene-diakinesis (57 percent undeveloped mother cells and 43 percent dyads; after this degeneration set in). Ribonucleic acid proved most favorable at a concentration of 25 mg/lit. Anthers excised at leptotene-zygotene showed 4 percent undeveloped mother cells, 16 percent at diplotene-diakinesis, and 80 percent dyads after 1 day; after 2 days there were 3 percent undeveloped mother cells, 76 percent dyads, and 21 percent tetrads. When anthers were excised at diplotene-diakinesis, all the mother cells formed tetrads within 1 day (*8*) and after 2 days one-celled microspores were formed.

In both the plants the optimum development obtained was up to the formation of one-celled microspores, and the division of the microspore nucleus could not be induced even when the anthers were excised at the tetrad or the one-celled microspore stage.

As is evident from the foregoing, whole anthers including the wall layers and the tapetum were cultured on the agar medium. Nevertheless, the extra nucleic acids supplied stimulated the development of the microspores a great deal. From this it would seem that the added nucleic acids are replacing some function normally provided by the rest of the plant, apart from the one provided by the tapetal cells.

Ribonucleic acid has been reported to increase growth and cell division in plant tissues (*7*). Hildebrandt *et al.* (*7*) have concluded that the growth of marigold and tobacco tissues was "strikingly stimulated by both filtered and autoclaved RNA at concentrations of 400 and 4000 mg per liter, while DNA had no effect at low concentrations and was strikingly inhibitory with 4000 mg per liter" (*9*). It may be pointed out here that the supply of RNA from the follicular cells (comparable to the tapetum) to the germ cells (comparable to the microspore mother cells) seems to be very widespread in animals, and it has been suggested that "a large amount of RNA is the cause, or at least one of the causes of the starting of meiosis" (*10, 11*).

I. K. Vasil

Department of Botany,
University of Delhi, Delhi, India

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6. RNA, sodium salt, yeast nucleic acid; DNA, sodium salt, ex herring sperms. Both dissolved in double-distilled water with the help of 0.05*N* NaOH (7). Chromatographic tests showed that DNA used in my experiments is free from any contamination with kinetin, a cell division factor, usually obtained from DNA. Similarly, the RNA sample was free from any desoxyribose or free ribose. Only fresh samples of nucleic acids were used.
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8. The time taken by *Rhoeo discolor* anthers, excised at diplotene-diakinesis, to form tetrads was reduced by 50 percent in vitro—that is, tetrads were formed within 24 hours, whereas in nature the time required is 48 hours.
9. In the present study also, RNA proved to be much more useful than DNA. Further, it seems likely, especially in the light of Linskens' observations, that hydrolyzed nucleic acids may have a greater influence on the growth of excised anthers. A detailed report is in press (*J. Exptl. Botany*).
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