the radioactivity of the original nicotine molecule, and about 40 percent was located in carbons 3 and 4 of the pyrrolidine ring. Approximately 40 percent of the C^{14} in the nicotine was located in the pyridine ring.

Even though a 7-day period of metabolism was used with the radioactive acetate, only a small amount of randomization of the C^{14} in the synthesized nicotine occurred. These data also show for the first time the utilization of a specific precursor for the *de novo* synthesis of the pyridine ring in a higher plant (*13*).

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Nucleoprotein Constituents Stimulating Growth in Tissue Culture: Active Protein Fraction

Abstract. A new method has been developed for removing the nucleic acid portion of the nucleoprotein fraction which stimulates growth in tissue culture. Biological activity resides in the purified protein fraction, while the high-polymer nucleic acid fraction is inert. The active protein fraction contains extractable lipids which have no effect on the biological activity.

As was previously reported, the factors of high molecular weight in embryo extract which are needed for stimulation of certain types of tissue culture can be isolated with streptomycin as a protein precipitate both from embryo extract (*1*) and from adult tissues (*2*). These growth factors, which are active in both plasma and glass substrate tissue cultures (*3*), are obtained from the streptomycin precipitate in the form of a nucleoprotein fraction (NPF) (*1*). Since the nucleoprotein fraction is a mixture containing four electrophoretic components (*4*), further fractionation was attempted to identify the individual active growth principles.

In view of recent work on viruses (*5*) demonstrating biological activity in a specially prepared nucleic acid fraction of very high molecular weight, attempts were made to obtain similar nucleic acid fractions from the nucleoprotein fraction, although earlier, lower polymer nucleic acid preparations were inactive (*1*). Various new methods for isolating these high polymers (*6*) were tried with the nucleoprotein fraction and chick embryo homogenates; these resulted in nucleic acid preparations of varying polymer size, but all nucleic acid preparations were inactive in culture (*7*). Although most of the protein residues from these experiments were insoluble in salt solutions and inactive in cultures, some protein residues from these experiments partially redissolved and possessed growth-promoting activity when tested in tissue culture. This suggested the possibility of an active soluble protein fraction. Separation of soluble proteins from nucleic acids in the nucleoprotein fraction was attempted on the basis of an earlier observation (*7*) that a high concentration of phosphate buffer caused the nucleoprotein fraction to separate in this dense medium and to form a floating scum. Since high salt concentrations are frequently used to split off nucleic acids from proteins, the nucleic acid was presumably split off in this instance. More important, the floating material redissolved easily in buffers of low ionic strength (*7*).

On reinvestigation the flotate was indeed found to exhibit a typical protein absorption spectrum with a maximum of 278 mμ, in contrast to the spectrum of the nucleoprotein fraction, with a maximum of 260 mμ (*1*). This change suggested a loss of nucleic acid from the nucleoprotein fraction to form the protein fraction (PF); this was confirmed by chemical analysis for nucleic acid (*8*): NPF, 6.5 percent; PF, 0.8 percent (on a dry weight basis).

The growth-promoting activity of the protein fraction was tested on chick embryo heart fibroblasts in Carrel flask cultures, as previously described (*1*). The potency of the protein fraction was found to be unchanged from that of the nucleoprotein fraction, while the nucleic acid moiety was inactive (Table 1). The dose-response curves were alike, and similar to those published for the nucleoprotein fraction (*2*). Likewise, as already reported (*3*), no essential loss of biological activity was observed when a protein fraction was isolated from the nucleoprotein fraction of adult chicken spleen and tested on chick myoblasts in glass substrate cultures. The protein fraction from adult spleen appeared to be more active than the protein fraction from the embryo in this test system.

Table 1. Biological activity of protein fraction in plasma clot cultures of chick embryo heart fibroblasts.

Component	Amount (mg/ml)	Areal outgrowth at 7 days* (mm^2)
Serum control		8.0 ± 1.5
Embryo NPF	0.4	36.3 ± 6.1
Embryo PF	0.4	42.4 ± 5.4
Embryo nucleic acid	0.4	10.3 ± 3.3
Embryo delipidized PF	0.4	33.2 ± 4.7

* Averages, with standard error, for six flasks.

A routine procedure for isolation of protein fraction is as follows (all steps at 0°C): Clear fresh nucleoprotein fraction in Gey's solution is treated with 2 volumes of 3.3*M* potassium phosphate buffer at *p*H 7.8. The white turbid solution is allowed to stand for 1 hour, then is centrifuged for 20 minutes at 28,000*g* in the Servall centrifuge to concentrate the floating insoluble proteins as a flotate. The subnatant, containing nucleic acids and some protein, is discarded, and the flotate is further compacted by spinning for 30 minutes at 144,000*g* in the Spinco ultracentrifuge. The solid flotate is dialyzed for 48 hours against 2000 volumes of 0.1*N* sodium bicarbonate in which the protein fraction is stable. This step removes phosphate and dissolves much of the solid flotate. The contents of the dialysis sac are then spun for 1 hour at 144,000*g*, the floating lipid is removed with a capillary pipette, and the clear midfraction (in concentrations up to 20 mg/ml) is saved as the protein fraction.

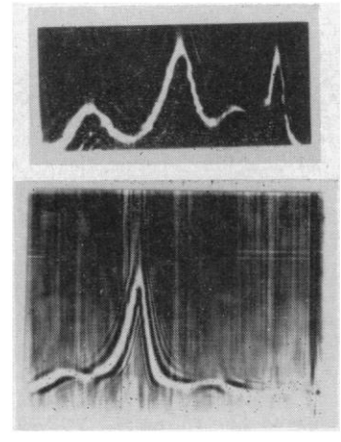

Fig. 1. Comparison of electrophoretic patterns of (top) nucleoprotein fraction (top) and protein fraction (bottom).

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*).

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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