

contralateral tectum receives retinofugal fibers.

No degeneration was observed along the ventrolateral surface of the caudal diencephalon and midbrain where a basal optic tract and nucleus have been described in other snakes (2-4).

These observations of the retinal efferent system in blind snakes differ in several respects with those reported for "higher" snakes (2-4). In *Typhlops* the ipsilateral contribution to the lateral geniculate nucleus is greater than in other snakes. The prominent nucleus lentiformis mesencephali, nucleus geniculatus, pretectalis, basal optic tract, and basal optic nucleus of "higher" snakes are not recognizable as distinct structures in *Typhlops*. The most striking difference is, however, the paucity in *Typhlops* of retinotectal connections which are abundant in "higher" snakes.

The major thalamic nucleus receiving tectal efferents in reptiles and birds is the large nucleus rotundus (9). The nucleus rotundus of the snakes *Natrix sipedon* (3) and *Thamnophis sirtalis* (4) is extremely small and is just barely distinguishable in some blind snakes (8). Thus it would appear that in these blind snakes the retinogeniculate and pretectal projections are sufficient to handle the limited reactivity to light which they demonstrate (5, 6).

In view of the supposedly primitive phylogenetic status of the blind snakes, the limited development of the retinotectal projection is notable since the tectum has been described as the dominant area for visually guided behavior in nonmammalian vertebrates (10). Perhaps in view of the above findings, the retinthalamic projection deserves greater attention in these forms.

The blind snake is potentially valuable as an example of a naturally occurring experiment in specialization. It would be of considerable interest to know whether the absent and reduced retinal projections correspond with absent or reduced features of visually dependent behavior and whether the retained projections contribute to functions which are held in common by other vertebrates.

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Application of the Elution Centrifuge to Separation of Polynucleotides with the Use of Polymer Phase Systems

Abstract. A flow-through centrifuge without rotating seals enables the use of polymer phase systems for chromatographic separation of macromolecules. The capability of the technique is demonstrated on partition of polynucleotides.

In recent years we have developed a number of arrangements for high-efficiency countercurrent separations. Best results were obtained by using a centrifuge to set up a multistage phase partition system in a coil containing thousands of turns of thin tubing. The flow-through coil planet centrifuge (1) and elution centrifuge (2) have both been shown to give high-efficiency separations of small molecules such as dinitrophenyl amino acids.

The versatility and usefulness of polymer phase systems for partition of macromolecules and particulates have been well documented (3-5). However, application of these systems to conventional liquid chromatography becomes difficult because of their aqueous/ aqueous phase composition. Hence, the partition technique at present relies upon the countercurrent distribution method, which requires a relatively long separation time and results in a considerable dilution of the sample. Below we report separation of poly-

nucleotides by using polymer phase systems in conjunction with our elution centrifuge. Advantages of the method are higher efficiency, shorter separation time, and minimum solute dilution. Stepwise or gradient elution with continuous monitoring and fractionation can be used as in conventional chromatographic systems.

The principle of the elution centrifuge is illustrated in Fig. 1. A cylindrical container, carrying the separation column, revolves in the horizontal plane while it rotates about its own axis at the same angular velocity to prevent twisting the flow tubes. Thus the system allows the effluent to move in and out through the running column without rotating seals. The centrifugal force produced by the planetary motion retains the stationary phase in a fine helical column while the mobile phase is steadily eluted. Consequently, solutes introduced locally are subjected to an efficient partition process resulting in chromatographic separation without influence from a solid support.

The polymer phase systems used in the present study have been introduced by Albertsson (4), consisting of 5 percent (by weight) dextran T 500 (Pharmacia), 4 percent (by weight) polyethylene glycol 6000 (Union Carbide), and 10 mM sodium phosphate of various compositions indicated in parentheses below. The phase mixture is gently degassed by suction and equilibrated at 18°C. The same temperature is maintained during the elution by cooling the apparatus.

Three column configurations, each made of Teflon tubing 5 m long and 0.55 mm in inside diameter, are examined for the operational requirements of countercurrent chromato-

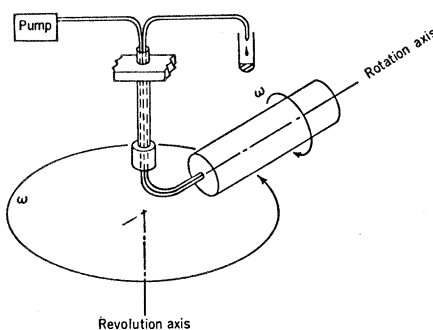


Fig. 1. Principle of the elution centrifuge. A cylindrical holder, carrying a separation column, revolves around the central axis (revolution axis) of the apparatus while it rotates about its own axis (rotation axis) to prevent twisting the flow tubes.

phy, that is, retention of the stationary phase and partition efficiency. The straight helix column is prepared by winding a tube onto four rigid cores (each 12 cm long and 3 mm in diameter) in series and mounted on the column support close to and nearly parallel to the rotation axis. The twisted column is prepared by folding a tube in two and twisting it along its length to make a ropelike configuration. The twisted tube is then wound around the

column support. The coiled helix column is made by winding a tube onto a flexible core, 50 cm long and 3 mm in diameter, which is in turn coiled onto the column support. Each column is placed into the column holder at a location remote from the revolution axis to minimize the effect of the Coriolis force previously analyzed (2).

With the use of these columns and a phase system (NaH_2PO_4), retention volumes of the stationary phase are

measured under various speeds of revolution at a flow rate of 2.8 ml/hour. The results are shown in Fig. 2A, where the percent of the retained stationary phase (open circles for the upper phase and closed circles for the lower phase) is plotted against the speed of revolution. All columns show a sufficient amount of retention of the lower phase above 1000 rev/min (250g).

To study column efficiency, stepwise elutions of polyuridylic acid (poly U) are performed. The sample is first eluted with the upper phase (NaH_2PO_4) at 1500 rev/min for 1 hour followed by the second elution (NaH_2PO_4 : $\text{Na}_2\text{HPO}_4 = 1:3$), the eluates being monitored with an LKB Uvicord II at 254 nm. Figure 2B shows the results of the experiments. The first elution eliminates most of the impurities present in the sample, while the second elution (indicated by arrows) elutes poly U as a single peak. The sharpness of the elution peak obtained from the coiled helix column clearly indicates the highest efficiency of the column types tested.

To test the general capability of the method, the partition of polynucleotides is studied by using both stepwise and gradient elution with a coiled helix column (6 m long, 0.55 mm inside diameter, and 1.5 mm helix diameter). Each polynucleotide sample is dissolved into a phase mixture (NaH_2PO_4) at a concentration of 0.12 g per 100 ml, and 0.1 ml (stepwise elution) or 0.15 ml (gradient elution) is charged at each run. In order to eliminate impurities present in the sample solution, the elution is carried out in two steps. The sample is first eluted with the upper phase (NaH_2PO_4) for about 1 hour, eliminating most of the impurities while retaining the polynucleotide in the column. This is followed by a second elution with the upper phase of various sodium phosphate compositions or an exponential gradient (between NaH_2PO_4 and Na_3PO_4).

Figure 3 shows chromatograms of polyuridylic acid (poly U), polycytidylic acid (poly C), polyadenylic acid (poly A), and polyinosinic acid (poly I) obtained by the second elution with the indicated phase compositions. The peaks from different polynucleotides can be clearly resolved with either stepwise or gradient elution techniques. The minor peaks in the gradient elutions presumably represent impurities.

We have demonstrated the feasibility

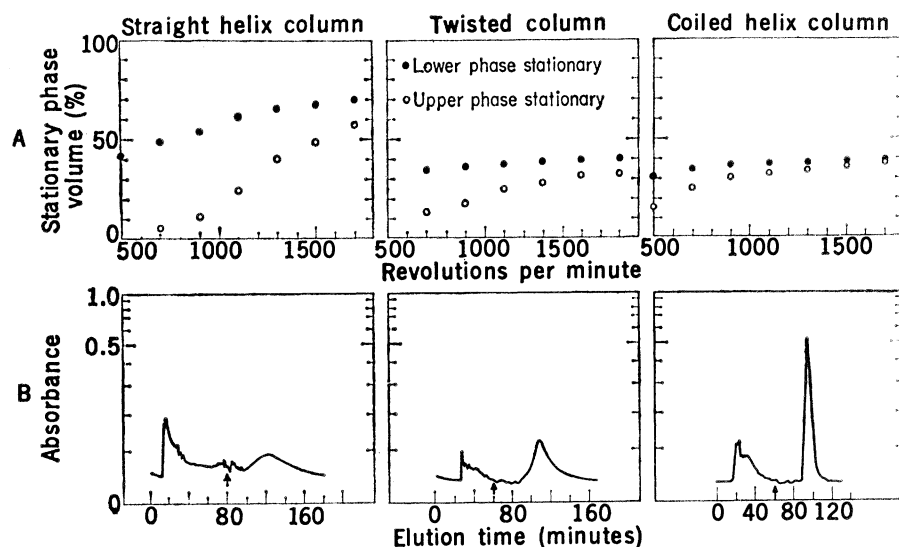


Fig. 2. Performance of three types of separation column. (A) Retention of the stationary phase. All three columns show sufficient retention of more than 30 percent above 1000 rev/min, if the lower phase is stationary. (B) Column efficiency examined on stepwise elution. The first elution eliminates impurities while the second elution, indicated by arrows, elutes the poly U sample. Sharpness of the peak obtained by the coiled helix column indicates the highest efficiency.

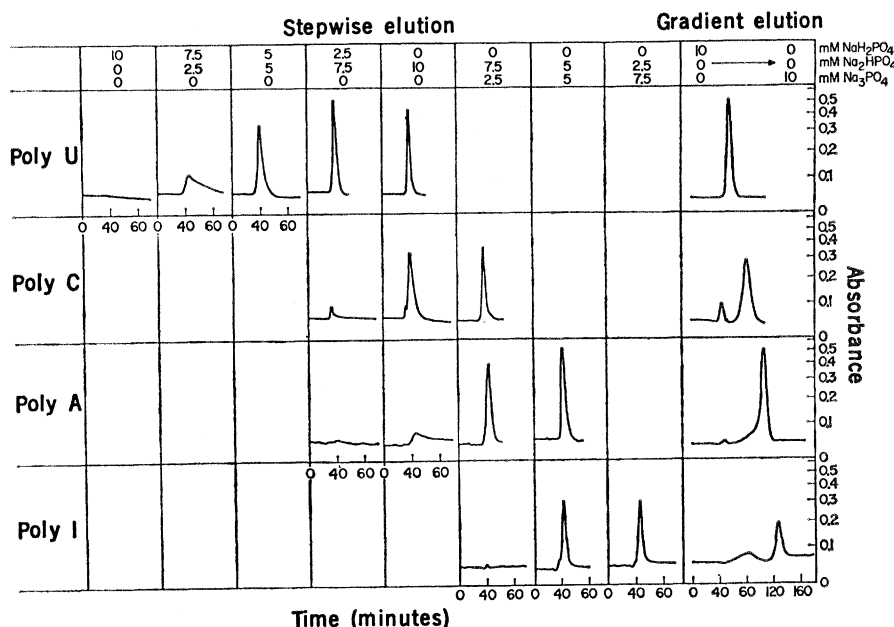


Fig. 3. Countercurrent chromatograms of polynucleotides with stepwise (left) and gradient (right) elutions, using the phase systems composed of 5 percent (by weight) dextran T 500, 4 percent (by weight) polyethylene glycol 6000, and 10 mM sodium phosphate of the indicated compositions.

of sharp separation by using the polymer phase systems in conjunction with the elution centrifuge. With further improvements in apparatus and the use of shallower gradients, separation of biological macromolecules having only small differences in partition coefficient may be possible.

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Monocular Spatial Distortions Induced by Marked Accommodation

Abstract. Contraction of the ciliary muscle during marked accommodation causes the leading edge of the retina to advance as much as 0.5 centimeter. Near the posterior pole of the eye, the upward and downward extensional strains on the retina should be reasonably balanced. In the horizontal meridian an asymmetry is introduced because of the nasal location of the optic nerve head. Observers were asked to bisect the space between two parallel lines while fixating a movable line lying near the midpoint of the two lines. The test was conducted with the target far from and near the subject, in the horizontal and vertical meridians, and was repeated with accommodation paralyzed by a cycloplegic agent. Marked accommodation induced significant spatial distortions in the horizontal meridian. The effect is largely retinal.

Contraction of the ciliary muscle during marked accommodation allows the eye lens to increase in convexity and also causes the leading edge of the retina to advance forward. The retina, attached to the choroid, rides forward with ciliary muscle contraction. This forward displacement of the retina has been measured in a number of ways. Hensen and Voelckers (1) recorded the movement of the head of a needle anchored in the sclera, its tip in the choroid, with each accommodative movement in an intact dog's eye. They found the anterior choroidal displacement at the equator amounted to approximately 0.5 mm and diminished on approaching the optic nerve. Van Alphen (2), working with cats, observed a forward movement of the entire choroid through small holes in the sclera at the posterior pole, the equator, and several points in between. Moses (3), with human subjects, photographed the position of the scalloped margin of the anterior retina, the ora serrata, made visible by transillumination. He found that the ora advanced about 0.05 mm with each diopter of accommodation. The retina is attached to the choroid at the ora serrata.

It follows that with the forward

movement of the choroid with accommodation the retina would become elongated anteriorly over the incompressible fluid spherelike vitreous body. Enoch (4) has calculated an increase in retinal area of 30 mm² with ten diopters of accommodation. Near the posterior pole of the eye, the upward and downward extensional strains on the retina caused by marked accommodation and the advance of the choroid should be reasonably balanced (Fig. 1C). However, across the horizontal meridian the strains on the retina will be asymmetrical because of the nasal location of the optic nerve head; the retina is fixed in position at this point of exit of the optic nerve from the eye. One would then expect there to be a graded retinal advance across the horizontal meridian during strong accommodation from a maximum at the ora to zero at the optic nerve. This statement in no way defines the nature of that gradient.

Because under particular conditions each retinal point has associated with it a direction in visual space (5), any unevenness in retinal stretch in the horizontal meridian should result in a change in the apparent relative position of objects in space (6). Disruptions in

space perception due to accommodative retinal elongation should be demonstrable when a monocular bisection experiment is conducted. This test requires an individual to bisect the space between two lines while fixating the bisecting line. The classical technique for this experiment has its origin in the work of Kundt and Münsterberg (7). They found that there is typically some asymmetry—that is, errors in the placement of the bisecting midpoint—in unaccommodated bisection judgments. Kundt reported that subjects overestimate the nasal visual field, thereby physically setting the temporal field larger for the match, while Münsterberg described the reverse asymmetry.

In accordance with what is known about accommodative retinal elongation, we would predict that a monocular bisection experiment in the horizontal meridian near the posterior pole may be altered by marked accommodation, while we would predict little or no effect when the same property is measured in the vertical meridian. To test our hypothesis, a series of bisections were made by several nearly emmetropic subjects of college age, all with normal, uncorrected vision (8). Using one eye at a time, subjects steadfastly fixated a short movable black bar and indicated when it was exactly midway between the two fixed parallel black bars separated by 22°. The background was a uniformly illuminated white cardboard surface. This bisection experiment was conducted both vertically and horizontally, and in each orientation judgments were compared with the target far from the subject and near him. The tests, with the target far from the subject—208 cm away from him—called for only about 0.5 diopter of accommodation. The tests with the target near the subject were made with the stimulus display placed just beyond the nearest point at which the subject could still hold the target in focus; these tests employed nearly maximum accommodation (8 to 13 diopters). The luminance in (millilamberts) of the near target, 1.75 log units, was greater than that of the far target, 1.2 log units. This was done in order to keep the stimulus, the retinal illuminance, constant, to compensate for the decrease in pupil size with accommodation. Retinal illuminance (in trolands) was high enough (3.1 log units) to provide minimal variability in accommodation (9).