

Fig. 1. Effect of 2-diethylaminoethanol on excised tomato roots grown for 6 days from 10-mm tips. Each point is the mean of nine roots. The horizontal lines show the growth in the control.

Table 1. The effect of either choline (experiment 1) or 2-dimethylaminoethanol (DMAE) (experiment 2) on inhibition by 2-diethylaminoethanol (DEAE). The values for experiment 1 are means of nine roots; for experiment 2 the values are the means of 19 roots in the control and low DEAE series and of nine roots in the high DEAE series.

Additions			Feature measured	
DEAE (µM)	$\begin{array}{c} \text{Cho-}\\ \text{line}\\ (\mu M) \end{array}$	DMAE $(\mu M)$	Main axis (mm)*	Lateral length (mm)†
Experiment 1				
0	0		133.1	200.5
40	0		79.4	202.2
40	.04		81.2	191.6
40	.12		81.5	175.1
40	.4		88.6	218.8
40	1.2		128.4	161.9
40	4.0		96.2	196.1
40	12.0		87.1	183.0
Experiment 2				
0		0	127.4	150.1
40		0	69.7	147.8
40		3.2	83.7	157.1
40		4.0	84.1	158.3
40		8.0	87.7	153.2
40		12.0	77.8	172.4
200		0	36.3	84.8
200		3.2	43.8	109.6
200		4.0	47.1	112.5
200		8.0	50.2	130.0
200		12.0	53.3	104.6

\* Length of main axis per root. † Total length of ten basal laterals per root.

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crease in growth of lateral roots. In other words, the inhibition of apical dominance more than offset the inhibition of lateral growth by DEAE.

Because of the differences in the proportions of the lateral roots and the main axis, the difference in morphology was very striking between control roots and those grown in  $40-\mu M$  DEAE. The doseresponse curves obtained with DEAE are not simply a consequence of inhibition as such, because they were not obtained with the antimetabolites sulfanilamide, ethionine, thiouracil, and desthiobiotin when these were added to the basal medium used here. However, dichloroanisole, an antiauxin (9), gave curves very similar to those shown in Fig. 1 (10). This suggests (i) that the inhibitory effect of DEAE at low concentrations is due to an effect upon the hormonal system which inhibits growth of lateral roots and (ii) that differences in the morphology of root systems (11) may result from differences between the endogenous concentrations at which an inhibitor affects the growth metabolism of terminal meristems and those at which it affects the metabolism upon which the hormonal, apical dominance depends.

Ethanolamine did not relieve the inhibition caused by DEAE at a concentration (40  $\mu M$ ) giving approximately 50 percent inhibition in growth of the main axis. The concentrations of ethanolamine tested ranged from 0.12 to 400  $\mu M$ . These concentrations were not inhibitory when supplied in the absence of DEAE.

Neither choline nor 2-dimethylaminoethanol increased the growth of clone R5 when supplied in the absence of DEAE. The effects of choline or 2-dimethylaminoethanol on inhibition by DEAE are shown in Table 1. Inhibition was relieved, to a greater or lesser extent, by either of these substances. The low level at which they are toxic to excised tomato roots precludes any attempts to determine whether the antagonisms are competitive or otherwise. However, the results presented here show that 2-diethylaminoethanol may be a useful inhibitor in the study of ethanolamine and choline metabolism in plants, and in the study of apical dominance in excised tomato roots.

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# **Partial Chromatographic** Separation of Pentose- and **Deoxypentosenucleic** Acids

Abstract. From a mixture of tobacco mosaic virus pentosenucleic acid (RNA) and calf thymus deoxypentosenucleic acid (DNA) (2 mg each), 73 percent of the RNA was separated free of DNA by discontinuous elution chromatography with phosphate buffers at pH 6.7 on columns of calcium phosphate.

The presently available procedures for the separation of pentosenucleic acids from deoxypentosenucleic acids in complex biological materials have been discussed by Chargaff (1), together with their individual advantages and limitations. Included among these is the method of Zamenhof and Chargaff (2), which involves the preferential adsorption of RNA on activated charcoal. The recent development of procedures for chromatographic separations of DNA on calcium phosphate columns (3-5) has prompted the extension of such techniques to mixtures of RNA and DNA. This we would like to report in this paper (6).

The results obtained for the attempted separation of tobacco mosaic virus RNA (7) from calf thymus DNA (Worthington), in a mixture of the two acids, by means of this chromatographic adsorbent material are presented in Fig. 1. Chromatography of 2.0 mg tobacco mosaic virus RNA alone (Fig. 1A) results in a series of peaks eluted by 0.12. 0.14, 0.16 and 0.20M phosphate buffer eluents at pH 6.7. The ratio of optical absorbance of 280 m $\mu$  to the optical absorbance at 260 mµ  $(A_{280}/A_{260})$  of these eluates was 0.46. That of the original RNA solution placed on these columns was 0.46.

When a mixture composed of 2.0 mg of tobacco mosaic RNA and 2.0 mg of DNA is chromatographed (Fig. 1B), a series of major peaks, as evidenced by the corresponding  $A_{260}$  values of the eluates in tubes 31 to 120, appears. The eluates in tubes 31-79 contain RNA, since the  $A_{280}/A_{260}$  values are within the limits of  $0.46 \pm 0.01$ . Further, no significant amounts of DNA were found by the Keck method (8) in any of these eluates. The DNA appears in the tubes beyond No. 79, mixed with the remainder of the RNA (that is, 27 percent) not accounted for in the eluents up to tube No. 79. The value of  $A_{490}$  of the DNA-indole "chromosol" produced by Keck's method parallels in percentage the value of  $A_{260}$  of respective eluates in tubes 70 to 112. This is shown by the shaded portions on this chromatogram (Fig. 1B). The distinct break in the nature of the peaks eluted after tube 79 is reflected in the change in the ratio  $A_{280}/A_{260}$  from 0.46 to  $0.545 \pm 0.01$ . The latter value is compatible with that of solutions of the DNA sample used (0.54).

From the data shown in Fig. 1B, the amount of RNA separated free of DNA was calculated to be 73 percent of the

total amount of RNA present in the initial mixture. These experiments were performed in duplicate and have been repeated with 1.0 mg of RNA in the place of 2 mg, with essentially the same results. In contrast to these results on calcium phosphate columns, it has been reported (9) that RNA was not resolved from a mixture of RNA and DNA on columns of ECTEOLA-cellulose.

It is to be noted from Fig. 1 that RNA (2.0 mg) desorbed from the column much earlier (that is, at lower eluent concentrations) in the presence of DNA than it did in the absence of DNA (2 mg). One reasonable explanation of this finding implies that, as the full capacity of the column is approached (but not necessarily attained) by the load of 4 mg of mixed nucleic acids, the RNA is preferentially displaced by the DNA. This



Fig. 1. A, Chromatography of tobacco mosaic virus RNA (2.0 mg). B, Chromatography of a mixture of tobacco mosaic virus RNA (2.0 mg) with calf thymus DNA (2.0 mg) on columns of modified calcium phosphate (1 by 7.5 cm) at 6° C. The solvent (4 ml volume) in which the nucleic acids were applied to the columns was 0.005M sodium phosphate buffer at pH 6.7; rate of elution, 4.8 ml/hr; tube volume 4.8 ml; discontinuous gradient elution. Eluents and phosphate buffers (pH 6.7) at molarities indicated. The value of  $A_{200}$  for each eluate is plotted according to the scale on the left. Values of bars,  $(A_{200}/A_{200})$  are plotted according to the scale on the right. In B, the darkly shaded areas represent respective concentrations of DNA expressed in terms of  $A_{400}$  of the DNAindole "chromosol" plotted according to the scale at the left. For concentrations of DNA (in terms of  $A_{200}$ ) in the range between 0.40 to 1.15, the ratio  $A_{400}$  of the DNA-indole "chromosol" to  $A_{200}$  of a DNA solution was  $1.12 \pm 0.02$  for any particular concentration of DNA. Recovery of DNA: 82 percent of that placed on column.

same displacement phenomenon also has been observed in the chromatography of proteins and of polynucleotides in the presence of DNA (10).

In experiments briefy reported elsewhere (5), bovine plasma albumin was cleanly separated from calf thymus DNA when 20 mg of the former and 2.0 mg of the latter were chromatographed as a mixture on columns of this absorbent (11).

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# **Centrifugal Arousal**

### in the Olfactory Bulb

Abstract. The electrical activity of the olfactory bulb was recorded in awake, unrestrained cats with electrodes permanently implanted. It was found that any kind of sensory stimulation producing alertness or arousal brought about the appearance of bursts of rhythmic activity, the magnitude of which was related to the degree of alertness of the cat.

Some centrifugal fibers terminating around second-order sensory neurons, such as those of the retina and of the olfactory bulb, have been known for a long time, since Cajal's anatomical descriptions (1). However, it has only recently been shown that stimulation of these fibers may modify the electrical activity of those neurons. Electrical stimu-