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THE APPLICATION OF ANION-EXCHANGE RESINS TO THE CHROMATOGRAPHIC SEPARATION OF YEAST RIBONUCLEIC ACID FROM ITS SMALLER DEGRADATION PRODUCTS

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THE APPLICATION OF ANION-EXCHANGE RESINS TO THE CHROMATOGRAPHIC SEPARATION OF YEAST RIBONUCLEIC ACID FROM ITS SMALLER DEGRADATION PRODUCTS

By René A. Bolomey and Leon Wish

Ion exchange and adsorption chromatography have become very useful tools for the separation of closely related substances. The separation of rare earths[1,2,3,4,5] from each other by ion-exchange techniques and the resolution of mixtures of cis-trans caratanoid isomers[6,7,8,9] into individual components may be cited as noteworthy examples.

The chemical composition and the structure of nucleic acids and of their degradation products indicate that these substances can, for the most part, be bound by both cation- and anion-exchange resins. Schwartz, et al.[10] reported preliminary experiments on the use of Amberlite IR-4 and IR-100 for the separation of adenine, guanine, uracil, etc. Cohn[11,12,13] successfully separated the purine and pyrimidine bases as well as the ribosides and monoribonucleotides from each other using cation- and anion-exchange resins.

Questions that remain to be answered concern the separability of the nucleic acid degradation products from the parent compound by means of ion-exchange resins and the quantitative recovery of the nucleic acid from the resins, the separability of ribo- from desoxyribonucleic acid, and the separability of the different polymers of ribo- or desoxyribonucleic acids from each other. The present investigation deals with the choice of suitable conditions for the purification and the quantitative recovery of a commercial ribonucleic acid.

Experimental

Sodium citrate buffer (0.1 M pH 6.0) was chosen as the over-all solvent for the work reported in this paper. This choice was based on the assumption

that nucleic acid would be stable at pH 6.0 and that the ionic strength of a 0.1 M solution of citrate would be high enough to completely elute the nucleotides before nucleic acid appeared in the eluate.

Adenylic and guanylic acids[14] were dissolved in the citrate buffer without further purification even though these compounds were found to be contaminated with the bases, ribosides and other nucleotides by paper chromatography and by ion exchange.

Yeast nucleic acid[14] was dissolved in the citrate buffer and dialyzed through a seamless cellulose membrane[15] for forty-eight hours against frequent changes of buffer in order to remove the smaller dialyzable components. The resulting nucleic acid solution was then filtered through a cintered glass funnel.

The concentration of nucleic, adenylic and guanylic acid solutions was so adjusted that one milliliter would possess an extinction value of 20 to 50 at 260 mμ. The concentration of these compounds in the eluate was determined at 260 mμ. with a Beckman quartz spectrophotometer in 1-cm. silica cells. The spectral purity of various aliquots of the eluate was followed at 5-mμ. intervals in the range of 235 to 300 mμ.

Inorganic phosphate was determined from radioactivity measurements of added ^{32}P in the form of phosphate. For each column experiment 5 μc. of ^{32}P was added to 0.2 ml. of a 0.2 M potassium dihydrogen phosphate solution contained in 1 ml. of the citrate buffer.

Duolite A-3[16], Dowex-2[17] and Amberlite IRA-400[18] were compared as anion exchangers. All resins were ground in a ball mill and screened. A mesh size of 100 to 200 was selected for the work reported in this paper. The resins were then thoroughly washed, under constant shaking, with water, 1 N hydrochloric acid, water, 1 N ammonium hydroxide, and again with water. The smaller resin

particles were discarded by decantation until the supernatant solution remained clear. Five grams of the air-dried resins were then equilibrated under agitation, with frequent changes of the buffer. The slurry was then transferred to a column 30 cm. long and 1 $cm.^2$ in cross sectional area.

The columns were operated at a flow rate of 2.4 to 3.0 ml. per hour. Walter[19] states that boundaries characteristic of equilibrium adsorption are obtained only if the time of passage of the solution through the column is greater than the half time of the reaction by a factor of at least 100.

Discussion

Equilibrium data obtained on the solutions prepared as mentioned above indicated that the compounds should be eluted in the order: inorganic phosphate, adenylic acid, guanylic acid and finally nucleic acid. Moreover, they indicated that although a good separation of adenylic and guanylic acids could not be expected with 0.1 M citrate, these acids could be separated as a group from the nucleic acid. They also indicated that a stronger eluting agent than 0.1 M citrate was required to elute the nucleic acid in a reasonable volume. On the basis that a high salt concentration will repress Donnan membrane equilibrium, the citrate was made to contain 10% sodium chloride and tried as an eluting agent for the nucleic acid.

Typical results obtained with nucleic acid and Duolite A-3, Amberlite IRA-400 and Dowex-2 are shown in Figs. 1, 2 and 3 respectively. With all three resins no elution occurred when dialyzed nucleic acid was eluted with citrate. In some experiments, up to 1500 ml. of citrate were passed through the column with no detectable change in the optical density at 260 mμ. in the eluate. When the citrate buffer employed for the elution was made to contain 10% sodium chloride the optical density of the solution at 260 mμ. was raised sharply. The material

in this fraction could be precipitated with hydrochloric acid and the precipitate could be dissolved in pH-6 citrate buffer. After dialysis against frequent changes of citrate buffer to remove the excess salt the nucleic acid showed the same behavior as did the original material when placed on a fresh column.

The shape of the elution curve of nucleic acid (Figs. 1, 2 and 3, especially Fig. 2) appears at first glance to agree with the theoretical type of curve derived by De Vault[20] for a nonlinear adsorption isotherm. This agreement is fortuitous and is in reality due to the fact that the front part of the curve is crowded on the front of the stronger eluting agent. As a rule, an elution curve follows a normal curve of error[21] which reflects the randomness of adsorptive phenomena. When an eluting solvent is replaced by another, such as one having a stronger eluting power than the former, a new set of conditions affecting the adsorption isotherm is established[20] and the adsorbent bed may not be long enough to obtain complete development of the chromatographic band.[22] It is possible that a normal elution curve would have been obtained had longer columns been employed.

The recoveries (based on extinction values) of nucleic acid eluted from Duolite A-3 (Fig. 1) were of the order of 60 to 70% in the fraction 10 to 150 ml. When the elution was continued with more of the salt-containing buffer the optical density at 260 mμ. approached zero asymptotically indicating that very large volumes of eluant would be required to significantly increase the yield. This phenomenon was even more pronounced with Amberlite IRA-400 (Fig.2). The front edge of the band was sharper and the lower part of the tail was more trailing than was the case with Duolite A-3. The recoveries of nucleic acid eluted from the Amberlite resin were of the order of 25% in the fraction 10 to 150 ml. In contrast to these findings the resin Dowex-2 gave an elution curve (Fig. 3)

Figure 1.

Elution curve of Schwarz's yeast nucleic acid from Duolite A-3.

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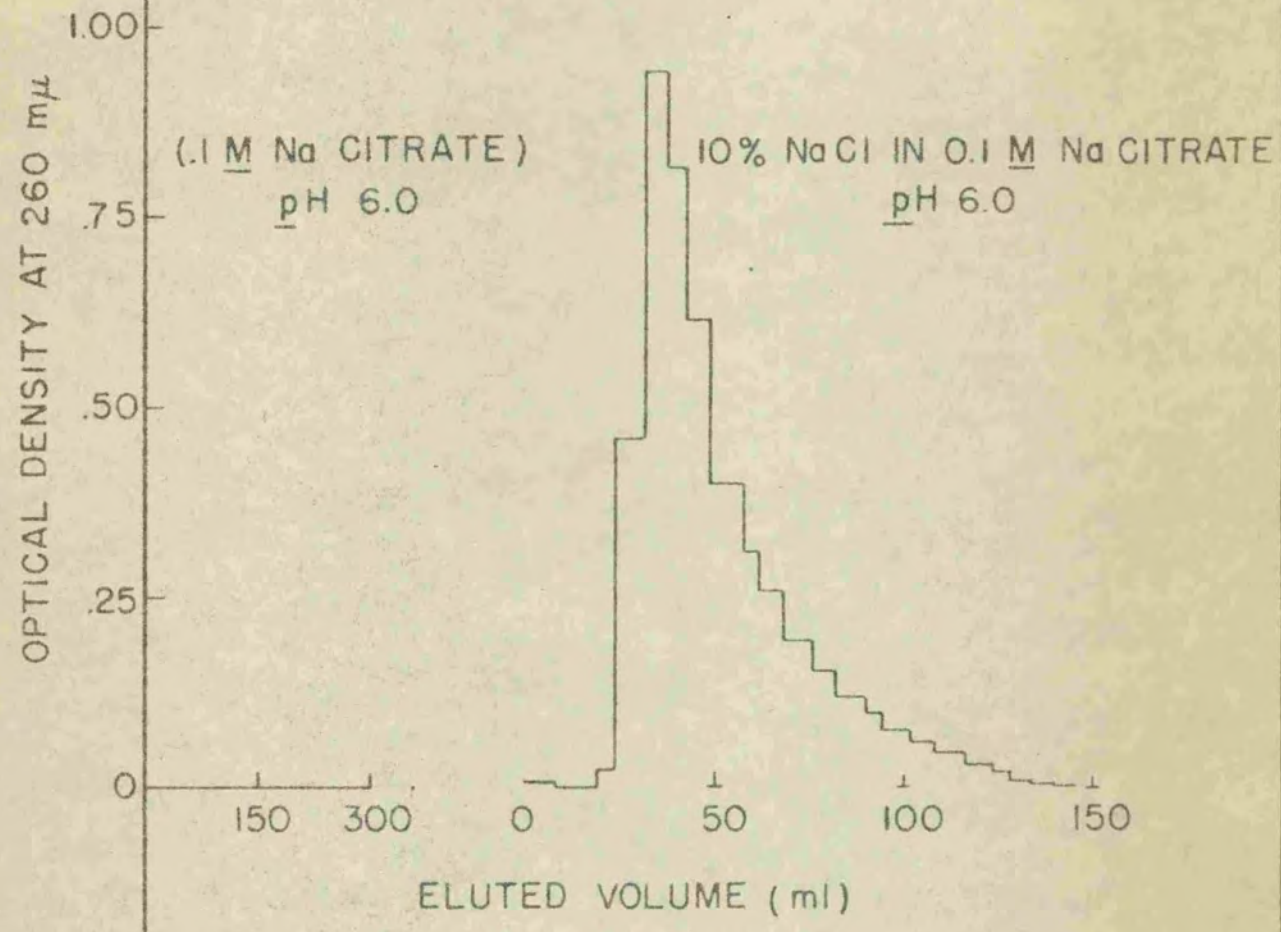

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Figure 2

Elution curve of Schwarz's yeast nucleic acid from Amberlite IRA-400.

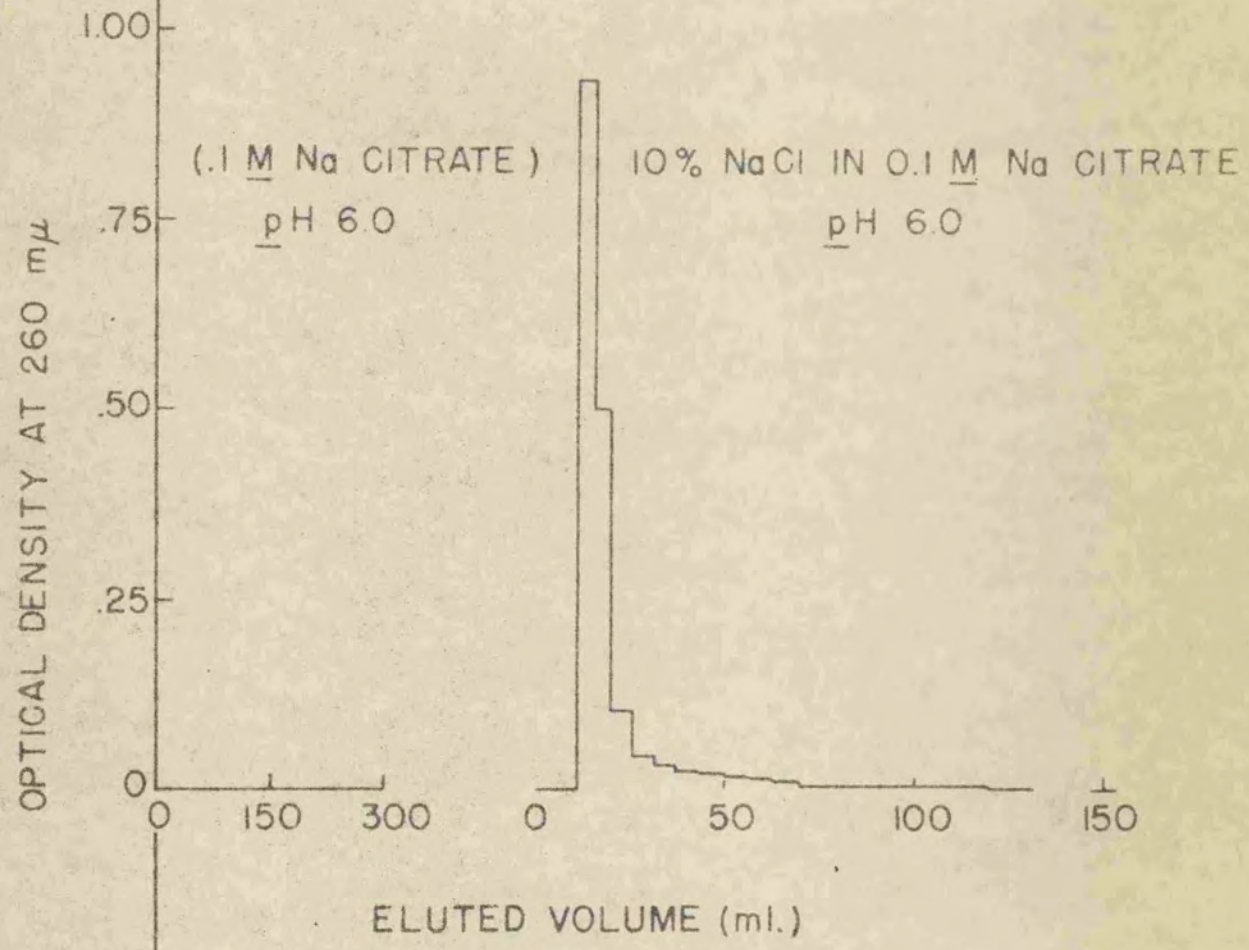

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Figure 3.

Elution curves of various nucleic acid constitutents from Dowex-2.

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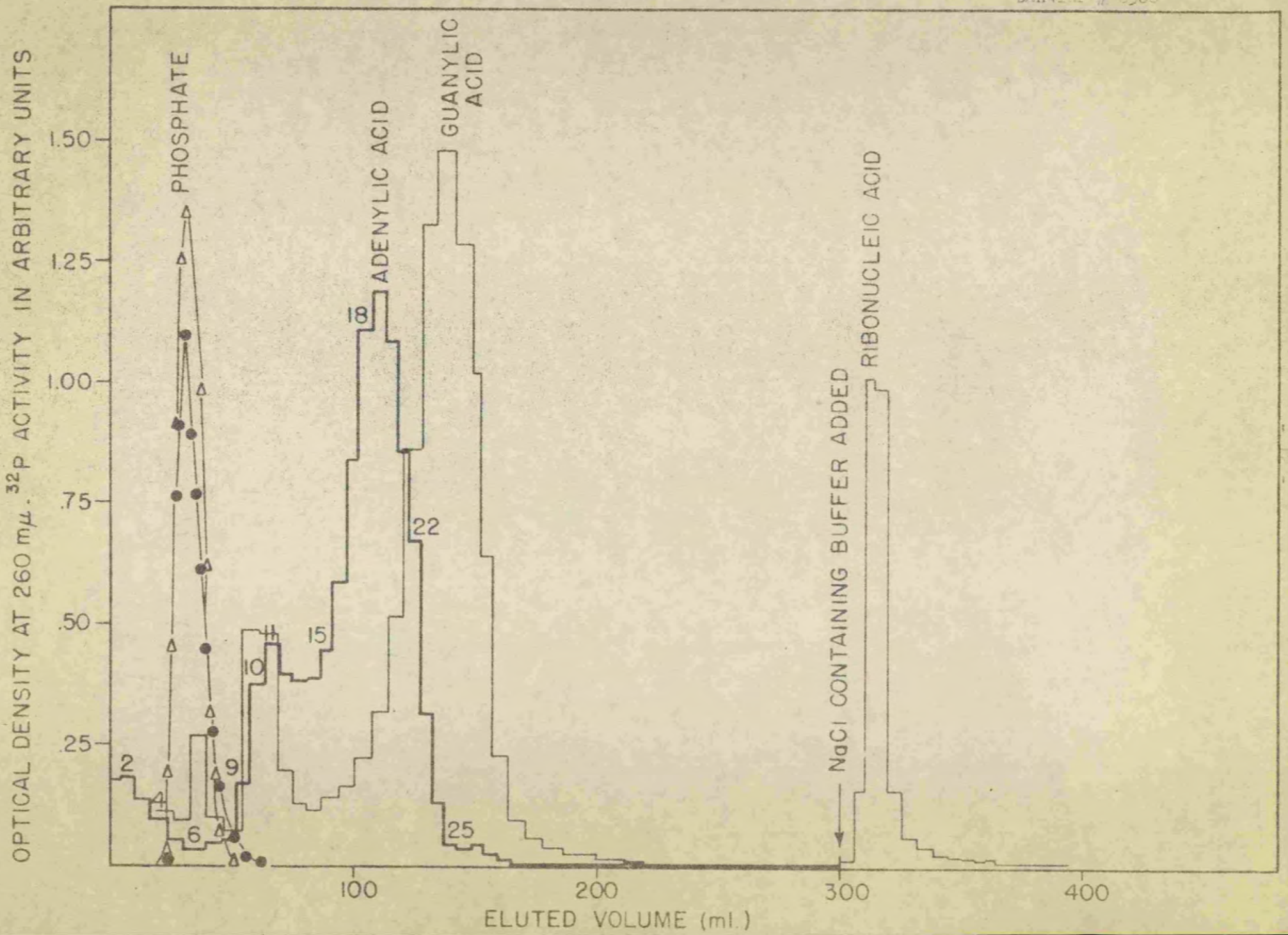

that was less skewed and approached in shape the form expected of equilibrium conditions and linear adsorption isotherm. With this resin the elution of nucleic acid was quantitative (96 to 102%)with small volumes (6 to 63 ml.) of salt buffer.

It was realized from the start that the ionic strength of the citrate as well as the pH selected were not optimal for the separation of the smaller nucleic acid degradation components from each other. Preliminary experiments indicated that lower ionic strength than that given by 0.1 M citrate would have been better for this purpose and that acetate appeared to be better than citrate. These experiments were not carried further as others[23] in our laboratory were doing work along these lines.

The conditions chosen for the work reported here, however, were ideal for the separation of phosphoric, adenylic and guanylic acids from the macro molecules. As Dowex-2 resulted in good recoveries for nucleic acid, this resin was studied further. A mixture of the radiophosphate, adenylate and nucleate solutions was passed through a fresh column and eluted with 300 ml. of citrate buffer (Fig. 3). The phosphate was eluted in the 23- to 60-ml. fraction. This elution band was followed by one (40 to 90 ml.) containing an impurity in the adenylic acid then by one (60 to 170 ml.) containing the adenylic acid itself (see Fig. 4 for absorption curves). The last 130 ml. of eluate were free of material detected by optical density measurements at 260 mμ. Similarly a mixture of the radiophosphate, guanylate and nucleate solutions was passed through another column and eluted with 300 ml. of citrate buffer (Fig. 3). Inorganic phosphate appeared in the 20- to 50-ml. fractions. The fractions 25 to 50 ml. and 40 to 100 ml. contained impurities present in the guanylic acid. The fractions 80 to 220 ml. contained the guanylic acid. No optically absorbing material was found

in the last 80 ml. that were eluted. In both cases, nucleic acid appeared in the eluate after the sodium chloride buffer was added to the column.

Absorption Spectra. - If we consider that an ion-exchange resin, previously equilibrated with buffer, is adsorbing a solute by an exchange mechanism we may expect that the buffer concentration in the first few ml. of the eluate will increase to an extent that is proportional to the number of new ions adsorbed by the resin. This will tend to increase the background value in the first eluted fraction to a greater or lesser extent depending on the extinction value of the buffer in the wave length region employed for the measurements. Any nonadsorbing spectral impurity present in the adsorbate will also contribute to this effect as well as will any spectral impurity normally adsorbed on the resin providing this impurity survived the preconditioning treatment of the resin and providing further that this impurity is exchanged by the adsorbate ions.

The adsorption curves presented in Fig. 4 were obtained on the citrate eluate of the phosphate-adenylate-nucleate mixture from Dowex-2 shown in Fig. 3. The number on the curves of Fig. 4 refers to the aliquot numbers shown in Fig. 3. The shape of curves 2, 4 and 6 with the exception of the band in the neighborhood of 270 to 290 mμ. are characteristic of a difference in citrate concentration between the blank and a citrate sample of somewhat higher concentration than the blank. Curves 9, 10 and 11 (especially 10 and 11) do not show the citrate influence as markedly as did the previous ones, but indicate the presence of a fair amount of material absorbing maximally in the neighborhood of 250 mμ. Curves 15, 18 and 22 are characteristic of adenylic acid while curve 25 nearly corresponds to the buffer blank employed for the optical measurements and for the elution. The ratio $D\lambda_{max.}/D\lambda_{min.}$ for dialyzed Schwarz nucleic acid was 2.69 and that for an Eimer and Amend sample was 2.38. The ratio for nucleic acid eluted from Dowex-2 was 1.9 to 2.0. When the eluted sample (after precipitation with dilute hydrochloric

Figure 4.

Absorption curves of various eluted aliquots from the Adenylic acid -- Dowex-2 curves.

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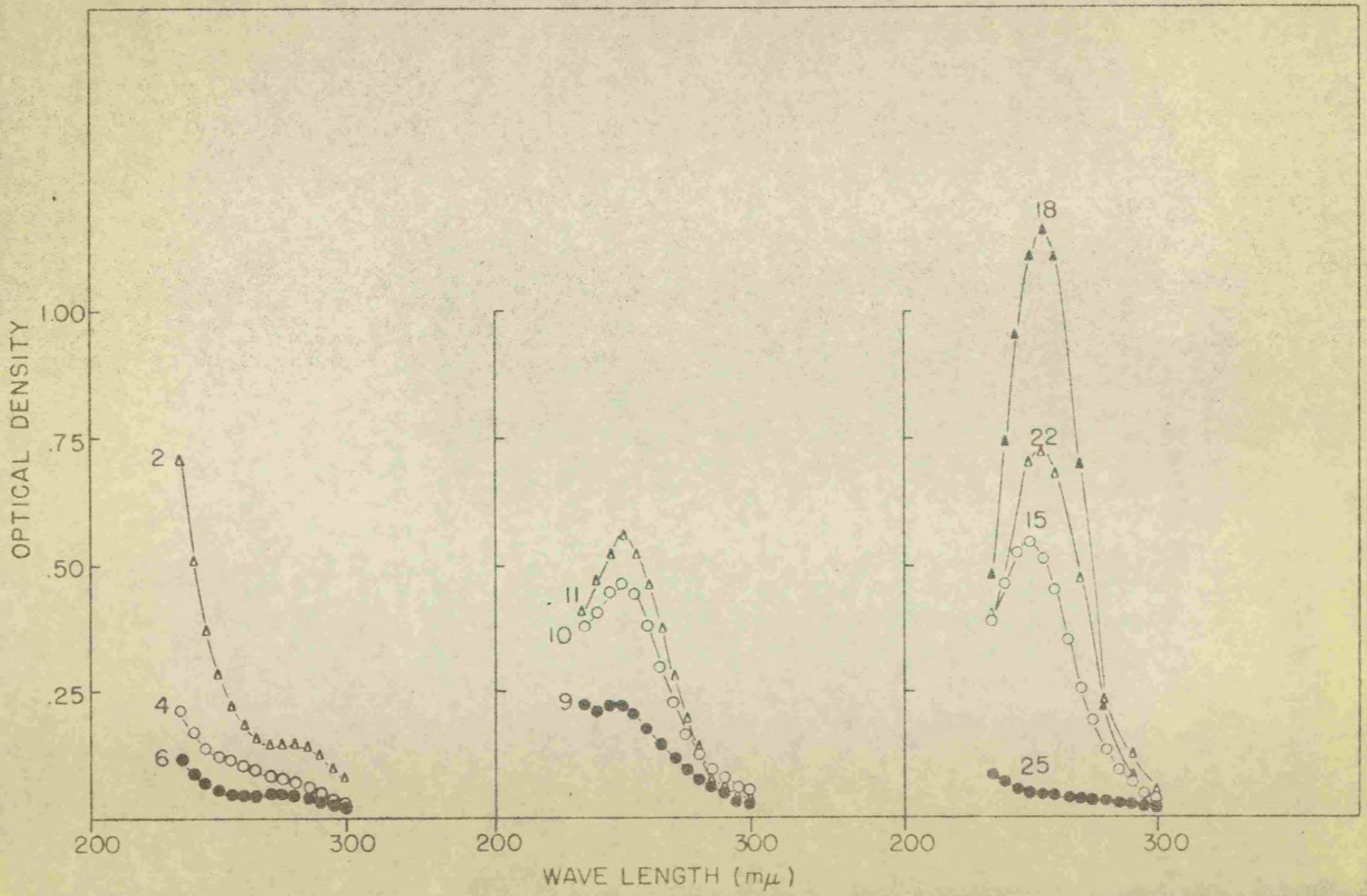

acid, washing once with water, drying by washing with 95% ethanol and desiccation *in vacuo* over the weekend) was dissolved in citrate buffer (*p*H 6.0) the ratio was 2.60.

Summary

It has been possible to separate phosphoric, adenylic and guanylic acids from ribonucleic acid, and to quantitatively recover the ribonucleic acid by chromatography on the anion-exchange resin Dowex-2.

The resins Amberlite IRA-400 and Duolite A-3 were also investigated and were found to result in low recoveries of the adsorbed ribonucleic acid.

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