THE SEPARATION OF DESOXYPENTOSENUCLEIC ACIDS AND PENTOSENUCLEIC ACIDS

by

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The analysis of desoxypentosenucleic acids by the method of paper chromatography (CHARGAFF[1]; MARKHAM AND SMITH[2]; SMITH AND WYATT[3]; LALAND, OVEREND AND WEBB[4]) has shown that the nucleic acids from different sources contain different proportions of their constituent bases, adenine, guanine, cytosine, thymine and 5-methylcytosine. In view of these results, much attention has been paid to the problem of the isolation and analysis of nucleic acids from numerous diverse sources. In the case of certain animal tissues (calf thymus, herring roe etc.) there has been no difficulty in obtaining desoxypentosenucleic acids essentially free from pentosenucleic acids by using the valuable method of MIRSKY AND POLLISTER[5]. In other cases, however, such as extraction of the nucleic acid from rye (LALAND, OVEREND AND WEBB[6]) and particularly from certain bacterial sources, this method was ineffective and the alternative methods used gave nucleic acids which were mixtures of desoxypentose and pentose types.

Several methods have been used to separate the two types of nucleic acid, but most of these were tedious and not satisfactory. Digestion with ribonuclease or deoxyribonuclease has been used to remove one type of nucleic acid from a mixture (CHARGAFF AND ZAMENHOF[7,8]), but this method leaves behind an enzyme-resistant core which may have some influence on the analytical results and physical measurements of the residual nucleic acid. SMITH AND WYATT[3] have used digestion with sodium hydroxide to remove pentosenucleic acid from bacterial desoxypentosenucleic acids. These authors have shown that the proportion of bases in desoxypentose nucleic acid was not altered by this procedure, but the method causes extensive degradation of the desoxypentosenucleic acid and thus renders it useless for many purposes. Attempts have also been made to separate the nucleic acids by means of their calcium salts (CHARGAFF AND ZAMENHOF[7]; MCCARTY AND AVERY[9]) but no clear cut separation was obtained.

This paper gives details of a convenient method for the separation of the two types of nucleic acid by fractionation of the cetyltrimethylammonium and calcium salts. In addition an alternative method is described by which pentosenucleic acid (PNA) is removed from desoxypentosenucleic acid (DNA) by means of activated charcoal (according to CHARGAFF AND ZAMENHOF[8]) and the PNA subsequently eluted from the charcoal with 15% phenol at pH 7–7.5.

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EXPERIMENTAL

Analytical methods

Nitrogen and *Phosphorus* estimations were carried out as described in the previous paper (JONES[10]).

Pentose

Pentose estimations were carried out by the method of EULER AND HAHN[11] using purified yeast nucleic acid as standard. By reducing the final volume of the solution from 10 ml to 1 ml, and using the micro-cells on the "Spekker" absorptiometer, it was possible to estimate 0.05 mg of PNA with an accuracy of about 5%.

Desoxypentose

The DISCHE diphenylamine reaction[12] was used to estimate desoxypentose. The standard was a purified sample of thymus nucleic acid.

It was not considered that these colour reactions gave absolute values for the PNA and DNA contents of the various samples. This was because these reactions may not have been strictly quantitative for the estimation of a very small amount of one nucleic acid in the presence of a large amount of the other. Also, as both methods estimate only the carbohydrate combined with the purine bases, and as the purine composition of nucleic acids differ, an absolute value for the amount of one nucleic acid could not be obtained by comparison with nucleic acid from another source. These errors would be unimportant, however, in purified DNA where there was present less than 5% PNA to be estimated, and similarly for the estimation of a small quantity of DNA in purified samples of PNA.

Source of nucleic acids

The nucleic acids of *Mycobacterium tuberculosis*, *Mycobacterium phlei* and *Sarcina lutea* were isolated as previously described[10]. The nucleic acid of wheat germ was kindly supplied by Dr S. G. LALAND, and that from mouse sarcoma by Dr D. L. WOODHOUSE. Both specimens had been isolated by the method of MIRSKY AND POLLISTER.

The purification of the nucleic acid of Sarcina lutea

The bacterial nucleic acids isolated by the use of cetyltrimethylammonium bromide (Cetavlon) (JONES[10]) had low nitrogen and phosphorus contents, indicating the possible presence of impurities. This was particularly the case with the DNA of *Sarcina lutea* which contained about 7% phosphorus and 12% nitrogen. This nucleic acid was purified as follows:

A solution of the nucleic acid (10 mg) (N, 12.1%; P, 7.1%) in 1 *M* sodium chloride (10 ml) containing 1% Cetavlon was diluted to 0.3 *M* with distilled water, and the suspension centrifuged. The precipitate was separated, and further purified by two reprecipitations from 1 *M* sodium chloride solution by dilution with water, and then dissolved in 1 *M* sodium chloride, precipitated with ethanol, and dissolved in distilled water. The solution was dialysed until free from sodium chloride, and freeze-dried to give nucleic acid (6.85 mg) which contained N, 13.6%; P, 7.9% (E_{260} 1.10: 0.06 mg/ml).

The addition of ethanol to the 0.3 *M* sodium chloride supernatant liquid gave a fraction which was dissolved in distilled water, and the solution dialysed. After freeze-drying a white solid (1.5 mg) was obtained, which contained N, 8.5%; P, 3.2% and had E_{260} 0.390 (0.06 mg/ml).

It was apparent that this procedure removed a considerable amount of impurity from the nucleic acid. Further purification was achieved by precipitation of the nucleic acid in the presence of calcium ions.

A series of tubes was made up containing nucleic acid (0.5 mg/ml), calcium chloride (0.05 *M*) and ethanol (0–40%). The solutions were centrifuged at 0° C. Carbohydrate in the supernatant liquid was measured by the carbazole reaction[13] and nucleic acid estimated by measuring the optical density at 260 mμ.

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This experiment indicated that nucleic acid was precipitated in preference to polysaccharide material at 20% ethanol concentration. A similar experiment carried out at constant ethanol concentration, but varying calcium concentration indicated that 0.5-1.0 *M* was the best concentration for the purification of the nucleic acid.

The application of this second purification procedure to the nucleic acid of *Sarcina lutea* gave a final product which contained N, 14.2%; P, 8.4% and had E_{260} 1.20 (0.06 mg/ml). A second impurity (12% of the original nucleic acid) was isolated which contained N, 3.3%; P, 1.8% and had E_{260} 0.180 (0.06 mg/ml).

No purification of the nucleic acids of *Myco. tuberculosis* or *Myco. phlei* was obtained by these methods.

Attempted fractionation of nucleic acids with ethanol

Direct fractionation of nucleic acids with ethanol did not achieve an effective separation of the two types of nucleic acid due to the extremely sharp precipitation of the material as the ethanol concentration was increased. The same effect was obtained when nucleic acid was precipitated in the presence of 0.1 *M* Ca^{++} but in this case much less ethanol was required (30% conc.). A more gradual separation of the nucleic acids was obtained by fractionation of the material with increasing concentrations of Ca^{++} in the presence of a constant ethanol concentration (20%). The method was investigated to see whether separation of the two types of nucleic acid could be achieved.

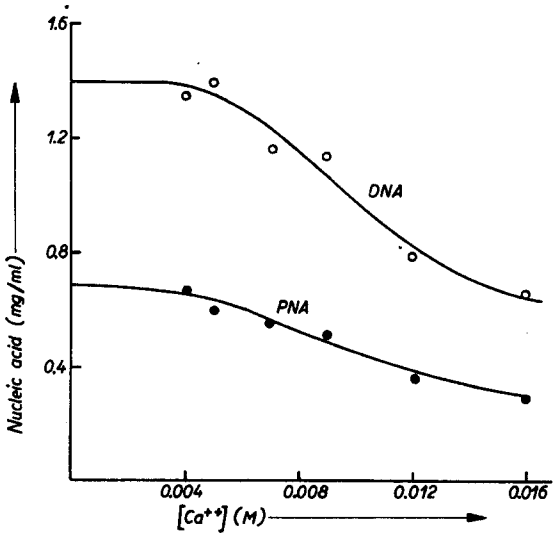

Fig. 1. Precipitation of the nucleic acid of *Myco. tuberculosis* with Ca^{++} in the presence of 20% ethanol.

The nucleic acid (16 mg) of *Myco. tuberculosis* was dissolved in acetate buffer, pH 6.2, ionic strength, 0.01 (4 ml) and an aliquot (0.25 ml) introduced into each of a series of eight tubes. Ethanol, acetate buffer and calcium chloride solution were added so that the final volume in each tube was 0.5 ml. The ethanol concentration was constant (20%) and the calcium chloride concentration varied from 0.004 *M* to 0.016 *M* over the range. The tubes were centrifuged at 0° C and then an aliquot (0.15 ml) removed from each tube, diluted to 5 ml and the total nucleic acid determined by measurement of the optical density at 260 mμ. A further 0.1 ml sample was removed from each tube for the determination of the PNA. The results (Fig. 1) indicated that the two nucleic acids were precipitated almost simultaneously and therefore no separation was achieved.

Precipitation of nucleic acids with metallic ions

Certain cations such as Cu^{++}, La^{+++} and Pb^{++} precipitated both thymus DNA and yeast PNA, but other cations such as Ca^{++}, Ba^{++} and Mn^{++} precipitated yeast PNA

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but not thymus DNA. An attempt was made, therefore, to separate the two types of nucleic acid by the use of an appropriate cation (*cf.* CHARGAFF AND ZAMENHOF[7]).

The nucleic acid (12 mg; PNA, 11%) of *Myco. phlei* was dissolved in distilled water (10 ml) and 2 ml samples saturated with calcium chloride, barium chloride and manganous chloride respectively. After 24 h at 0° C, the tubes were centrifuged at 7,000 g for 30 mins. The total nucleic acid of the supernatant liquid was estimated by measurement of the optical density at 260 mμ. A second sample (1 ml) of the supernatant liquid was taken and the nucleic acid precipitated by the addition of ethanol (1 ml). The precipitate was redissolved in distilled water (0.1 ml) and the PNA estimated. The results are summarised in Table I.

TABLE I

Solution saturated with	% *Pentosenucleic acid*	
	Supernatant	*Precipitate*
Calcium chloride	8	90–100
Barium chloride	10	28
Manganous chloride	6	54

Thus, although an essentially pure PNA was precipitated with calcium chloride, only a small percentage of the PNA separated in this way and, therefore, the method was not suitable for the separation of the nucleic acids.

Fractionation of nucleic acids using cetyltrimethylammonium bromide (Cetavlon)

Nucleic acids readily formed, with Cetavlon, a complex which was soluble in strong sodium chloride solutions (*ca.* 1 *M*) but sparingly-soluble in more dilute solutions (*ca.* 0.3 *M*). In order to determine whether Cetavlon could be used to separate DNA and PNA, the solubilities of the Cetavlon complexes of the nucleic acids of *Myco. tuberculosis, Myco. phlei, Sarcina lutea*, wheat germ and mouse sarcoma, were investigated as follows:

Nucleic acid (15 mg) was dissolved in distilled water (5 ml) and sodium chloride (290 mg) and Cetavlon (100 mg) added. The solution was maintained at 0° C and aliquots (0.5 ml) added to each of a series of eight tubes which contained mixtures of sodium chloride and distilled water so that the volume of liquid in each tube was 1.5 ml and the final sodium chloride concentration range of the series was 0.67–0.33 *M*. The solutions were kept at 0° C for 30 minutes, and then centrifuged at 0° C (3,600 r.p.m. for 15 minutes). The total nucleic acid in the supernatant liquid was estimated by dilution of a sample (0.3 ml) to 5 ml and measuring the optical absorption at 260 mμ. The PNA estimation was carried out using a 0.5 ml sample. With the nucleic acids which contained less than 20% PNA the sample was quantitatively evaporated to dryness in the centrifugal freeze-dryer, redissolved in distilled water (0.1 ml) and the EULER and HAHN reagents added to make a final volume of 1 ml.

From these results the DNA and PNA contents of the supernatant liquids were calculated and are shown in Figs. 2–6. The curves represent the percentage DNA of the system remaining in solution (I), and the percentage PNA remaining in solution (II). Fig. 7 shows the solubility of the Cetavlon complex of the nucleic acid of *Myco. phlei* at 20° C.

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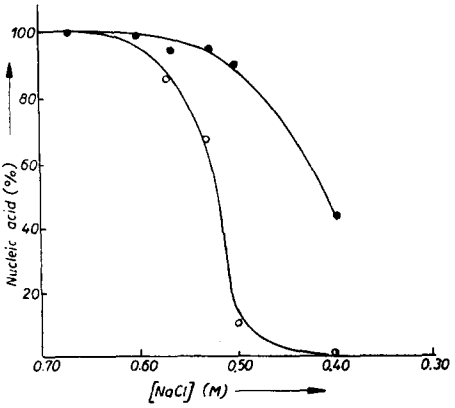

Fig. 2. Nucleic acid of *Myco. tuberculosis* (temp. o° C)

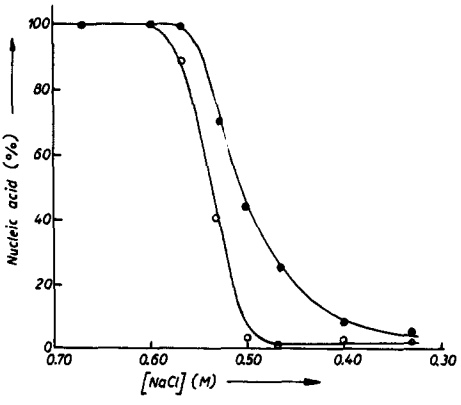

Fig. 3. Nucleic acid of *Myco. phlei* (temp. o° C)

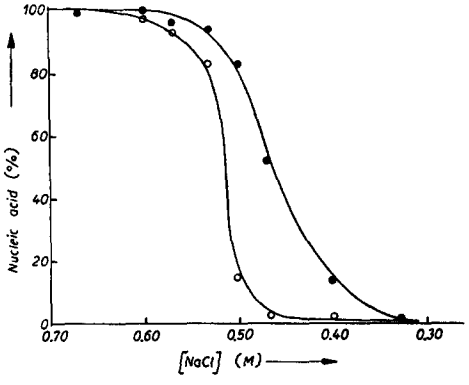

Fig. 4. Nucleic acid of *Sarcina lutea* (temp. o° C)

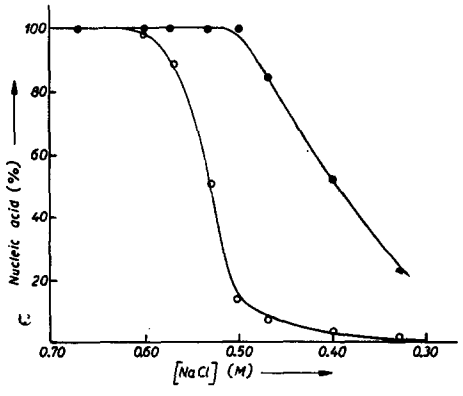

Fig. 5. Nucleic acid of wheat germ (temp. o° C)

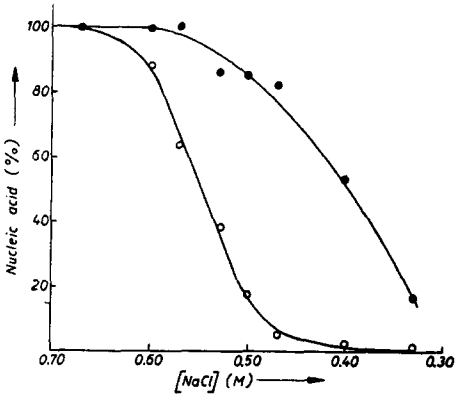

Fig. 6. Nucleic acid of mouse sarcoma (temp. o° C)

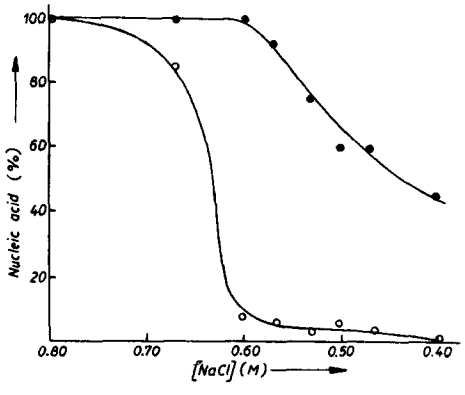

Fig. 7. Nucleic acid of *Myco. phlei* (temp. 20° C)

Solubility of the Cetavlon complexes of nucleic acids in aqueous sodium chloride.

○——○ % of the total DNA remaining in solution

●——● % of the total PNA remaining in solution

It was apparent, therefore, that there was a considerable difference in the solubility of the Cetavlon complexes of the two types of nucleic acid and that a separation was possible by fractionation at 0.5 *M* sodium chloride at 0° C or in the case of the nucleic acid of *Myco. phlei* at 0.60 *M* sodium chloride at 20° C.

Separation of the nucleic acids of Myco. tuberculosis

The nucleic acid (44 mg; PNA, 30%) isolated from *Myco. tuberculosis*, was dissolved in distilled water (15 ml) and the solution adjusted to 1 *M* with solid sodium chloride. Cetavlon (260 mg) was added, the solution cooled to 0° C and mixed with an equal volume of distilled water (cooled to 0° C). The mixture was allowed to stand for 30 minutes and then centrifuged at 0° C. The precipitate was redissolved in 1 *M* sodium chloride and reprecipitated as above with an equal volume of water. The resulting precipitate was again redissolved in 1 *M* sodium chloride, and the nucleic acid precipitated as the sodium salt by the addition of ethanol (3 vols). The precipitate was dissolved in distilled water, sodium chloride added and the nucleic acid reprecipitated by the addition of ethanol (3 vols). The precipitate was washed with 85% ethanol (five times), dissolved in distilled water, dialysed and the solution freeze-dried to give DNA (28 mg) which contained N, 12.4%; P, 7.1% and PNA, 1.6%.

The 0.5 *M* sodium chloride supernatant liquids were combined and diluted with an equal volume of distilled water. The precipitate thus obtained was treated as above to give a PNA fraction (7.4 mg) which contained N, 12.3%; P, 6.8% and DNA, 5.1%.

In a similar manner the nucleic acid of *Sarcina lutea* (20 mg; PNA, 38%) was separated into DNA (8.6 mg; N, 13.4%; P, 7.9% and PNA, 2.8%) and a PNA fraction (7.2 mg; DNA, 22%). Wheat germ nucleic acid (40 mg; PNA, 4.8%) gave a DNA (28 mg) which contained 1.1% PNA (N, 12.9%; P, 7.9%) and mouse sarcoma nucleic acid (40 mg; PNA, 15%) gave a DNA (28 mg) which contained 1.9% PNA (N, 12.9%; P, 7.6%).

Separation of the nucleic acids of Myco. phlei

This separation was carried out at 20° C because when carried out at 0° C the DNA fraction still contained 5–6% PNA. It appeared, however, that a much better separation would be obtained at 20° C (see Fig. 7).

Nucleic acid (128 mg; PNA, 20%), isolated from *Myco. phlei*, was dissolved in distilled water (60 ml) and the solution adjusted to 1 *M* with solid sodium chloride. Cetavlon (1.2 g) was dissolved in the solution and distilled water (40 ml) added, the temperature being kept at 20° C. The DNA fraction (95 mg) was isolated as previously described (N, 13.2%; P, 7.6%; PNA, 0.7%). The PNA (24 mg) fraction contained N, 13.7%; P, 8.0%; DNA, 7.1%.

The purification of pentose nucleic acids

Certain of the pentosenucleic acids isolated by the Cetavlon method still contained an appreciable amount of DNA. These were further purified by precipitation of the calcium salts from aqueous solution. It was found that the best purification was obtained with 1 *M* calcium chloride. The nucleic acid concentration was very important, and the optimum varied with the composition of the nucleic acid. With nucleic acids containing 80–90% PNA the optimum concentration was 4–5 mg/ml. Under these conditions the

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calcium salt of PNA was precipitated preferentially. The purification was carried out as follows:

Pentosenucleic acid (12 mg; DNA, 7.1%) from *Myco. phlei*, partially purified by the Cetavlon method, was dissolved in distilled water (3 ml) and calcium chloride (330 mg) added at room temperature. The precipitate was centrifuged off (7,000 g for 15 minutes), the precipitate washed with water (two portions of 1 ml), and dissolved in 0.01 *M* sodium citrate. The solution was dialysed and then freeze-dried to give PNA (7.4 mg) which contained 4.5% DNA. The addition of ethanol (2 vols) to the supernatant liquid from the calcium precipitation gave a small fraction (1.9 mg) which contained 17% DNA.

Using this method a purified PNA of *Sarcina lutea* also was obtained which contained 2.4% DNA.

Adsorption of nucleic acids by activated charcoal

For these experiments, Norit SX25 (N.V. Norit-Vereeniging, Verkoop Centrale, Amsterdam: Haller and Phillips Ltd. London) was used, as the grades recommended by CHARGAFF AND ZAMENHOF[8] were unobtainable in this country. From the work of these authors it appears that PNA is adsorbed by charcoal at certain salt concentrations in preference to DNA. The optimum concentration of sodium chloride for this preferential adsorption of PNA on Norit SX25 was determined as follows:

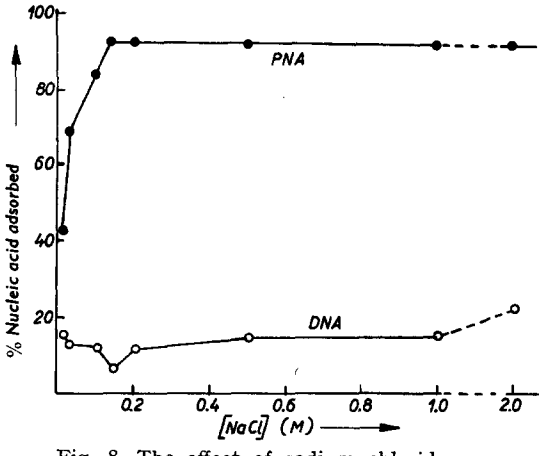

Fig. 8. The effect of sodium chloride concentration on the adsorption of DNA and PNA of *Sarcina lutea* on Norit SX25

Norit SX25 was washed in running tap water for 18 h, and then twice with 2 *M* sodium chloride (8 vols) and once with 0.14 *M* sodium chloride (10 vols). A series of nine tubes containing the nucleic acid of *Sarcina lutea* (PNA, 35%; conc. 0.8 mg/ml), washed Norit SX25 and sodium chloride, was set up so that the concentration of sodium chloride varied from 0–2 *M* over the range. The tubes were shaken at 2° C for 60 minutes, and then centrifuged at 10,000 r.p.m. for 20 minutes. The clear supernatant liquids from each tube were removed and aliquots analysed for total nucleic acid and PNA by the usual methods. The results (Fig. 8) indicated that the optimum salt concentration for preferential PNA adsorption was 0.14 *M*.

The amount of Norit used for the adsorption was also important, as the presence of too much resulted in the adsorption of appreciable quantities of DNA. For a nucleic acid of the above composition, at a concentration of 1 mg/ml, 1/15th vol of Norit was found to be the optimum.

Isolation of DNA from the nucleic acid of Sarcina lutea

A solution of the nucleic acid (10 mg) in 0.14 *M* sodium chloride (10 ml) was cooled to 2° C and shaken with the requisite amount of washed Norit SX25 for 1 h at 2° C.

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Source	Recovery	Cetavlon-calcium					
		PNA			DNA		
		N %	P %	DNA %	N %	P %	PN/ %
Myco. tuberculosis	80 %*	12.3	6.8	5.1	12.4	7.1	1.6
Myco. phlei	78 %	13.7	8.0	4.5	13.2	7.6	0.7
Sarcina lutea	58 %	13.8	7.9	2.4	13.4	7.9	2.8
Mouse sarcoma	74 %*	—	—	—	12.9	7.6	1.9
Wheat germ	75 %*	—	—	—	12.9	7.9	1.1

* Purification of PNA with calcium not carried out.

The suspension was centrifuged at 10,000 r.p.m. for 30 minutes and the DNA precipitated from the clear supernatant liquid by the addition of ethanol (3 vols). The fibrous precipitate was collected with a glass rod and washed with 85% ethanol until free from sodium chloride. It was then dissolved in distilled water, and the solution freeze-dried to give DNA (5.4 mg) which contained N, 14.2%; P, 8.4% and PNA, 2.4%.

Elution of PNA from Norit SX25

Attempts to elute the adsorbed PNA from Norit SX25 by means of aqueous solutions of various ionic strengths and pH values were unsuccessful, as were those using aqueous pyridine or sodium lauryl sulphate at pH 2–10. An aqueous solution of phenol, adjusted to pH 7–7.5, however, was effective in eluting most of the PNA adsorbed on the Norit. Solutions containing above 15% phenol were effective, and elution occurred equally well at 20° C or 0° C.

Separation of nucleic acids using Norit SX25

A solution of the nucleic acid in 0.14 *M* sodium chloride (1 mg/ml) was treated with 1/15th vol of washed Norit SX25 at 2° C as previously described. The suspension was centrifuged, and DNA isolated from the supernatant liquid as usual. The Norit was washed with distilled water, and then suspended in fifteen times its own volume of a 15% aqueous suspension of phenol (pH 7–7.5) and shaken for 8–10 h at 2° C. The suspension was then centrifuged at 10,000 r.p.m., and Norit washed with 15% phenol (pH 7–7.5) and the combined supernatant and washings brought to 1 *M* with sodium chloride. The nucleic acid was precipitated by the addition of ethanol (3 vols) and the precipitate redissolved in 1 *M* sodium chloride, reprecipitated with ethanol, and washed several times with 85% ethanol to remove phenol. It was finally dissolved in distilled water, dialysed, and the solution freeze-dried.

This separation was carried out on the nucleic acids of *Sarcina lutea*, *Myco. phlei* and mouse sarcoma. The results of these experiments and comparative experiments using the Cetavlon method of separations are summarised in Table II.

DISCUSSION

The great similarity in properties of the two types of nucleic acid, in their native

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II

Separation

Norit SX25						
Recovery	*PNA*			*DNA*		
	N %	*P* %	*DNA* %	*N* %	*P* %	*PNA* %
—	—	—	—	—	—	—
75%	11.8	6.5	4.5	13.0	7.5	5.6
78%	14.0	8.0	2.1	14.2	8.4	2.4
70%	11.2	6.5	2.0	12.8	6.9	1.0
—	—	—	—	—	—	—

states, has made their separation by a mild method rather difficult. By utilising the difference in solubility of the Cetavlon salts, however, a convenient separation of the two types of nucleic acid has been achieved. Deoxypentosenucleic acids have been produced which were sufficiently free from pentosenucleic acids for chemical analysis. As the products were fibrous solids which produced highly viscous solutions, it did not appear that extensive degradation had occurred. The pentosenucleic acids produced by the above method were purified further by their preferential precipitation as their calcium salts. In order for this precipitation to be effective, it was necessary to remove most of the DNA by the Cetavlon method, or else a high proportion of the PNA remained associated with the DNA.

This investigation has confirmed the findings of CHARGAFF AND ZAMENHOF[8] that PNA could be adsorbed on activated charcoal, in preference to DNA. The new discovery that the PNA could be eluted from the charcoal by 15% phenol at pH 7–7.5 has enabled this method to be used for the separation of the two types of nucleic acid. This method was superior to the Cetavlon-calcium method in that it was simpler in operation, but it had the disadvantage that it was difficult to remove completely the charcoal from the products.

It appears, therefore, that the methods described above will enable DNA and PNA to be obtained free from each other in a relatively undegraded state, and, therefore, will facilitate the determination of the chemical composition and physical properties of nucleic acids from bacteria and other sources.

The authors are grateful to Imperial Chemical Industries Ltd. for the gift of a sample of Cetavlon and for financial assistance, and to the Medical Research Council for a grant in aid of expenses.

SUMMARY

Pentosenucleic (PNA) and desoxypentosenucleic acid (DNA) have been separated from each other by two mild methods. In the first method the cetyltrimethylammonium (Cetavlon) salts of the nucleic acids were fractionated with 0.5 *M* sodium chloride at 0° C when the DNA was preferentially precipitated. The PNA fraction was isolated from the supernatant liquid, and in some cases further purified by precipitation of the calcium salts. In the second method PNA was preferentially adsorbed on charcoal (Norit SX25) from 0.14 *M* sodium chloride (*cf.* CHARGAFF AND ZAMENHOF[8]) and subsequently eluted by means of 15% phenol at pH 7–7.5. The PNA and DNA of *Myco. tuberculosis, Myco. phlei, Sarcina lutea,* wheat germ and mouse sarcoma have been separated from each other by these methods.

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RÉSUMÉ

Les acides pentose- et désoxypentosenucléiques ont été séparés par deux méthodes douces. Dans la première, les sels de cétyltriméthylammonium (Cetavlon) des acides nucléiques étaient fractionnés à 0° à l'aide de chlorure de sodium 0.5 *M*, dans lequel l'acide désoxyribonucléique est le moins soluble. La fraction pentosenucléique était isolée du liquide surnageant et, dans quelques cas, purifiée ensuite par précipitation des sels de calcium.

Dans la seconde méthode, l'acide pentosenucléique était adsorbé sur charbon (Norite SX 25) d'une solution 0.14 *M* de chlorure de sodium, puis élué à l'aide de phénol 15% et pH 7–7.5.

Les acides pentosenucléiques et désoxypentosenucléiques de *Myco tuberculosis*, de *Myco phlei*, de *Sarcina lutea*, du germe de blé et d'un sarcome de souris ont été séparés par ces méthodes.

ZUSAMMENFASSUNG

Pentosenukleinsäure (PNS) und Desoxypentosenukleinsäure (DNS) wurden durch zwei milde Methoden von einander getrennt. Bei der ersten Methode wurden die Cetyltrimethylammonium-(Cetavlon)-salze der Nukleinsäuren mit 0.5 *M* Natriumchlorid bei 0° fraktioniert, wobei die DNS vorzugsweise ausgefällt wurde. Die PNS-fraktion wurde von der überstehenden Flüssigkeit abgetrennt und in einigen Fällen weitergereinigt durch Ausfällen der Calciumsalze. Bei der zweiten Methode wurde die PNS vorzugsweise an Kohle (Norit SX25) aus 0.14 *M* Natriumchlorid (vgl. CHARGAFF UND ZAMENHOF[8]) adsorbiert und darauf mit 15%-igem Phenol bei pH 7–7.5 eluiert. Die PNS und die DNS von *Myco. tuberculosis*, *Myco. phlei*, *Sarcina lutea*, Weizenkeimlingen und Mäusesarkom wurden durch diese Methoden voneinander getrennt.

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