An Electrophoretic Investigation of the Mixture of Oligonucleotides Formed by the Enzymic Degradation of Deoxyribonucleic Acid by Deoxyribonuclease

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The aim of the present work has been the investigation of the mixture of oligonucleotides formed by the action on deoxyribonucleic acid of the deoxyribonuclease isolated from pancreas by Kunitz (1948). Recent investigations of the nucleic acids concerning the analysis of the relative amounts of purine and pyrimidine bases set free on complete hydrolysis demonstrated the existence of different nucleic acids both in the deoxyribonucleic (DNA) and ribonucleic acid (RNA) series when isolated from different sources (Chargaff, Vischer, Doniger, Green & Misani, 1949; Vischer & Chargaff, 1948; Wyatt, 1950). That differences even exist between the DNA's of closely similar types of bacteria is also certain, due to the remarkable work of Avery, MacLeod & McCarty (1944) and Boivin, Delaunay, Vendrely & Lehoult (1945) on the transforming factors of pneumococci and *Escherichia coli* respectively.

The need for further investigation of these substances hardly requires stressing, but, for example, it may be mentioned that no satisfactory method for the isolation of the mononucleotides of the deoxy series yet exists. In addition little is known about the type of bonds joining these units into the chains of the nucleic acid molecule. Although it would certainly be preferable to investigate a nucleic acid specimen of known molecular homogeneity, some useful information may be obtained by studying hydrolysates of preparations which are actually mixtures of more than one nucleic acid. In fact certain preparations of DNA such as those of Hammarsten (1924) and Gulland, Jordan & Threlfall (1947) appear to be homogeneous when examined both by electrophoresis (Stenhagen & Teorell, 1939; Creeth, Jordan & Gulland, 1949) and by ultracentrifugation (Pedersen, 1940; Cecil & Ogston, 1948) and these results are supported by the recent work of Jungner, Jungner & Allgen (1949) on the dielectric behaviour of these substances. However, much further work is required before homogeneity can be regarded as established.

Despite this situation it seemed worth while investigating DNA as at present available both in order to develop suitable techniques, and because at least preliminary information bearing on molecular structure might be obtainable. Because investigations of intact nucleic acids have shown the DNA's as a class to have larger molecules than the RNA's, perhaps due to the milder conditions used for isolation, this investigation has been confined to thymus DNA.

As even slightly acid conditions lead to the destruction of mononucleotides by hydrolysis of the purine-deoxyribose bonds, and as the internucleotide bonds are inconveniently stable to alkali, the only possibility seemed to be to make use of enzymic hydrolysis. This approach was facilitated by the various studies which have already been made on the breakdown of DNA by deoxyribonuclease. Thus, in addition to specification of the optimum conditions for the activity of this enzyme, the work of Fischer, Böttger & Lehmann-Echternacht (1941), McCarty (1946) and Kunitz (1950) has made it clear that by its action DNA is converted into a mixture of oligonucleotides. Measurement of the average diffusion constant of this mixture indicated mean molar sizes corresponding to units of four (Fischer *et al.* 1941) or more (Kunitz, 1950) mononucleotides. Titration data show the liberation after complete enzymic hydrolysis of one acid group per four atoms P. Thus Fischer *et al.* (1941) have suggested that on the average the hydrolysate consists of tetranucleotides. Another important characteristic of the action of this enzyme is its failure to liberate inorganic P.

In the present work electrophoresis of the enzymic hydrolysate was carried out in jelly. After one such separation (Exp. 3) pieces of the jelly were cut out and an attempt was made to estimate the diffusion constants of the oligonucleotides contained in each. Another separation (Exp. 5) was conducted in a specially broad trough so that sufficient material could be obtained for quantitative analysis of the purine and pyrimidine bases present in those bands selected for analysis.

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METHODS

Materials. Deoxyribonucleic acid was prepared according to Hammarsten (1924) from calf thymus.

Deoxyribonuclease, crystalline material prepared according to Kunitz (1948) was purchased from the Worthington Biochemical Laboratory, Freehold, New Jersey, U.S.A.

The mononucleotides were commercial samples obtained from the National Biochemical Corporation, New York, with the exception of the sodium uridylate which was prepared in the laboratory. The diphosphopyridine nucleotide was kindly supplied by Dr J. B. Neilands.

Difco Bacto agar was set as a 1 % aqueous jelly and after cutting into small pieces was washed for 3 days in running tap water. The lumps of agar were then drained and stored in the cold room. This material will subsequently be referred to as washed 1 % agar. Washing the agar in this way served to remove most of the diffusible material and resulted in much stiffer jellies being formed at any concentration of agar.

Enzymic degradation of DNA. 500 ml. of a 0·2 % (w/v) solution of DNA was brought to pH 6·5 by the addition of 0·1 N-NaOH. $MgSO_4$ was added to make the solution $0{\cdot}3 \times 10^{-3}$ M with respect to Mg^{++}. Next 5 mg. enzyme were added and the solution was kept at 37° for 24 hr. The pH was maintained at 6·5 by the occasional addition of N-NaOH. No buffer could be used because of the subsequent electrophoresis. Finally, the digest, which no longer gave more than a very slight opalescence when acidified, was concentrated *in vacuo.*

Methods of electrophoresis. Electrophoresis was always conducted in agar jelly. Agar rather than silica jelly was used because it permits the passage of large molecules (Gordon, Keil, Šebesta, Knessl & Šorm, 1950) and it was desired to compare the mobility of the oligonucleotides with that of the original DNA. Agar is also convenient because solutions suitable for estimation in the Beckman quartz spectrophotometer can be made by melting pieces of the jelly with suitable volumes of water.

For all the separations except the preparative one (Exp. 5) a flat glass trough similar to that described by Consden, Gordon & Martin (1946) was used. Its internal dimensions were $85 \times 15 \times 0{\cdot}78$ cm. For the preparative experiment a similar trough having internal dimensions of $60 \times 60 \times 0{\cdot}78$ cm. was used. To ensure an adequate supply of buffer solution at the electrodes, branched tubes ending in four equally spaced glass taps were provided at each end of the jelly. In this work the jelly was never covered by a glass lid.

After the work had been in progress for some time it was found that ultraviolet-absorbing substances can be seen in the trough if a trace of a fluorescent substances is originally incorporated in the jelly which is then illuminated with ultraviolet light. This technique is also sufficiently sensitive to detect bands of protein in agar, if the proteins contain the usual proportions of tyrosine and tryptophan. The source of ultraviolet light was a Mineralight lamp giving maximum emission at 258 mμ. (Chargaff, Magasanik, Doniger & Vischer, 1949).

Owing to the range in which dissociation of the amino groups of the purine and pyrimidines present in the nucleotides occurs, useful mobility differences between such components are only to be expected at rather low pH values. On the other hand, care has to be exercised to avoid acidities at which hydrolysis of purine deoxyribose bonds can occur or at which there may be a possibility of precipitation. Because of these considerations most of the present work was carried out at pH 3·7. In addition a few experiments were carried out in acetate buffer at pH 4·4–4·6. As can be seen from Figs. 2 and 6 separation is not so good at pH 4·4 as at pH 3·7, but this had to be accepted in the case of the preparative experiment as the only buffers available which were suitable for the lower pH range also gave precipitates with mercury.

Examination of agar slab after electrophoresis. After examination with the ultraviolet lamp a row of suitably spaced rectangular cuts ($0{\cdot}8 \times 2{\cdot}0$ cm.) were made with a metal cutter along the length of the jelly. Next the pieces of jelly were lifted out of the cuts with a special spatula and placed in a series of test tubes. After the addition of 5 ml. water to each the jelly was melted. The solutions were then cooled and estimated by measurement of their extinction coefficients in the Beckman quartz spectrophotometer. Fortunately, between 248–310 mμ. no absorption due to fluorescein could be detected. Perhaps the greatest source of error in the values thus obtained results from losses of small pieces of jelly inadvertently allowed to remain in the trough.

Analysis for purines and pyrimidines. The solutions containing the oligonucleotides were evaporated to dryness *in vacuo.* The purines and pyrimidines were set free by hydrolysis with anhydrous formic acid as described by Wyatt (1950). For this purpose the oligonucleotides were dissolved in sufficient formic acid for the concentration of oligonucleotides to be approximately 20 mg./ml. The hydrolysis was carried out in 0·5 ml. portions in sealed tubes in an air bath at 175° for 30 min. After hydrolysis and centrifugation the black flakes were washed three times with a total of 6 ml. of warm formic acid. The combined supernatants were evaporated to dryness *in vacuo* and the excess formic acid removed by repeated evaporation after the addition of water.

The quantitative analysis of purine and pyrimidine bases was carried out by means of a chromatogram of Dowex 50 which was developed with 2 N-HCl as described by Cohn (1949). For this purpose each dry residue after the evaporation of formic acid was dissolved in 5 ml. 2 N-HCl and applied to the top of a Dowex column, height 90–100 mm., diameter 9 mm. Each chromatogram was developed and analysed as described by Cohn. Fractions containing the same base were quantitatively combined and the amount of base in each solution thus formed was determined by its light absorption in the Beckman quartz spectrophotometer. The calculations were carried out with constants which had been obtained previously by Reichard (1949*b*).

EXPERIMENTAL

Exp. 1. *Electrophoresis of DNA, adenylic, cytidylic, guanylic and uridylic acids at* pH 3·7

Washed 1 % agar (300 g.), 4 g. sodium hydroxide 21 g. citric acid and 4 mg. fluorescein were added to 700 ml. water and brought just to the boil. The solution was cooled to 60° and poured into the 15 cm. wide trough. The jelly thus formed was 0·78 cm. thick. After setting, a $5{\cdot}0 \times 0{\cdot}9$ cm. piece of jelly was cut out of the slab at a distance of 20 cm. from the cathode end of the trough. The gutter thus formed was filled with 3·5 ml. of a solution containing the same concentration of agar as that of the rest of

the jelly, 10 mg. each of DNA, adenylic, cytidylic and guanylic acids and sodium uridylate. No sodium citrate was added. Owing to the unavailability of the mononucleotides of the DNA series mononucleotides from RNA were used. It was assumed that compared with DNA-nucleotides no difference of mobility would be observable and that uridylic acid would move at practically the same rate as thymidylic acid. Impurities present in the mononucleotides no doubt account for the small extra peaks in Fig. 1.

The pH of the solution of the mononucleotides was so close to that of the rest of the jelly that no adjustment was necessary. Next the ends of the jelly were cut away and electrophoresis was carried out as described by Consden *et al.* (1946) and by Gordon *et al.* (1950). The solution at the electrodes was 0·1 M-sodium citrate at pH 3·7 First a potential gradient of 2·3 V./cm. was applied for 2 hr. and then 3·7 V./cm. for a further 15 hr. At the end of this period the jelly was examined as described above, light absorptions being noted at 240, 248, 262, 270 and 310 mμ. In this way the components responsible for each band were readily identified. The results thus obtained are shown in Fig. 1.

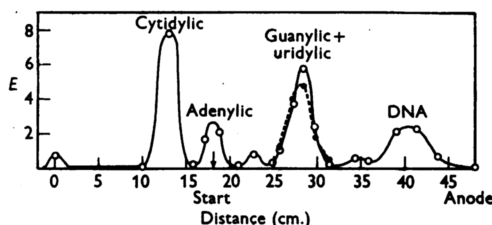

Fig. 1. Electrophoresis of DNA, adenylic, cytidylic, guanylic and uridylic acids at pH 3·7. ——, Light absorption at 262 mμ.; - - - -, light absorption at 248 mμ.

Exp. 2. Electrophoresis of enzymic hydrolysate of DNA and DNA at pH 3·7

The apparatus and jelly were the same as in Exp. 1. In this experiment, however, two 5·0 × 0·9 cm. gutters were cut out so that both DNA and a sample of the enzymic hydrolysate could migrate side by side along the trough. Into one was placed the usual agar solution containing 0·1 M-sodium citrate at pH 3·7 in which had been dissolved 21 mg. of DNA. During dissolution the temperature of this solution was never allowed to exceed 70°. The second was filled with a solution containing the oligonucleotides produced by the hydrolysis of 77 mg. DNA. In this and subsequent experiments where the enzymic hydrolysate was investigated the jelly in the inlay did not contain any buffer, the aim being always to keep the osmotic pressure of this part of the jelly as low as possible to prevent sweating. The pH was adjusted by the addition of N-citric acid. Electrophoresis was conducted for 4 hr. at 2·3 V./cm. and then for a further 19 hr. at 5·7 V./cm. Finally the jelly was examined as usual. By means of the ultraviolet lamp it was apparent that while most of the material had moved towards the anode, two extremely faint bands had moved towards the cathode. The results of the absorption measurements are shown in Fig. 2.

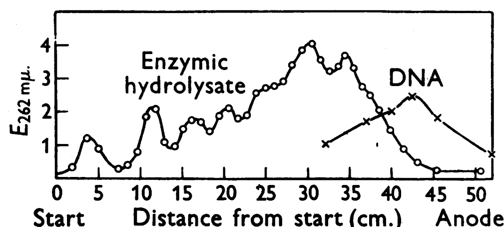

Fig. 2. Electrophoresis of enzymic hydrolysate of DNA and DNA at pH 3·7.

Exp. 3. Electrophoresis of enzymic hydrolysate of DNA at pH 3·7 *and diffusion measurements*

The apparatus and jelly were the same as in Exp. 1. The gutter was 11 × 0·9 cm. It was filled with 7·7 ml. of a solution at pH 3·7 containing agar and the oligonucleotides produced by the hydrolysis of 170 mg. of DNA. Electrophoresis was carried out as in the last experiment. Then the jelly was examined with the results shown in Fig. 3. As in Exp. 2, two faint bands were seen to have moved towards the cathode.

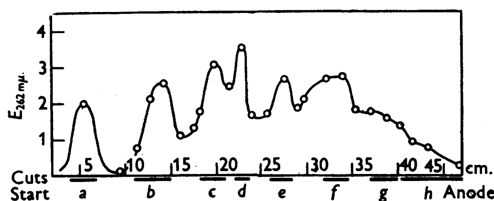

Fig. 3. Electrophoresis of enzymic hydrolysate of DNA at pH 3·7 (Exp. 3).

Using the information thus obtained eight sections were cut out from the jelly in the positions shown in Fig 3. After weighing and melting, each fraction was made up to 30 ml. by the addition of a hot solution of agar containing 750 g. washed agar/l. and 0·1 M-sodium citrate. These solutions were then transferred to flat-bottomed bottles of 4·0 cm. internal diameter. The concentration of agar was chosen partly because it gave jellies of adequate strength for the diffusion experiment, and partly because measurement of the thickness of the jelly in the trough at the end of the electrophoresis indicated that it had decreased to give a jelly of about this concentration. Thus, although different amounts of extra jelly were added for the resetting of each fraction, the final agar concentration in each bottle was nearly the same.

After complete setting, the fractions, together with one bottle containing only 0·1 M-sodium citrate agar and another which in addition contained approximately 7 mg. of cytidylic acid (ribose), were transferred to a thermostat air oven held at 27°. 1 hr. was allowed for temperature equilibration and then 30 ml. of the 0·1 M-citrate buffer was added to each bottle. The oven was equipped with stirrers which were now started so that the buffer solution

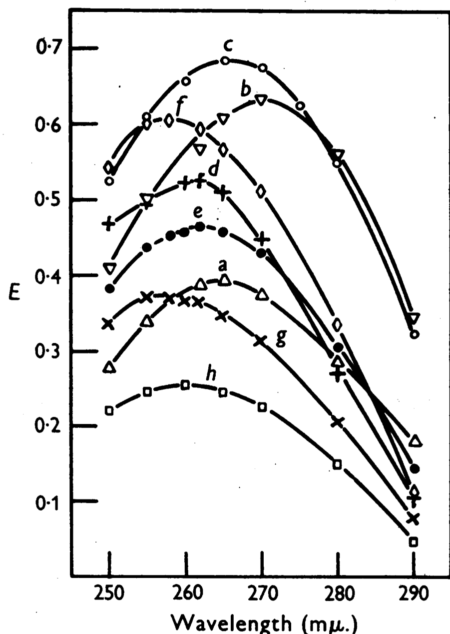

Fig. 4. Ultraviolet absorption of fractions obtained by electrophoresis of enzymic hydrolysate of DNA (Exp. 3).

over each jelly was kept in motion. After a further 1, 2, 3, 4 and 5 hr. respectively small portions of the solutions were examined in the Beckman spectrophotometer and quickly returned to the bottles. Finally, in order to ascertain the concentration of ultraviolet-absorbing material which must have been present in the jelly at the start of the experiment, each bottle was emptied into a beaker, the jelly was melted and made up to 400 ml. with 0·1 M-sodium citrate. The light absorption of these solutions was measured at a number of wavelengths; the curves thus obtained being shown in Fig. 4. As a control experiment approximately similar amounts (7–10 mg.) of adenylic, guanylic, cytidylic and uridylic acids, diphosphopyridine nucleotide, DNA and unfractionated enzymic hydrolysate were set in agar in the bottles and allowed to diffuse into 0·1 M-citrate buffer. Measurements were again made after 1, 2, 3, 4 and 5 hr. The results of all these diffusion measurements have been calculated in the manner suggested by Friedman & Kraemer (1930) and are shown in Table 1.

Table 1. *Diffusion constants of fractions obtained by electrophoresis of enzymic hydrolysate of DNA (Exp. 3) compared with those of mononucleotides, coenzyme I and intact DNA*

Substance	Diffusion constant (sq.cm./sec. $\times 10^{-6}$)
DNA	0·25
Coenzyme I	4·9
Adenylic acid	6·2
Guanylic acid	6·0
Cytidylic acid	6·0
Uridylic acid	6·8
Enzymic hydrolysate	3·8
Fraction *a*	4·3
Fraction *b*	3·7
Fraction *c*	4·5
Fraction *d*	4·7
Fraction *e*	3·4
Fraction *f*	3·5
Fraction *g*	3·0
Fraction *h*	3·9

Exp. 4. *The electrophoresis of the enzymic hydrolysate at* pH 4·6

The apparatus and jelly were the same as in Exp. 1 except that 0·2 M-sodium acetate at pH 4·6 replaced the citrate buffer in the jelly and 0·3 M-acetate buffer at pH 4·6 was used at the electrodes. The use of a more concentrated buffer solution at the electrodes was found to be advisable at this pH to prevent the jelly becoming swollen with liquid near the anode. Unless this was done the area of swollen jelly extended during the electrophoresis and sometimes interfered with the location of the substances.

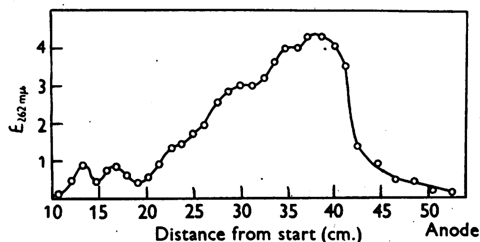

Fig. 5. The electrophoresis of the enzymic hydrolysate of DNA at pH 4·6.

Next a gutter was cut out and a sample of the enzymic hydrolysate in agar at pH 4·6 was added just as in Exp. 2. Electrophoresis was then conducted for 8·5 hr. at 2·3 V./cm. and then for 13·5 hr. at 5·7 V./cm. Finally the jelly was examined as usual, the curve obtained being shown in Fig. 5.

Exp. 5. *Large scale electrophoresis of the enzymic hydrolysate at* pH 4·4

The large trough was filled with 2·8 l. of solution made up as in Exp. 1 except that 0·2 M-sodium acetate replaced the citrate. After spaces had been

made for the electrodes at the ends of the trough a gutter 58 × 0·9 cm. was cut out at a distance of 6 cm. from the cathode edge of the jelly and parallel with it. This was then filled with 37 ml. of the usual agar solution containing the oligonucleotides produced by the hydrolysis of 1 g. of DNA. Electrophoresis was conducted for 5 hr. at 4·1 V./cm. and then for a further 16 hr. at 6·7 V./cm. Probably because the concentration of oligonucleotides and salt in the inlay was too high, considerable 'sweating' occurred in this region. As much as possible of this free liquid was removed. After the electrophoresis the jelly was examined with the results shown in Fig. 6. By means of this information and making use of the ultraviolet lamp to ascertain that the bands had not become curved, pieces of jelly were cut out as shown in Fig. 6.

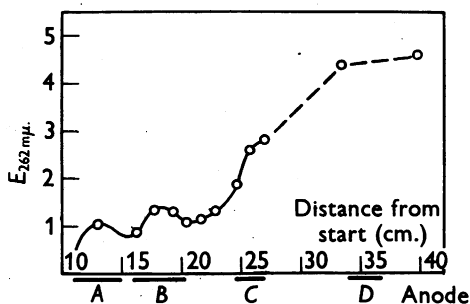

Fig. 6. Large scale electrophoresis of enzymic hydrolysate of DNA at pH 4·4 (Exp. 5).

Each section of agar was allowed to stand in 500 ml. of water for 24 hr. In this way the greater part of the salts and oligonucleotides diffused out. The solution was filtered off and extraction was repeated with a further 500 ml. of water. The combined solutions were then evaporated *in vacuo* to a volume of about 10 ml.

In order to free the oligonucleotides from salts and from almost all the agar they were precipitated with Hg^{++}, a method which has been applied by Reichard (1949*a*) to the preparation of ribomononucleotides. Following this method after adjusting the pH to 2·5 with 5 N-nitric acid, mercuric nitrate was added. The resulting precipitates were allowed to stand for 48 hr., washed and freed from mercury by treatment with hydrogen sulphide. The completeness of precipitation with mercury was checked by measuring the light absorption at 262 and 310 mμ. of the supernatant and washings. No indication of the presence of free purines or pyrimidines was obtained. Finally the purine and pyrimidine contents of the solutions were estimated by the method described above, and the results are given in Table 2, together with an analysis of the original DNA.

Exp. 6. *Electrophoresis of the enzymic hydrolysate at* pH 7 *in a strong jelly*

This experiment was carried out similarly to the electrophoresis of the enzymic hydrolysate described in Exp. 2 except that the jelly was made by

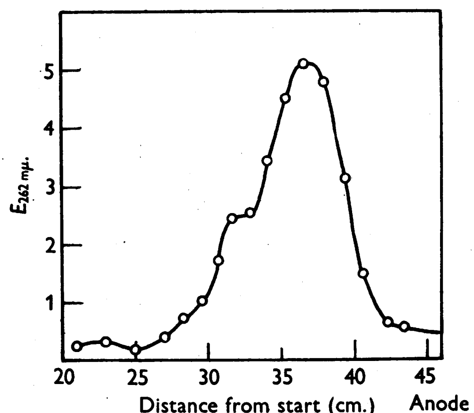

Fig. 7. Electrophoresis of enzymic hydrolysate of DNA at pH 7 in a strong jelly.

melting 1 % washed agar without the addition of water. 0·1 M-sodium phosphate buffer at pH 7 was used in the jelly and at the electrodes. The results obtained are shown in Fig. 7.

DISCUSSION

Little difficulty was experienced in adapting for the present purpose the experimental conditions used previously for the electrophoresis of proteins

Table 2. *Amounts of purine and pyrimidine bases found after hydrolysis and chromatography of fractions obtained by electrophoresis of enzymic hydrolysate of DNA* (*Exp.* 5)

(*R* = molar ratio of cytosine to the other bases.)

	Thymine		Cytosine		Guanine		Adenine	
	mg.	*R*	mg.	*R*	mg.	*R*	mg.	*R*
DNA	0·77	1·22	0·55	1·00	0·69	0·92	0·75	1·12
Fraction *A*	0·21	0·16	1·15	1·00	0·10	0·06	0·93	0·66
Fraction *B*	0·61	0·44	1·21	1·00	0·16	0·10	0·19	0·13
Fraction *C*	1·04	0·55	1·64	1·00	0·62	0·28	1·18	0·66
Fraction *D*	1·50	1·82	0·75	1·00	0·70	0·68	1·01	0·99

(Gordon *et al.* 1950). However, an initial complication was encountered when an attempt was made to investigate intact DNA at pH 5. Perhaps not surprisingly, in view of the strong tendency of DNA to form jellies, part of the ultraviolet-absorbing material originally set in the inlay scarcely moved from this position; another part of the DNA, however, moved towards the anode in the manner expected. It would be of interest to ascertain whether any real fractionation of DNA can be achieved in this way. Presumably because DNA cannot form jellies as easily at pH 3·7 this type of behaviour was not observed in any of the later experiments. However, even at this pH value the DNA did not give as regular a band as those obtained from substances of lower molecular weight. Thus observation with the ultraviolet lamp of DNA which had moved a considerable distance through the jelly, sometimes revealed a single band with irregular front and back. Such bands also caused considerable reduction in thickness of the jelly, and it seems likely that the observed irregularities were due to some such cause rather than to any possible inhomogeneity of the DNA. This type of behaviour probably occurred in Exp. 2 as is evidenced by the very flat shape of the DNA band. Despite this bad shape there is no doubt that only a small proportion of the fastest moving molecules of the enzymic hydrolysate moved as fast as did the DNA itself. The most probable explanation of this behaviour is the compactness of the intact DNA molecule. Thus it seems that this factor must more than counterbalance those likely to tell in the opposite sense. These latter presumably must include the extra negative charges introduced on enzymic hydrolysis and any tendency of the DNA to be held back by the jelly either by absorption or by a molecular sieve effect (Synge & Tiselius, 1950). That these two latter effects are negligible in agar at least for the enzymic hydrolysate was shown in Exp. 6 in which a very strong jelly was used.

The main result of the electrophoresis of the enzymic hydrolysates in their division into a number of distinct bands. The variation of the relative mobilities of some of these with pH is immediately apparent if the results obtained at pH 3·7 and 4·6 are compared. Thus at the higher pH the two slower moving bands are obviously less well resolved from the main bulk of the absorbing material. Even if all these bands finally prove to contain more than one molecular species the possibility of analysing the mixture produced by the enzyme begins to emerge. Another deduction which can be made directly from the results of the electrophoreses is that only very little guanine or adenine, if any, is split off as such by the action of the enzyme. This follows from the observation in Exp. 2 of two extremely faint bands moving towards the cathode. In Exp. 3 two similar bands were observed; judged by their appearance with the ultraviolet lamp they were only a little stronger. A more important difference between the two enzymic hydrolysates is, however, immediately apparent on comparison of Figs. 2 and 3, i.e. the much greater heights of the slower moving bands in the latter. Just possibly these differences may be due to the fact that the second hydrolysate was made with a different batch of deoxyribonuclease; the enzyme was not subjected to any purification after purchase. It seems more likely, however, that the differences may be due simply to inadequate control of the conditions during the hydrolysis.

Owing to the very incomplete separation of all but the two slowest moving bands the attempt to ascertain the bases present in each was restricted to these two. Cuts *C* and *D* in Fig. 5 were made for control purposes and to gain general information about the type of separations taking place. Unfortunately as shown in Fig. 5 even bands *A* and *B* in this experiment were not completely separated from one another. If this be taken into account it seems likely that the main component present in band *A* contains only cytosine and adenine in equimolar proportions. The analytical figures actually obtained could thus be explained as being due to overlapping with band *B* which was found to contain thymine and cytosine in the molecular ratio of about 1:2. Another simple explanation would be that band *A* contains at least two oligonucleotides, one containing cytosine and adenine in the molecular ratio 2:1, the other adenine and thymine in the ratio 1:1. However, this hypothesis seems unlikely as a dinucleotide of adenine and thymine containing two phosphoric acid groups would be expected to have a rather higher electrophoretic mobility than that of band *A*. The mobilities of bands *A* and *B* make it extremely unlikely that these bands consist of mixtures of mononucleotides. Only thymidylic acid could be present in band *B*, but the additional presence of cytosine in this band makes it much more likely that both are parts of an oligonucleotide. The presence of guanine in both these bands and adenine in band *B* can no doubt again be explained as being due to contamination with contiguous bands.

The diffusion measurements referred to above are also relevant to the question as to whether bands *A* and *B* contain mainly di- and tri-nucleotides or on the other hand substances of much higher molecular weight. Owing to the presence of agar these measurements could not be made in the usual diffusion cell and cannot therefore be compared with those obtained by Kunitz (1950) on the unfractionated hydrolysate of DNA. As the method employed here was originally worked out for non-electrolytes no claim is made that the figures obtained (Table 1) are of absolute significance. In addition the rather high blank values given by agar itself are a source of

inaccuracy. Despite these facts two conclusions seem to be legitimate. They are that there is no great difference in respect to the diffusion constants of the electrophoretically slower and faster moving components of the enzymic hydrolysate and that the diffusion constants of bands *A* and *B* are not inconsistent with those that might be expected for di- and tri-nucleotides. As mixtures containing dinucleotides have been found to be separable by means of paper chromatography (Hummel & Lindberg, 1949) it would seem worth while to test out the suitability of this technique for the further analysis of the fractions obtainable by electrophoresis.

SUMMARY

1. Deoxyribonucleic acid has been hydrolysed by means of the deoxyribonuclease of ox pancreas.

2. The mixture of oligonucleotides thus formed has been subjected to electrophoresis in agar jelly.

3. The incorporation of a trace of fluorescein in the jelly has made possible the visualization of bands of ultraviolet-absorbing material.

4. After electrophoresis appropriate sections have been cut out of the jelly and the diffusion constants of the materials contained in each has been estimated.

5. The ratios of the purine and pyrimidine bases present in such cuts have been estimated after hydrolysis and separation on an ion-exchange chromatogram.

6. The evidence thus obtained suggests that the main components of the two slowest moving bands are respectively dinucleotide(s) of adenine and cytosine and trinucleotide(s) containing one residue of thymine and two residues of cytosine.

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Improved Nomograms for Manometer Constants

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Dixon (1945) published a set of nomograms for the rapid determination of the constants of Warburg and Barcroft manometers. They have proved useful in practice and have saved much time and calculation, but could only be used for one, or at the most two, fixed temperatures. For work at other temperatures it was necessary either to construct new nomograms or to determine the constants by calculation.

Burris (1949) later devised a nomogram for Warburg manometers which could be used for any temperature up to 45°, but it was inconveniently complicated and required three separate settings of the straight edge (in addition to the calculation of v_G from V and v_F) to determine each constant. No such nomogram has been produced for Barcroft (differential) manometers.