STUDIES ON NUCLEOPROTEINS*

IV. PREPARATION OF THE DEOXYRIBONUCLEOPROTEIN AND FRACTIONATION OF THE DEOXYRIBONUCLEIC ACID OF WHEAT GERM

by

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In previous papers of this series[1-4] we have discussed certain properties of native and artificially prepared nucleohistones and the use of these complexes and of similar compounds with globin[3] or synthetic polyelectrolytes[4] for the fractionation of a variety of deoxyribonucleic acids. We have shown that the fractional extraction or dissociation of such products with salt solutions of increasing strength yields, usually in the presence of a chloroform phase, a series of deoxyribonucleic acid fractions of regularly graded composition, in which progressively falling proportions of guanine and cytosine and progressively rising proportions of adenine and thymine are accompanied by the maintenance of the unity relationships that appear to be characteristic of all deoxyribonucleic acids[5,6]. We submit here observations on the isolation of the deoxyribonucleoprotein of wheat germ together with studies on the composition of deoxyribonucleic acid fractions prepared by the fractional dissociation of the nucleoprotein itself and of artificially prepared nucleic acid-histone complexes. As the nucleic acid of wheat germ is known to contain not inconsiderable quantities of a fifth nitrogenous constituent, 5-methylcytosine[7-11], studies on the distribution of this base in the several nucleic acid fractions are of some interest in providing an additional criterion of differentiation. We shall deal below with other inferences from this work.

For reasons that will be discussed later, a brief study of the nucleotide composition of the pentose nucleic acid of wheat germ is included.

EXPERIMENTAL

Procedures and materials

The methods for the characterization and analysis of the nucleoprotein preparations have been outlined before[1-3]. Other analytical methods and most of the procedures for the quantitative estimation of the nitrogenous constituents and for the study of the sugar component have been

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listed previously[12]. Among minor modifications may be mentioned that the filter paper used in all chromatographic separation experiments was Whatman No. 1; and that for the hydrolysis with conc. formic acid the heating period at 175° was 40 minutes, the bomb tubes in their safety shields being immersed in a temperature-constant bath of silicone fluid 550 (Dow Corning). Several hydrolysis experiments were also performed with 7.5 *N* perchloric acid as the hydrolyzing agent[13].

Several chromatographic procedures were used for the determination of 5-methylcytosine, in which the solvent system consisting of a mixture of 6 volumes of *n*-butanol and 1 volume of 0.1 *N* ammonia, first applied by Dr. G. BRAWERMAN[8] and mentioned previously[14], was employed. In one arrangement, 0.03 to 0.04 ml portions of the hydrolysate (7 mg nucleic acid in 0.5 ml 98 % formic acid) were delivered to the papers which then were neutralized in an atmosphere of gaseous ammonia and irrigated with water as the solvent; this effected the separation of the purines from the pyrimidines[15,16]. The air-dried papers were then subjected to chromatography with butanol-NH_3, either in a second dimension or, after the purine zone had been cut off, in the arrangement described previously[15]. Analytically more satisfactory was the unidimensional chromatography of 0.04 to 0.05 ml portions of the hydrolysate with butanol-NH_3 on 52 cm long sheets for 36 hours. In this manner, all bases except guanine could be determined at the same time; the latter was estimated separately in portions containing about 6 γ of guanine.

Histone was prepared from calf thymus by the procedure of CRAMPTON *et al.*[2]. Wheat germ was obtained from the Gordon Wheat Germ Co., New York, N.Y., and stored in the cold until used.

Isolation of deoxyribonucleoprotein

All operations were carried out in the cold. We describe a typical experiment. A suspension of 300 g of fresh unprocessed wheat germ in 1 l of petroleum ether (b.p. 30–60°) was shaken for 8 hours and the material extracted twice more with 700 ml portions of the same solvent, each time for 24 hours, and dried in the air. The partially defatted wheat germ was stirred for 5 minutes in a high-speed mixer with 900 ml of a saline-citrate solution (pH 7.5; 0.1 *M* NaCl, 0.05 *M* sodium citrate) and after centrifugation (15 minutes, 3600 × *g*) resuspended in 800 ml of saline-citrate and stirred mechanically for one hour. The process of centrifugation and washing was carried out 6 times. The wash fluids gave no reaction for deoxyribose (diphenylamine), but showed strong pentose reactions (orcinol).

The washed residue, from which no deoxyribonucleoprotein could be extracted at this step with distilled water, was mixed at a high speed for 2 minutes with 650 ml of cold 10% aqueous NaCl[17]. The supernatant liquid obtained by centrifugation at 25,000 × *g* for 10 minutes was centrifuged in a Spinco centrifuge at 78,000 × *g* for 45 minutes*. A slight sediment giving strong reactions with diphenylamine and orcinol was discarded and the supernatant solution (600 ml) subjected to dialysis against 6 liters of 0.15 *M* saline-citrate for 40 hours. The nucleoprotein, which had precipitated during this time, was collected by centrifugation, washed several times with saline-citrate, and dissolved in 200 ml of *M* NaCl. Dialysis and dissolution were repeated 3 more times. The white product thus obtained, which was not soluble in the absence of electrolytes, contained less than 1 % of ribonucleic acid; it was used without drying in the fractionation experiments to be described below. The yield amounted to about 0.4% of the fresh wheat germ; the weight ratio of protein to nucleic acid was around 1.6; the absorption at 260 mμ corresponded to an ε (P) value of 6500. The two nucleoprotein preparations subjected to fractionation are in the following designated 1-NP and 2-NP.

* In another preparation, a single centrifugation (one hour at 25,000 × *g*) was substituted for the two runs.

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Preparation of deoxyribonucleic acid[§]

All operations were carried out in the cold. 300 g of wheat germ were treated in a high-speed mixer with 750 ml of ethanol for 8 minutes. After the addition of 750 ml of ether the mixture was stored for 90 minutes and the solvent then removed by suction. The suspension of the residue in 800 ml of saline-citrate was stirred for 1 hour and centrifuged at 3600 × *g* for 15 minutes. Washing and centrifugation were repeated 9 times under the same conditions. The washed wheat germ then was treated in a high-speed mixer with 750 ml of 10% NaCl for 2 minutes and the extract freed of debris by being passed through a Sharples supercentrifuge at 50,000 r.p.m. The threads produced by the injection of the clarified extract into 2 volumes of 95% ethanol were collected by spooling and washed in 80%, and stored under 95%, ethanol. A considerable amount of granular material, which precipitated at the same time, was also used in the subsequent isolation of nucleic acid.

The fibers, pressed free of alcohol and cut into small pieces, were dissolved with stirring in 500 ml of dist. water and the solution was treated with "Duponol", and the sodium deoxyribonucleate isolated, according to the procedure of KAY *et al.*[18] The air-dried fibers weighed 1.64 g, but were still contaminated with 16% ribonucleic acid. The latter was removed by adsorption on activated charcoal[19]. The solution of the substance in 1640 ml of 0.14 *M* NaCl was stirred with 84 ml of washed "Norit A" for 2 hours, clarified in the Sharples centrifuge, brought to a *M* NaCl concentration, and injected into one volume of ethanol. The fibers, washed with alcohol and acetone and dried in air, weighed 0.8 g. They contained 8.7% P, had an ε (P) of 6100 at 260 mμ and were free of ribonucleic acid (orcinol) and protein (biuret). The specific viscosity divided by the molarity of the solution with respect to P[1,2] was $\eta_{sp}(P) = 620$ (solution in 0.1 *M* NaCl containing 16.2 γ P per ml; 30°; Ostwald-Fenske viscosimeter, outflow time for 0.1 *M* NaCl 69.2 seconds).

The distribution of nitrogenous constituents, based on 7 individual hydrolysis experiments, is listed in Table I together with a series of previous data from the

TABLE I

COMPOSITION OF TOTAL SODIUM DEOXYRIBONUCLEATE OF WHEAT GERM*

Reference No.	Moles per 100 g-atoms P**						Molar ratios					
	A	*G*	*C*	*MC*	*T*	Actual recovery	$\frac{A+T}{G+C+MC}$	$\frac{Pu}{Py}$	$\frac{A}{T}$	$\frac{G}{C+MC}$	$\frac{C}{MC}$	$\frac{T}{MC}$
Present work***	28.1 (0.1)	21.8 (0.1)	16.8 (0.2)	5.9 (0.1)	27.4 (0.3)	97.3	1.25	1.0	1.03	0.96	2.85	4.64
7	26.5	23.5	17.2	5.8	27.0	84.5	1.15	1.0	0.98	1.02	2.96	4.65
8	27.3	22.7	16.8	6.0	27.1	96.2	1.19	1.0	1.01	1.00	2.80	4.52
9	27.1	20.2	19.6	5.7	27.4	83.0	1.20	0.9	0.99	0.80	3.44	4.81
10	26.8	23.2	16.7	5.3	28.0	99.5	1.21	1.0	0.96	1.05	3.15	5.28
11	27.8	22.2	16.3	6.2	27.5		1.24	1.0	1.01	0.99	2.63	4.44

* Abbreviations used: A, adenine; G, guanine; C, cytosine; MC, 5-methylcytosine; T, thymine; Pu, purines; Py, pyrimidines.

** The mean proportions of each constituent have been corrected for a 100% recovery. The actual recovery is listed in the seventh column.

*** The figures in parentheses denote the standard errors.

§ We wish to thank Dr. G. BRAWERMAN for acquainting us with many unpublished observations made in the course of his work[8].

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literature. Several pertinent molar ratios are also included. The agreement between the present figures and most of the preceding results[7,8,11] is satisfactory.

Nature of the sugar component

Specimens of the nucleic acids from wheat germ and calf thymus were treated in parallel. The procedures for the release of the sugar moiety and for its demonstration by chromatography have been described previously[20,21]. In all solvent systems, the deoxy sugar liberated from the wheat germ nucleic acid occupied the same position on the chromatograms as 2-deoxyribose obtained from the calf thymus preparation.

Fractionation of wheat germ deoxyribonucleic acid

Presentation of results. The deoxyribonucleoprotein preparations from wheat germ are designated NP, the artificially prepared complexes between histone and wheat germ deoxyribonucleic acid are designated HN, in each case preceded by an arabic numeral denoting the particular starting material. The separated nucleic acid fractions are numbered consecutively with roman numerals. Thus, 2-NP-IV stands for Fraction IV obtained by the fractional extraction of nucleoprotein preparation No. 2.

Fractional extraction of nucleoprotein gels. The wheat germ nucleoprotein preparations 1-NP and 2-NP described above were used in fractionation studies which followed the procedures employed previously for calf thymus nucleohistone[1-3]. The products were dissolved in 300 ml of 0.5 *M* (Prep. 1-NP) or 0.4 *M* NaCl (Prep. 2-NP) and the solutions treated in a high-speed mixer with 150 ml of chloroform-pentanol (4:1, v/v) for 3 minutes. The supernatant fluid collected by centrifugation (3600 × *g*) was removed and the gel extracted stepwise with NaCl solutions of increasing concentration, each treatment in the high-speed mixer (3 minutes) being followed by centrifugation. The collection of all fractions up to *M* NaCl was performed on the same day; the gel then was mixed with 1.7 *M* NaCl and kept overnight: a treatment that yielded no more than minute additional quantities of nucleic acid.

The proportions of original total nucleic acid found in the extracts, which are indicated in Table II, were determined by extinction measurements in the ultraviolet. The total recovery in the separated fractions amounted to 98.3 and 91.2% of the nucleic acid present in Preparations 1-NP and 2-NP, respectively. For the isolation of the nucleic acid fractions, each extract was adjusted to 2.6 *M* NaCl by the addition of salt, and the solutions, after being kept in the cold for 3 hours, were injected into 2 volumes of 95% ethanol. The fibers were collected and the substances recovered after dialysis and lyophilization in the usual manner. Purity checks included P determination and diphenylamine, orcinol and biuret tests. The purine and pyrimidine composition of the separated fractions is listed in Table II which also includes a summary of some of the molar relationships.

Fractional dissociation of histone nucleate gels. For the fractionation of 600 mg of the preparation of the sodium deoxyribonucleate of wheat germ, a 0.1% solution in distilled water was brought to 0.55 *M* NaCl by the addition of solid salt. The treatment with histone (weight ratio of protein to phosphorus, 15.1) and the removal and collection of fractions followed the previously described technique[3]. Analytical and other experimental data yielded by this experiment are given under 1-HN in Table II. About 88% of the nucleic acid submitted to fractionation were recovered in the form of 6 fractions.

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TABLE II

COMPOSITION OF DNA FRACTIONS OBTAINED BY FRACTIONAL EXTRACTION OF WHEAT GERM NUCLEOPROTEIN GELS (NP) AND OF COMPLEXES BETWEEN WHEAT GERM DNA AND HISTONE (HN)*

Preparation	Fraction No.	NaCl molarity	% of DNA in starting material***	Moles per 100 g-atoms P** A	G	C	MC	T	Molar ratios (A+T)/(G+C+MC)	Pu/Py	A/T	G/C	C/MC
1-NP	I	0.5	16.1	27.0	23.0	17.2	6.9	25.9	1.12	1.00	1.04	0.95	2.49
	II	0.55	13.5	27.4	23.6	16.4	6.5	26.1	1.15	1.04	1.05	1.03	2.52
	III	0.6	12.8	27.4	23.4	16.5	6.0	26.6	1.18	1.03	1.03	1.04	2.75
	IV	0.65	12.8	27.6	23.0	16.9	5.1	27.4	1.22	1.02	1.01	1.04	3.31
	V	0.7	9.3	28.0	22.5	17.1	5.5	26.9	1.22	1.02	1.04	1.00	3.11
	VI	0.75	12.6	28.0	22.6	16.9	5.2	27.3	1.24	1.02	1.02	1.02	3.25
	VII	0.8	7.5	29.1	20.8	16.4	5.2	28.4	1.36	1.00	1.02	0.96	3.15
	VIII	0.9	11.1	29.9	20.2	15.4	4.6	29.8	1.49	1.01	1.00	1.01	3.35
2-NP	I	0.4	8.6	26.2	25.5	16.8	6.4	25.1	1.05	1.07	1.04	1.10	2.62
	II	0.45	4.7	25.5	25.9	17.9	6.6	24.1	0.98	1.06	1.06	1.06	2.71
	III	0.5	13.7	26.7	24.1	16.9	6.1	26.3	1.12	1.03	1.01	1.05	2.77
	IV	0.55	12.8	27.5	23.4	16.1	5.6	27.4	1.22	1.04	1.00	1.08	2.88
	V	0.6	10.5	29.3	22.2	16.1	5.1	27.3	1.30	1.06	1.07	1.05	3.16
	VI	0.65	12.3	29.0	22.7	16.0	4.9	27.4	1.29	1.07	1.06	1.08	3.27
	VII	0.75	12.7	29.1	21.6	15.8	4.8	28.7	1.37	1.03	1.01	1.05	3.29
	VIII	0.8	6.5	30.2	21.0	15.0	4.4	29.4	1.48	1.05	1.03	1.08	3.41
	IX	0.9	5.9	30.0	21.2	15.5	4.1	29.3	1.45	1.05	1.02	1.08	3.78
1-HN	I	0.55	10.6	24.9	24.4	17.8	7.9	25.0	1.00	0.97	1.00	0.95	2.25
	II	0.65	19.4	27.4	21.9	17.2	6.2	27.3	1.21	0.97	1.00	0.93	2.77
	III	0.75	23.0	28.1	21.4	16.5	5.7	28.3	1.29	0.98	0.99	0.96	2.89
	IV	0.85	19.2	28.6	20.7	16.4	5.7	28.6	1.34	0.97	1.00	0.94	2.88
	V	0.95	11.2	28.6	21.4	15.6	5.4	29.0	1.36	1.00	0.99	1.02	2.89
	VI	2.6	4.5	28.8	22.7	15.2	5.5	27.8	1.30	1.06	1.03	1.09	2.76

* Compare Table I for abbreviations.

** The mean proportions of each constituent have been corrected for a 100% recovery. The actual total recovery ranged from 94 to 101%.

*** Additional fractions were obtained from 1-NP at 1.7 *M* NaCl (2.6% of DNA in starting material) and from 2-NP at *M* NaCl (2.0%) and 1.7 *M* NaCl (1.5%). The following proportions of the nucleic acid present in the starting material thus were secured as separated fractions: 1-NP, 98.3%; 2-NP, 91.2%; 1-HN, 87.9%.

Distribution of 5-methylcytosine. The uneven distribution of 5-methylcytosine in the various deoxyribonucleic acid fractions, to which attention has been drawn at previous occasions[1,22], is shown in Table III. In addition, Table II includes data on the molar ratio of cytosine to 5-methylcytosine.

TABLE III

DISTRIBUTION OF 5-METHYLCYTOSINE IN FRACTIONS OF WHEAT GERM DEOXYRIBONUCLEIC ACID*

Preparation	5-Methylcytosine as mole % of cytosine + 5-methylcytosine in fractions I	II	III	IV	V	VI	VII	VIII	IX
1-NP	28.6	28.4	26.7	23.2	24.3	23.5	24.1	23.0	
2-NP	27.6	26.9	26.5	25.8	24.0	23.4	23.3	22.7	20.9
1-HN	30.7	26.5	25.7	25.8	25.7	26.6			

* Compare Table II for description of fractions.

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Pentose nucleic acid

Composition. The nucleotide composition of the total pentose nucleic acid of wheat germ was determined by the procedure fully described in recent papers from this laboratory[23-25]. The results, derived from several independent preparations and hydrolysis experiments, are assembled in Table IV.

TABLE IV

WHEAT GERM PENTOSE NUCLEIC ACID: NUCLEOTIDE COMPOSITION AND MOLAR RELATIONSHIPS*

Moles per 100 moles nucleotide		*Molar ratios*	
Adenylic acid	22.4	A/U	1.08
Guanylic acid	29.2	G/C	1.06
Cytidylic acid	27.6	Pu/Py	1.07
Uridylic acid	20.8	6-Am/6-K	1.00
		(A + U)/(G + C)	0.76

* Abbreviations used: A, adenylic acid; G, guanylic acid; C, cytidylic acid; U, uridylic acid; Pu, purine nucleotides; Py, pyrimidine nucleotides; 6-Am, compounds carrying an amino group in 6 position (A, C); 6-K, compounds carrying a keto group in 6 position (G, U).

Search for 5-methylcytidylic acid. No indication of the presence of a ribonucleotide containing 5-methylcytosine was obtained. When, after hydrolysis with NaOH, the mixture of the sodium salts of the nucleotides was subjected to chromatography in *iso*butyric acid-ammonium *iso*butyrate (pH 3.6)[26], only the three usual spots giving dark shadows in U.V. light were seen, namely, in descending order, guanylic plus uridylic acids, cytidylic acid, adenylic acid. A bright blue fluorescing spot, moving about 40% faster than adenylic acid showed no characteristic absorption. In analogy to the behavior of the deoxyribonucleotides in this solvent[27], 5-methylcytidylic acid should have moved faster than adenylic acid. In the ammonium sulfate-*iso*propanol solvent[28] the fluorescent spot was the slowest moving one, above 3'-adenylic acid, but no new component was seen.

When the potassium salts of the nucleotides, prepared by hydrolysis with KOH, neutralization with perchloric acid and centrifugation, were subjected to hydrolysis with N H_2SO_4 for one hour at 100°, a mixture of purines and pyrimidine nucleotides resulted. Three solvent systems[29] were used in one-dimensional or, in various combinations, in two-dimensional chromatography, namely, *iso*butyric acid-NH_3, *n*-butanol-NH_3 and *iso*propanol-HCl. Here, too, only the zones corresponding to adenine, guanine, and cytidylic and uridylic acids could be discerned. The fluorescent zone had disappeared. The separated components were examined for their absorbance ratios at several characteristic wave lengths[30,31]. Good agreement with the published values was found in all cases except that of uridylic acid. But it is hardly likely that 5-methylcytidylic acid could have accompanied uridylic acid in both the *iso*butyric acid and *iso*propanol solvents.

DISCUSSION

In this paper the fractionation of a deoxyribonucleic acid preparation of plant origin is described for the first time. The most troublesome impurity in studies of this sort

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is the presence of ribonucleic acid which is especially hard to avoid in deoxyribonucleic acids from plant cells[6]. While several studies have, in the past, dealt with preparations from wheat germ[7-11,17,32], attention to the presence of this contaminant appears to have been paid only in a few instances[7,8,11,32] and deliberate attempts to eliminate it are even rarer. In the preparations under study here this difficulty has been overcome.

The investigation of the deoxyribonucleoproteins of wheat germ is far from completed. We hope to be able to return to it on a later occasion, especially as regards the protein, or more probably the proteins, with which the nucleic acids are associated. In its dissociability by salt and in other properties the wheat germ nucleoprotein resembles calf thymus nucleohistone; it is not improbable that it, too, contains basic proteins. It differs, however, from the latter in not being extractable from the cell with distilled water.

Table I, in which our results on the composition of wheat germ deoxyribonucleic acid are compared with previous data from the literature, is of some interest: it shows the agreement to be, on the whole, quite good, despite wide divergences in the isolation methods and the yield, and even the state of preservation, of the nucleic acid preparations. The cytosine value reported in one paper[9] is unusually high, that for 5-methylcytosine rather low in another instance[10]. But the ratio of the molar sum of adenine and thymine to that of guanine, cytosine and 5-methylcytosine (the "dissymmetry ratio") is around 1.2 in all cases; and, even more significantly, the figure reported for 5-methylcytosine only ranges from 5.7 to 6.2 mole % in those analyses that were based on paper chromatography.

As regards the nature of the sugar constituent of the wheat germ nucleic acid, attention may be drawn to the study included here which makes it likely that the deoxy sugar, as in all other deoxypentose nucleic acids investigated thus far, was 2-deoxyribose.

The fractionation experiments summarized in Table II fully bear out previous findings in this laboratory on the fractionation of several deoxyribonucleic acids by the fractional dissociation of nucleohistones or protein nucleates[1,3,4]. What may be worth mentioning is the observation that the fractionation appears more efficient when carried out with the wheat germ nucleoprotein itself (NP) than with an artificially prepared histone nucleate (HN). It may be that in the first case one is dealing with a much larger number of preformed conjugated proteins, differing in ease of dissociation and in other properties, than occur in a histone nucleate complex. Whereas only Fraction 1-HN-I was significantly different from the average composition (Table I) and from Fractions 1-HN-II to VI, the trend of fractionation can hardly be missed in the experiments with Preparations 1-NP and 2-NP. All fractions show the unity relationships, but contain consecutively increasing quantities of adenine and thymine and decreasing quantities of guanine, cytosine and 5-methylcytosine.

The separation of deoxyribonucleic acids into a series of fractions of different base composition has been reported from three laboratories. We have employed the stepwise extraction of nucleic acid-polybase complexes with salt solutions of increasing strength in the presence of a chloroform phase[1-4]. BROWN AND WATSON[33] have eluted the nucleic acid from a histone-kieselguhr column with salt solutions of increasing concentration. LUCY AND BUTLER[34] have essentially followed our technique,

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except that time rather than NaCl concentration was the variable, extraction being carried out repeatedly with salt solutions of constant concentration (0.6 *M*). In each arrangement similar results were achieved: the nucleic acid was fractionally brought into solution or eluted; and these fractions differed in composition, the earlier cuts containing relatively more guanine and cytosine. As either the ionic strength or the duration of treatment at a constant ionic strength was increased, the fractions became progressively richer in adenine and thymine.

As to the possible factors operative in the fractional extraction process reference may be made to several previous discussions[1,3,4,6,33]. Though LUCY AND BUTLER[34] were led to conclude that the sodium chloride concentration in the extracting solution is not the variable that is essential for the fractionation, we do not believe that a revision of our point of view, as set forth previously, is called for.

A detailed consideration of the principles underlying the three fractionation procedures would not be profitable at the present time. All these procedures can, however, be similarly interpreted in the light of three assumptions. (a) The bonds between the nucleic acid and the polybase are largely electrostatic and hence dissociable by neutral salt. (b) These bonds are not all of the same strength. (c) The bonds formed by nucleic acid fractions rich in guanylic and cytidylic acids are weaker than those formed by fractions rich in adenylic and thymidylic acids.

It should be noted that the dissociation of an electrostatic bond by neutral salt is not an abrupt process: there is a range of ionic strength in which the electrostatic attraction between the nucleic acid and the polybase is diminished but still operative. The degree of dissociation of any particular bond will depend both on the intrinsic strength of the bond and on the ionic strength of the medium. As the ionic strength is raised, the bonds will dissociate; the polar groups involved will interact more freely with the solvent and the complex will become more soluble. Those portions of the complex that have the weakest bonds will be the most highly dissociated and the most soluble; at any single ionic strength high enough to initiate dissociation, they will be disentangled most easily and rapidly.

The fractionation procedures under comparison all are, it would seem, not dissimilar to the process of ion exchange separation. It is possible to separate different ionic species by elution with salt solutions of increasing concentration; sometimes separation may also be effected by prolonged elution with a salt solution of a single, properly chosen ionic strength: *i.e.*, either a concentration gradient[1-4,33] or a time gradient[34] may be applied. In both instances, however, the determining factors would appear to be the strength of the electrostatic bonding forces and the ionic strength of the eluting medium. Other factors undoubtedly are also of importance[6], *e.g.*, the molecular configuration of the partners and the existence of secondary valence links; but the general processes both of ion exchange separation and of the fractionation of nucleic acids are best understood in the context of electrostatic interaction.

A few words should, finally, be said about the distribution of the nitrogenous constituent present in the lowest, but still in quite considerable, concentration in wheat germ deoxyribonucleic acid, *viz.*, 5-methylcytosine. This pyrimidine was first shown by WYATT to occur in minute amounts in several deoxyribonucleic acids of animal origin and in larger quantities in the deoxyribonucleic acid of wheat germ[7,35]. As has been noted above, the 5-methylcytosine content of wheat germ deoxyribonucleic acid has been found remarkably uniform in several laboratories (Table I).

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It can, moreover, be seen that, in the light of the unity relationships characteristic of deoxypentose nucleic acids[5,6], 5-methylcytosine partly replaces cytosine: the molar sum of cytosine and 5-methylcytosine equals the molar concentration of guanine, both in the total preparation (Table I) and in the separated fractions (Table II). This must, however, not be taken to mean that the replacement occurs in a random fashion.

We had already shown in a study of the composition of fractions of calf thymus deoxyribonucleic acid that the distribution of 5-methylcytosine, which may be said to occur in this nucleic acid as a minor satellite (1.3 mole %), did not parallel that of cytosine: the ratio of cytosine to 5-methylcytosine was far from constant in the several fractions[1]. It appeared of interest to repeat this study with a nucleic acid in which 5-methylcytosine occurs in larger quantity. As can be seen in Table II and, especially, Table III, the results demonstrate again that the distribution of 5-methylcytosine relative to that of cytosine is not uniform, the percentage contribution of 5-methylcytosine to its sum with cytosine being highest in the earlier fractions. The data are not sufficiently numerous for a statistical test of significance; but the existence of a definite and unbroken trend in the disproportionation (see Tables II and III) makes us confident that the observation is valid.

We believe that in the synthesis of new deoxyribonucleic acid molecules by the cell a much more stringent selection of constituents must take place than would be suggested by the copying mechanism proposed by WATSON AND CRICK[36] which cannot be reconciled with some of the experimental observations. This selection mechanism may be presumed to operate in such a manner as to distinguish not only between purines and pyrimidines, between 6-amino and 6-keto compounds, between ribosides and deoxyribosides, but also between elsewhere modified constituents, *e.g.*, pyrimidines carrying or lacking a methyl or hydroxymethyl group in position 5; and it must be able to insert them into, or exclude them from, specific positions within the polynucleotide chain. We have touched upon some of these points before[3,6] and discussed them in more detail in a recent survey[37].

It is in this connection that the brief study of the composition of wheat germ pentose nucleic acid, which is included here, must be considered. The nucleotide distribution summarized in Table IV is a good example of the regularity stressed in recent publications, namely the occurrence in equal number of bases with 6-amino groups (adenine, cytosine) and bases with 6-keto groups (guanine, uracil)[25,38]. Whether it is more than accidental that there exists an inverse relationship between the dissymmetry ratios of the deoxyribonucleic acid (A + T/G + C + MC = 1.25) and of the pentose nucleic acid (A + U/G + C = 0.76) of wheat germ, and also between the corresponding single members of these expressions, remains to be seen.

We have been unable to find indications of the presence of 5-methylcytosine in the pentose nucleic acid of wheat germ. This is, perhaps, of some interest, not only in view of the numerous speculations on a direct interconversion of deoxypentose and pentose nucleic acids, but also for another reason. Wheat germ must possess a synthetic apparatus capable of introducing a considerable amount (around 6 mole %) of an unusual nitrogenous constituent, 5-methylcytosine or its deoxynucleoside or -nucleotide, into presumably specific places of the deoxyribonucleic acid chains. It is, therefore, noteworthy that this pyrimidine, which is closely related structurally to the ubiquitous constituent cytosine, is not found in the pentose

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nucleic acid. The conclusion is obvious that the distinctive aspects of the duplication of the nucleic acids within the cell, transcending by far what can be deduced from the well known analytical and structural regularities, still have eluded definition.

SUMMARY

The isolation, purification and composition of preparations of the deoxyribonucleoprotein and the deoxyribonucleic acid of wheat germ are described. The sugar constituent has been identified as 2-deoxyribose. In confirmation of previous findings, the separation of the deoxyribonucleic acid into a series of fractions showing characteristic differences in composition has been achieved by the fractional extraction of the nucleoprotein or, less effectively, of histone nucleate complexes. The distribution of 5-methylcytosine relative to that of cytosine is not uniform in the nucleic acid fractions.

The nucleotide composition of the pentose nucleic acid of wheat germ has been studied; 5-methylcytosine does not appear to occur in the latter.

The paper also discusses the processes of the fractionation of nucleic acids and considers some of the conclusions arising from the findings on the uneven distribution of 5-methylcytosine.

RÉSUMÉ

L'isolement, la purification et la composition de préparations de la désoxyribonucléoprotéine et de l'acide désoxyribonucléique du germe de blé sont décrites. Le sucre s'y trouvant a été reconnu être la 2-désoxyribose. En corroboration de conclusions antérieures la séparation de l'acide désoxyribonucléique en une série de fractions exhibant des différences caractéristiques dans leur composition a été accomplie au moyen de l'extraction fractionnée de la nucléoprotéine ou — mais moins effectivement — de complexes du nucléate de la histone. La répartition de la 5-méthylcytosine par rapport à celle de la cytosine n'est pas uniforme dans les fractions de l'acide nucléique.

La composition des nucléotides dans l'acide pentose nucléique du germe de blé a été étudiée; il ne paraît pas que la 5-méthylcytosine y existe.

L'article discute aussi les procédés de fractionnement des acides nucléiques et examine quelques conclusions qui s'imposent à propos de la répartition inégale de la 5-méthylcytosine.

ZUSAMMENFASSUNG

Die Bereitung, Reinigung und Zusammensetzung von Präparaten des Desoxyribonukleoproteins und der Desoxyribonukleinsäure aus Weizenkeimlingen wurden beschrieben. Der Zuckerbestandteil wurde als 2-Desoxyribose identifiziert. In Übereinstimmung mit früheren Ergebnissen konnte mittels der Fraktionier-Extraktion des Nukleoproteins oder, obwohl weniger wirkungsvoll, von Histonnukleatkomplexen die Aufspaltung der Desoxyribonukleinsäure in eine Reihe von Fraktionen erzielt werden, die charakteristische Zusammensetzungsunterschiede aufwiesen. Die Verteilung des 5-Methylcytosins ist im Vergleich zu der des Cytosins in den Nukleinsäurefraktionen nicht einheitlich.

Die Nukleotidzusammensetzung der Pentosenukleinsäure aus Weizenkeimlingen wurde ebenfalls untersucht; diese Nukleinsäure scheint kein 5-Methylcytosin zu enthalten.

Die Grundlagen der Fraktionierungsmethoden für Nukleinsäuren werden in dieser Arbeit erwogen und die Folgerungen diskutiert, die sich aus der uneinheitlichen Verteilung des 5-Methylcytosins ergeben.

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