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The Isolation of Deoxyribonucleic Acids from Rye and Wheat

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As part of a wide investigation of the chemical and physical properties of a range of deoxyribonucleic acids extracted under mild conditions from various sources, our attention has been directed to the problem of the isolation of deoxyribonucleic acids from vegetable tissues and grain. The presence of extractable amounts of deoxyribonucleic acid in such material was shown by Kiesel and Belozersky [1] who isolated from pea-germ a nucleoprotein which gave a positive Feulgen [2] reaction, and which contained thymine. However, owing to the drastic methods found necessary to disintegrate the pea-germ, marked degradation undoubtedly occurred during the isolation of the nucleoprotein. In attempts to overcome this difficulty, Feulgen, Behrens and Mahdihassan [3] separated nuclei from embryos of rye by grinding the latter under benzene in a specially constructed mill, followed by centrifuging the suspension in a mixture (S. G. 1.39) of carbon tetrachloride and benzene. From the nuclei thus obtained, a deoxyribonucleotide was isolated by a procedure which involved a preliminary extraction with boiling water, further extraction with sodium carbonate solution, and digestion with pancreatin. This method of Feulgen *et al.*[3] is obviously of limited application, since, apart from the disadvantages of the alkaline extraction, and the probable presence of deoxyribonuclease in the pancreatin preparation the use of organic solvents is liable to damage the nuclei (Glick [4]). Accordingly, alternative methods of extraction have been investigated. Following the work of Mirsky and Ris [5] in which cell nuclei were successfully disrupted by vigorous agitation in a high speed mixer, and of Overend and Webb [6] who isolated a deoxyribonucleic acid from *Haemophilus pertussis* cells disintegrated in a Mickle shaker, it appeared that a combination of these methods would permit the isolation of highly polymerised deoxyribonucleic acids from various grains. In this connection it should be

noted that Mirsky and Pollister [7] state that a deoxyribonucleohistone may be isolated by extracting with 1 *M* sodium chloride solution, wheat germ which had previously been extracted with petroleum-ether. The experimental details described leave much to be desired.

The present communication records the results obtained by the application of such methods to rye and wheat. From the latter source it was possible to obtain comparatively pure preparations of deoxyribonucleic acid. The isolation from rye was complicated by the apparent solubility of the initial deoxyribonucleoprotein in 0.14 *M* sodium chloride solution. Consequently it was difficult to separate the deoxyribonucleic acid from ribonucleic acid.

EXPERIMENTAL

1. Isolation of deoxyribonucleic acid from rye

The dry grain was crushed in a mill and the resulting powder (1.5 kg) was suspended in 100 g portions in 1 *M* sodium chloride solution (300 ml/100 g powder) and subjected to vigorous agitation in a high speed mixer. After 10 minutes the suspension was filtered through cheese-cloth to remove the hulls. The residue was re-extracted with 1 *M* sodium chloride solution (total volume 1200 ml) as above, and the combined filtrates were centrifuged to remove starch. The insoluble starch was washed at the centrifuge with 1 *M* sodium chloride solution (500 ml) and discarded. Ethanol (3 vols.) was added to the combined supernatent liquid and washings. (This solution was opalescent.) The precipitate formed was collected at the centrifuge after 18 hours at 0°. It was suspended in 1 *M* saline (300 ml) and vigorously agitated in the mixer for 5 minutes. The resulting viscous suspension was diluted with 1 *M* sodium chloride (200 ml) and then centrifuged. The clear supernatent (S_1) (See Scheme) was decanted and the residue re-suspended in 1 *M* saline (100 ml), homogenised in the mixer and again centrifuged. The supernatent liquid (S_2) was decanted and the solid residue mixed with glass (Ballotini) beads and disintegrated in the Mickle shaker. After dilution with 1 *M* saline (100 ml), the suspension was centrifuged. The supernatent liquor (S_3) was decanted off and the solid (R) was discarded. Dilution of each of the above solutions (S_1, S_2, and S_3) with water (6 to 10 vols.) (*cf.* Mirsky and Pollister [7]) failed to precipitate the deoxyribonucleoprotein. Consequently much ribonucleic acid accompanied the deoxyribonucleic acid throughout the subsequent stages of the preparation (*cf.* Table 1). Addition of ethanol (1.5 vols.) to S_1 afforded a fibrous precipitate (D_1-collected by winding around a glass rod) and a precipitate (D_2) composed of very short fibres and some non-fibrous material. This precipitate (D_2) separated rapidly and was isolated at the centrifuge after decanting the main bulk of the supernatent liquid. The latter on standing overnight, deposited more precipitate (D_3). Samples of D_1 and D_2 gave an intense blue colour with the Dische[8] diphenylamine reagent, which exhibited the characteristic absorption band (Max. at 5800 Å) (*cf.* Deriaz, Stacey, Teece and Wiggins [9]) confirming the presence of deoxyribonucleic acid. Subsequent operations were as shown in the accompanying scheme and require little further comment. The qualitative separation of fractions D_2A, D_2B, D_2C, D_3A and D_3B was carried out according to the method described above for the separations

Table 1. Deoxyribonucleic acid and ribonucleic acid content of rye fractions, as determined by the method of Schmidt and Thannhauser [10].

Fraction	*Deoxyribonucleic acid*	*Ribonucleic acid*
	mg P/100 mg material	
Residue H	0.0105	—
Residue T	0.007	—
Residue R	0.003	—
Nucleoprotein D_1	1.3	2.31
Nucleic Acid D_1A	1.88	2.62
» » D_2A	1.58	2.12
» » D_2B	1.36	2.98
» » D_2C	0.57	2.49
» » D_3A	1.07	1.98
» » D_3B	0.14	2.31
Fraction S_2	0.402	2.06
Fraction S_3	0.128	2.35

The figures in the table multiplied by 10 give as an approximation the percentage of deoxyribo- and ribo-nucleic acids in the fractions analysed

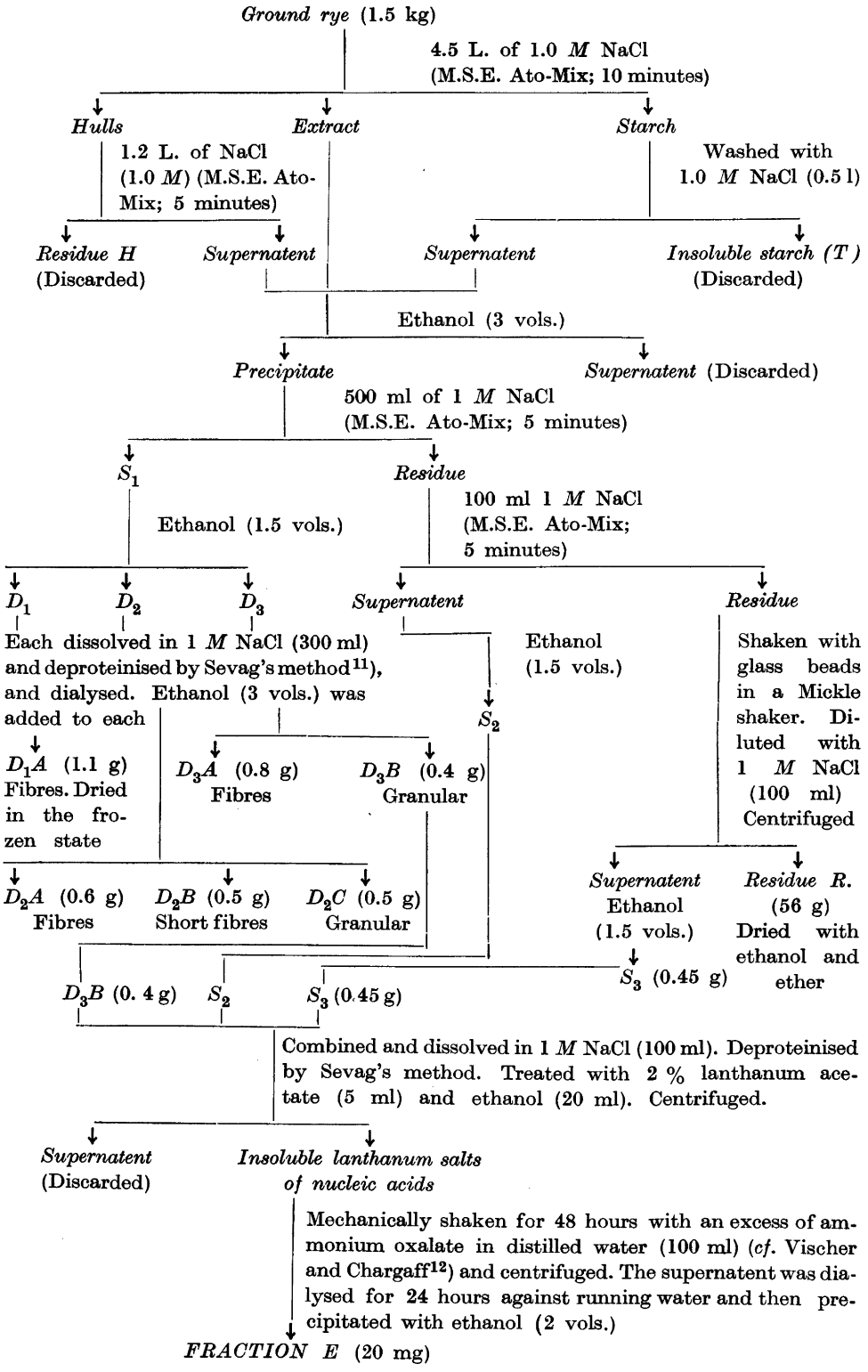
of fractions D_1, D_2, and D_3. The minimum volume of ethanol was used for precipitation at all stages, in order to minimise co-precipitation of polysaccharide material.

The analysis of the various fractions by the method of Schmidt and Thannhauser [10] gave the results recorded in Table 1. These indicate that the major part of the deoxyribonucleic acid of the rye was removed from the grain during the initial extraction and was concentrated in the fibrous precipitates. Attempts to concentrate the nucleic acids present in fractions S_2, S_3, and D_2B by first removing the protein and then precipitating the nucleic acids as lanthanum salts, gave insufficient material (Fraction E) to warrant further purification.

Purification of the crude deoxyribonucleic acid

Fractions D_1A, D_2A, D_2B and D_3A were combined and dissolved in distilled water (100 ml). Saturated calcium chloride solution (15 ml) was added and the precipitate of calcium phosphate removed at the centrifuge after 15 minutes. Addition of ethanol (2 vols.) to the clear supernatent solution resulted in the precipitation of fibres which were collected and dissolved in distilled water (80 ml) and 0.1 *M* veronal buffer (pH 7.8, 30 ml). To this solution 0.1 % (W/V) ribonuclease solution (previously heated to 100° at pH 4.0 to remove possible traces of deoxyribonuclease) and 1 : 1000 sodium ethylmercurithiosalicylate solution (14 ml) were added. The mixture was left at room temperature for 24 hours and the enzyme protein then removed by the Sevag [11] method. Five treatments with chloroform-octanol (9 : 1) served to remove the protein. The

Scheme of isolation.



aqueous solution was dialysed for 18 hours against running tap water, centrifuged and then added to ethanol (3 vols.). The resulting fibrous precipitate was removed rapidly from the small amount of granular material which accompanied it, washed with ethanol and dissolved in distilled water (10 ml) to give an extremely viscous solution. Absolute ethanol (14 ml) was added and the long fibres which immediately separated were removed on a glass rod. The fibres were dissolved in distilled water (30 ml) and re-precipitated with ethanol (75 ml). This procedure was repeated four times and the final product dried *in vacuo* in the frozen state. The product (0.5 g) dissolved extremely rapidly in water to give a viscous solution. It was free from protein (Sakaguchi and ninhydrin tests were negative) but contained some polysaccharide material as shown by the low analytical figures for nitrogen and phosphorus content, and the high reducing power value (as glucose) of the material after acid hydrolysis (see Table 2). The high content of polyribonucleotides remaining in the product (see Table 2) after its digestion with ribonuclease and subsequent dialysis, may be attributed to the fact that among the products from the action of ribonuclease on ribonucleic acid, is a relatively large non-diffusible polynucleotide fragment which is resistant to further action of the enzyme (Loring, Carpenter and Roll [13] *cf.* Overend and Webb [14]). It was not found possible to remove this polyribonucleotide from the deoxyribonucleic acid preparation, either by fractional precipitation with ethanol from aqueous solution, or by the precipitation of the nucleic acid as its insoluble lanthanum salt.

2. Isolation of deoxyribonucleic acid from wheat germ

From this material it was possible to isolate relatively pure preparations of deoxyribonucleic acid by the procedure outlined below. Except where stated to the contrary all operations were carried out in the cold room. In accordance with Mirsky and Pollister [7] it was found that a preliminary extraction of the wheat germ with petroleum-ether was essential in order to obtain workable amounts of the nucleoprotein. A suspension of the wheat germ (600 g) (supplied by Glaxo Laboratories Ltd) in petroleum-ether (b. p. 60—80°, 4 l) was kept at room temperature for 24 hours and then filtered. The dry solid was suspended in 0.14 *M* sodium chloride solution (5 l) and subjected to vigorous agitation in the high speed mixer. The suspension was kept for 24 hours and then separated by centrifuging. The supernatent liquid was decanted and the deposit homogenised with 1.0 *M* sodium chloride (2.5 l) in the mixer. After standing for 24 hours, this suspension was separated as before. The supernatent was diluted with seven volumes of water. The resulting precipitate which separated slowly was collected at the centrifuge after 1.5 hours, and transferred to 1.0 *M* saline (500 ml) when the main bulk of the material readily dissolved. The undissolved solid was removed (by centrifuging) and the clear solution obtained, was freed from protein by shaking seven times with chloroform-amyl alcohol (3.5 : 1) in the usual way. Addition of cold absolute ethanol (1.5 vols.) to the protein-free solution gave a fibrous precipitate which was collected at the centrifuge after 1.5 hours. A solution of the solid in distilled water (120 ml) was dialysed for 12 hours at 3° against distilled water (1600 ml) and then evaporated in the frozen state. The white powder (0.6 g) obtained, dissolved rapidly in water to give a viscous solution which was free from chloride ions and gave negative Sakaguchi and ninhydrin reactions. It contained 4.77 % of ribonucleic acid (see Table 2).

Table 2. Properties of deoxyribonucleic acid preparations from rye and wheat. Analyses performed on material dried in vacuo over phosphorus pentoxide at 80°.

Property	Source	
	Rye	Wheat
Ribonucleic acid content (method of Euler and Hahn [15])	16 %	4.77 %
Reducing value after acid hydrolysis	27 %	—
Phosphorus content (Allen's [16] method)	6 %	8.87 %
Nitrogen content (micro-Kjeldahl method)	10.3 %	13.66 %
N/P	1.70	1.55
Purine N/Pyrimidine N (method of Gulland, Jordan and Threlfall [17]	1.57	1.57
Ultraviolet absorption	λ max. 260 m/μ λ min. 230 m/μ	λ max. 260 m/μ λ min. 230 m/μ

SUMMARY

Deoxyribonucleic acids have been isolated from rye and wheat germ. Some characteristics of the products are reported.

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