A SEPARATION IN WEAK MAGNESIUM CHLORIDE SOLUTIONS OF NUCLEAR MATERIAL INTO TWO FRACTIONS WITH DIFFERENT PURINE AND PYRIMIDINE CONTENT

WITH A NOTE ON TWO UNKNOWN SUBSTANCES WITH A HIGH ABSORPTION IN THE ULTRA-VIOLET

by

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CHARGAFF *et al.*[1] and CRAMPTON *et al.*[2] have reported the separation of deoxyribonucleic acid (DNA) into fractions having different purine and pyrimidine content. The separation was performed on a preparation of deoxyribonucleoprotein (DNP) by treatment with amyl alcohol and chloroform followed by extracting with NaCl solutions of different concentrations. LUCY AND BUTLER[3] later obtained separation using only one NaCl concentration.

The investigation presented in the following article was made directly on nuclei isolated from calf thymus cells. The working hypothesis was that under suitable conditions the DNP fractions containing different types of DNA should show a difference in solubility.

Cell nuclei disintegrate and dissolve rapidly in distilled water or in NaCl solutions not stronger than 0.03 *M*. In order to be able to fractionate different material of the nuclei through a difference in solubility, the rate of solution must be slowed down considerably. A successful attempt, as reported below, was made by adding Mg ions in very low concentrations. Divalent ions were chosen because SCHNEIDER and co-workers[4,5] had found that Ca ions had a "hardening" effect on cell nuclei. From a paper by ANDERSSON[6] on the effect of various solutions on the nuclear envelope it was judged that Mg^{++} probably would have slightly greater effect than Ca^{++}. Magnesium was also chosen because it had previously been found to have a stabilising effect on DNP in NaCl solutions of higher concentrations[7].

METHOD OF PREPARATION

Immediately after the animal was killed, about 50 g of calf thymus, freed from blood vessels *etc.*, was frozen with carbon dioxide ice. It was then cut into pieces which were suspended in 250 ml ice cold 0.14 *M* NaCl + 0.01 *M* $MgCl_2$ by treatment in a homogenizer (type, Hollywood 51) for 150 seconds. The suspension was then centrifuged in a Spinco centrifuge model L, rotor 20 a 10,000 r.p.m. (max. $14{,}500 \times g$) for 30 minutes. The sediment was treated once more in the homogenizer, this time for

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50 seconds and in 200 ml of ice cold 0.14 *M* NaCl + 0.01 *M* $MgCl_2$. The subsequent centrifugation was performed in a smaller laboratory centrifuge (Wifug, type SP) at 4000 r.p.m. (max. 2200 × *g*) for 5 minutes. The sediment was then suspended in 100 ml of ice cold 0.14 *M* NaCl + 0.01 *M* $MgCl_2$, using a common glass rod as a stirrer. The suspension was again centrifuged in the laboratory centrifuge, this time at about 600–1000 × *g* (at the bottom of the tube) for 5 minutes. The two last steps were repeated once. Under the microscope the resulting sediment was found to consist almost exclusively of nuclear material, *i.e.* staining brown with aceto-orcein-fast-green. A few bluish green particles of cytoplasmic origin were, however, also observed. In the bottom of the centrifuge tubes some non-homogenized material was found. As this material packs together, it was easily removed when washing out the tubes for the two last described stirrings.

The washing of the nuclear material was now ended, and the extraction of the material was the next step. The sediment was stirred in 100 ml of dilute $MgCl_2$ for several hours and then centrifuged down again at 4000 r.p.m. for 5 minutes (max. 2200 × *g*). This procedure was repeated 15–20 times. In the first three extractions the concentration of $MgCl_2$ was 0.01 *M* $MgCl_2$; thereafter 0.0025 *M* solutions were used. The residue after all the extractions was found to give a very dark colour with aceto-orcein-fast-green and will hereafter be called "the blackstaining residue" or just "the residue".

To the different extractions NaCl was added to 0.9% and after at least five days the resulting precipitates were collected by centrifugation in the Spinco, model L, rotor 20, at 20,000 r.p.m. (max. 57,190 × *g*). The supernatants and the precipitates are identical with "the supernatants" and "the precipitates" referred to in the following.

In those cases, when, for the sake of comparison the preparation was performed without $MgCl_2$, NaCl solutions of the same ionic strengths were used, *i.e.* 0.17 *M*, 0.03 *M*, and 0.0075 *M* NaCl.

A COMPARISON BETWEEN THE TREATMENT OF CELL MATERIAL WITH AND WITHOUT ADDED MAGNESIUM IONS

The first steps, as pointed out above, consist of washing the nuclear material with physiological saline. If the homogenized cells are treated with 0.14 *M* NaCl + 0.01 *M* $MgCl_2$, the sediment after the last centrifugation in this stage will show some whole nuclei when investigated under the microscope (Fig. 1). If the preparation is made in 0.17 *M* NaCl (same ionic strength but no Mg ions), a careful investigation with the microscope will show no nuclei whatsoever. In the former ca se the number of nuclei increases considerably if instead of a homogenizer with rotating knives a Potter-Elvehjem piston homogenizer is used. The former type, however, can treat more material in one filling, and was considered sufficient for the present investigation.

The nuclear material which was washed with NaCl only, dissolves almost completely in five to eight portions of 0.03–0.0075 *M* NaCl, *i.e.* mostly as soon as the concentration of NaCl has been lowered enough to permit solution (about 0.03 *M* and lower). Three preparations were performed in this way without Mg^{++}. They were done as closely as possible in parallel with preparations with added Mg^{++}.

In the cases where Mg ions were added (seven preparations have up to now

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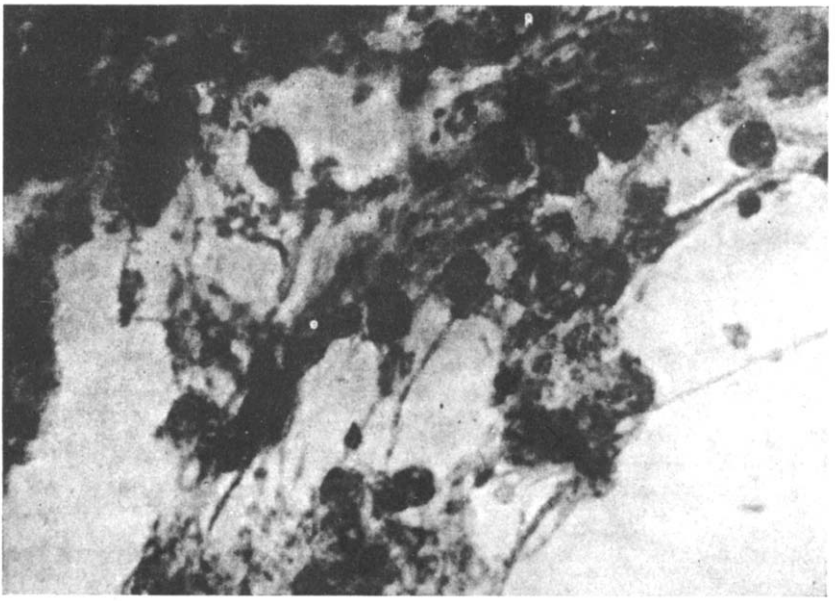


Fig. 1. A preparation from calf thymus, homogenized and washed 3 times with 0.14 *M* NaCl + 0.01 *M* $MgCl_2$. Stained with aceto-orcein-fast-green to a brown colour. × 1200.

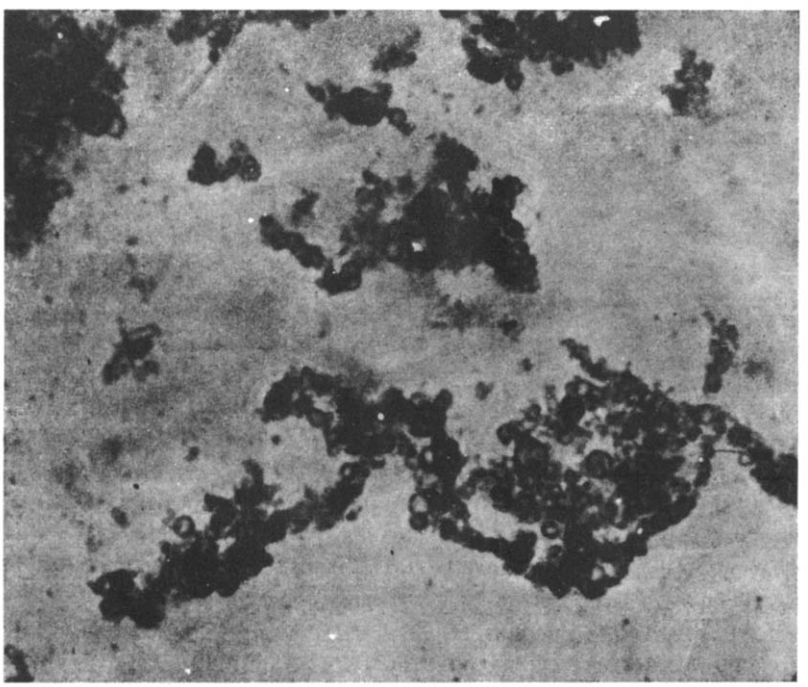
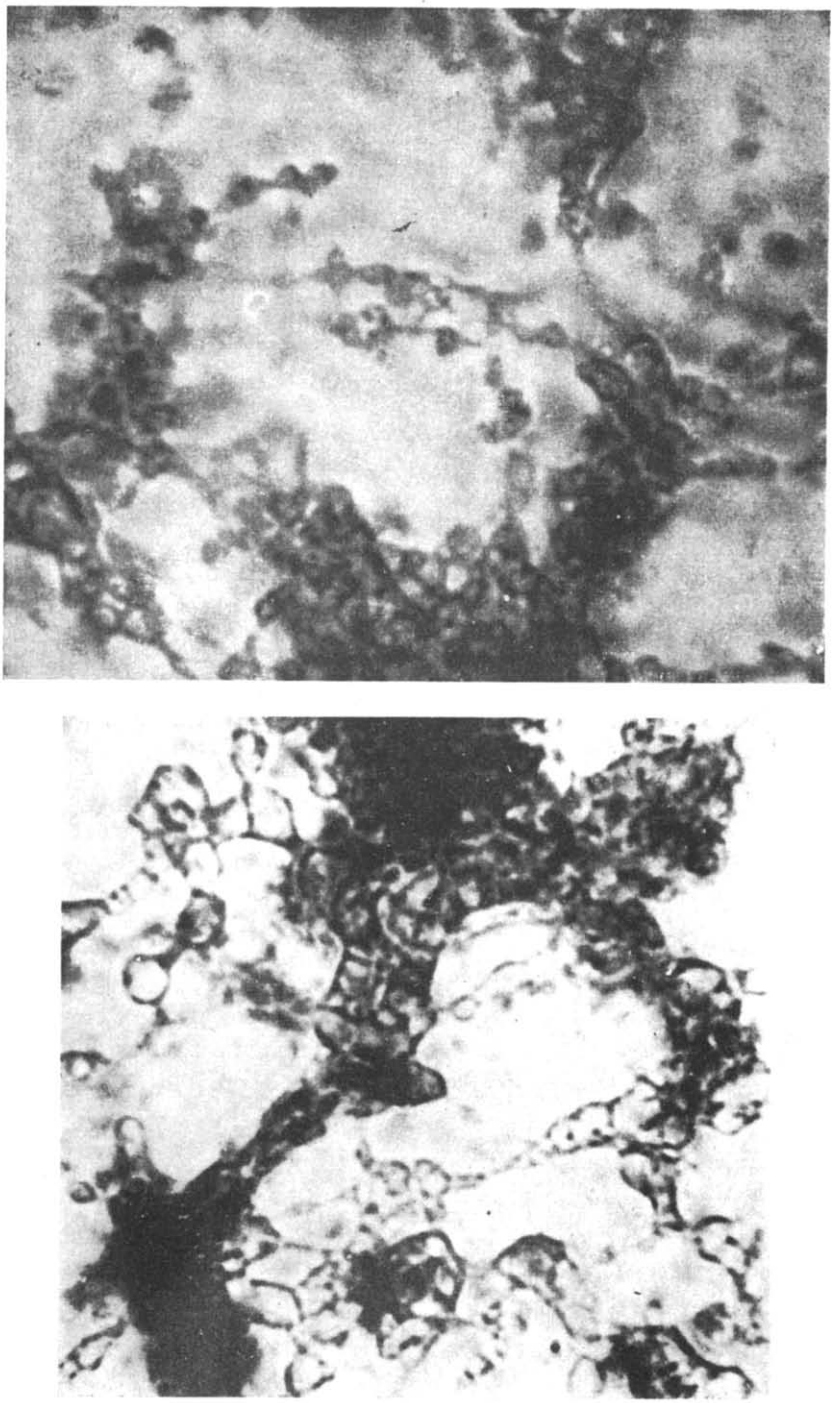


Fig. 2a, b, c. The material from calf thymus giving Fig. 1 after it has been extracted 3 times with 0.01 *M* $MgCl_2$, and then 5, 12, and 14 times respectively with 0.0025 *M* $MgCl_2$. Stained with aceto-orcein-fast-green to a brown or grey-brown colour. × 1200.



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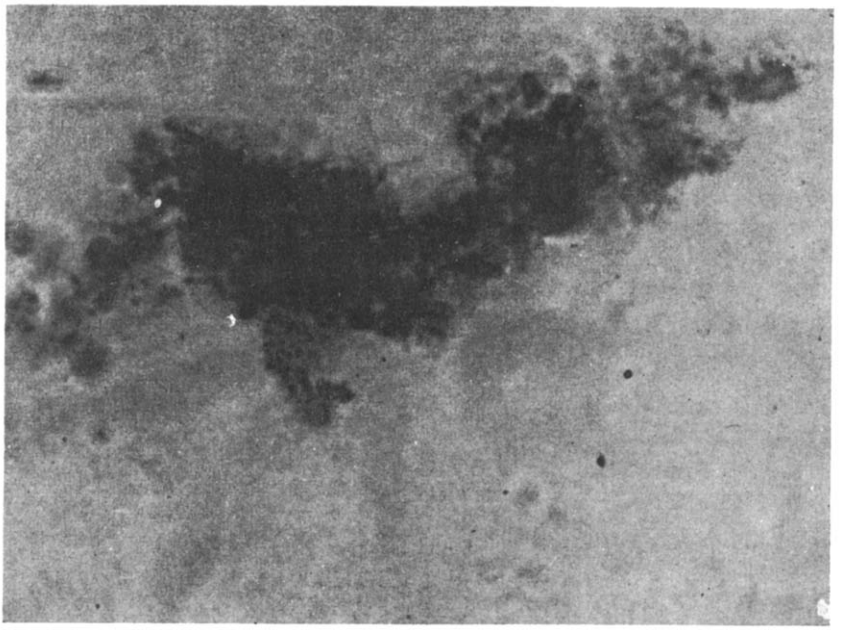

Fig. 3. The residue of the material from calf thymus giving Figs. 1 and 2 after 3 extractions with 0.01 *M* $MgCl_2$ and 17 times with 0.0025 *M* $MgCl_2$. Stained with aceto-orcein-fast-green to a black colour. × 1200.

been done in this way), the material went into solution more slowly. The type of the sediment obtained between extractions was also quite different. Without added Mg^{++}, the sediment was slimy and the supernatant was highly viscous and opalescent. With Mg ions, the sediment had nearly the character of a fine silt, and the supernatant was of low viscosity and nearly clear. This latter difference, however, is probably mainly due to the great difference in concentration of DNP.

The extractions were followed in the microscope; test samples were taken from the sediment and stained with aceto-orcein-fast-green. The different stages, as seen under the microscope may be broadly described as follows. At first, bundles of long brown-staining threads are seen, and the more or less intact nuclei disappear after the first extraction. The fibres, however, give place to small brown-staining bubbles, which become more and more unstable, stain less brown and instead assume a somewhat greyish colour as the extractions proceed (Fig. 2, a, b, c). Under the microscope the bubbles in the sediment after the last extractions disintegrate very easily, and the background is filled with a black staining material, in which a few fibres of brown staining material are seen. No bluish green staining material whatsoever can be detected at this stage. After about fifteen extractions the residue stained black (Fig. 3).

The dissolved material from the different extractions was precipitated by addition of NaCl to 0.9% as described under "preparation". In one case, the precipitates were collected and dissolved in 1 *M* NaCl. If distilled water was added to precipitate the DNP from this new solution, a maximal change in the precipitation curve[8] was obtained at 0.26 *M* NaCl. The curve is, in all essential details, identical with the one

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which was obtained from DNP prepared in the absence of $MgCl_2$, and also quite the same as the one already published[8].

A difference in the DNP prepared with and without Mg ions can be found in the magnitude of the U.V. absorption, concentration being calculated on the nitrogen content. For DNP prepared with NaCl only, the value of the extinction in a 1.0 cm cuvette per mg nitrogen per ml (E/N) was shown to fall between 67–70 at pH > 12. With Mg added during the preparation, the value is about 40. Tables I and II show this in greater detail.

TABLE I

Extinctions (cuvette 1.0 cm) for the precipitates dissolved in 10 ml 0.1 *M* NaOH (preparations number 108, 110, 112). Starting material 50 g calf thymus.

Extraction No.	*Extinction without $MgCl_2$*	*Extinction with $MgCl_2$*		*E/N without $MgCl_2$*	*E/N with $MgCl_2$*	
1	9.1	2.3	24.0	59	32	24
2	884.4	3.6	6.8	68	49	41
3	24.8	2.9	2.8	69	42	42
4	57.6	3.4	3.5	71	35	45
5	175.0	3.9	5.1	73	36	42
6	26.0	3.9	3.5	72	33	42
7	31.6	9.2	3.6	76	33	42
8	8.6	8.8	8.2	(50–70)	37	(60)
	almost no residue	further 3 extr.	further 6 extr.	—	average 34 of 3	average 47 of 6
Sum of extinction	1217.1	71.4	totally 73.1 + black-staining residue			

TABLE II

Extinctions for the supernatants (100 ml, near neutrality) (preparation number 108 and 110). Starting material 50 g of calf thymus.

Extraction No.	*Extinction × 10**		*E/N*	
	without $MgCl_2$	*with $MgCl_2$*	*without $MgCl_2$*	*with $MgCl_2$*
1	43.4	6.1	35	10
2	129.8	3.6	45	9
3	9.0	3.4	33	11
4	11.9	5.1	30	17
5	18.5	4.6	36	15
6	7.9	3.6	30	27
7	3.7	11.8	—	22
8	1.2	12.4	—	27
9	—	17.2	—	50
10	—	17.2	—	55
11	—	15.8	—	50
Sum of extinction	225.4	100.4		
Sum × 1.33*	299.8	133.5		

* In order to facilitate a comparison with Table I the extinction values are multiplied with 10 (difference in volume) and the sum of the values with 1.33 (difference in pH).

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When $MgCl_2$ is used, there is some increase in the amount of absorbing material found in the last extraction. The increase is especially marked for the supernatants.

For convenience the time of extraction was not always the same. Two three-hour extractions during the day were followed by one overnight for 15 hours. In one case (prep. No. 113), each extraction was continued for about 24 hours. After 22 extractions, still only 10% of the absorbing material had gone into solution. Almost no brown staining material was seen after 19 extractions.

The value of E/N for the black-staining residue of one preparation (No. 107) was measured in 1 *M* NaOH. The value found was 35–39, with some uncertainty, however, arising from an unavoidable opalescence probably consisting of denatured protein. The collected precipitates from the same preparation (No. 107) gave also an E/N quotient 35–39, some uncertainty arising this time from unspecific absorption, when the maximal extinction is determined.

The dry weight of the black-staining material collected from 100 g of thymus was 4.0–3.5 g. There are, however, great losses during the preparation. KAY *et al.*[9], for instance, calculated the amount of DNA to be 2.6–3.0 in 100 g thymus. In Tables I and II, about half the absorbing material should then have been accounted for in the case when only NaCl was used. In the cases when $MgCl_2$ was added most of the absorbing material is to be found in the black-staining residue. This would mean, however, about the same amount accounted for or slightly more. The losses may seem to be heavy, but it must be remembered that much material has to be lost in the first washings if one wants to get rid of as much cytoplasmic material as possible.

The amount of absorption in the black-staining residue at 260 mμ compared with that of the material which goes in solution is between 8 to 1 and 9 to 1.

CONTENT OF PURINES AND PYRIMIDINES

The relative content of purines (guanine, adenine) and of pyrimidines (cytosine, 5-methyl-cytosine, thymine) was determined chromatographically on paper with 65% *iso*propyl alcohol and 2 *N* HCl following the procedure of WYATT[10]. The chromatograms were performed with ascending liquid phase, and the time was approximately 36 hours for each run. The paper used was Munktell OB. In this way a very good separation of the purines and pyrimidines mentioned was obtained. The position of the different spots on the paper was determined using a television set with an U.V. sensitive camera (RCA, TV eye). The spots were cut out and extracted for 24 hours with 5 ml 0.1 *N* HCl. The subsequent measurements of the U.V. absorption of the extracts were made in a Beckman spectrophotometer making a reading every five mμ between 340 and 400. A piece of the same paper near to each spot was cut out and the extract used as a blank. In the calculations the values of the molecular extinctions given by WYATT were used.

The starting hydrolysis was performed in 85% formic acid for 45 minutes at 175° in a sealed glass tube. After evaporation to dryness on a water bath a few drops of 1 *N* HCl were added, and after some hours the chromatograms were started.

Analyses were performed for one preparation (No. 112, total number of extractions 14) on the precipitates from extractions Nos. 1, 3, 5, 7, 9, 11, 13, and on the black-staining residue and for another preparation (No. 107, total number of extractions 17) on the precipitates from extractions No. 9 and 17 and on the residue. It

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may be pointed out that the last extractions in both cases were made on a material, which under the microscope did not differ from the black-staining residue. Analyses were also performed on DNA isolated from both the collected precipitates of two preparations (No. 107 and 113) and from the residue of one preparation (No. 113).

The preparation of the analytical data will be given in three parts. First the values from the earlier extractions (3, 5, 7, from No. 112 and 7 from No. 107) will be given and after that the data for the residues after a special treatment with HCl. The analyses of the later extractions and of the residues not treated with HCl presented a more complicated picture and will therefore be discussed separately.

a. Contents of purines and pyrimidines in the precipitates from the earlier extractions

The analytical data of the precipitates from the earlier extractions are given in Table III. As very little material was available, the measurements on the separate extractions were performed directly on hydrolysed DNP, without trying to isolate the DNA first. If the chromatograms are sprayed with ninhydrin, it will be seen that the amino acids move faster than the purines, but they apparently add somewhat to the U.V. absorption of the pyrimidines. The unspecific absorption of thymine in these runs was also fairly high. As the values for guanine and adenine are more reliable, their molecular ratios were calculated separately to the sum of two, *i.e.* for two P in the DNA. The less accurate and certainly too high values for the pyrimidines have also been calculated to the sum of two. As the quotient of purines to pyrimidines has been calculated without division into two groups of the original ratios, using values directly calculated to the sum of four, it gives the magnitude of the "correction" introduced by the former way of calculation. The ratios used to determine the expression (A + T)/(G + C) are the same directly calculated ratios. Here, however, the difference between using the differently calculated values of purines and pyrimidines is very small as there is one purine and one pyrimidine value both in the denominator and numerator.

Two DNA samples were prepared from a mixture of the first eight fractions of preparations No. 107 and of preparation No. 113. In the first case the method of KAY *et al.*[9] with detergent, was used and in the second the procedure of GULLAND *et al.*[11], with amyl alcohol and chloroform, was followed. In both cases the yield seemed to be very low. The chromatograms were of a good quality for both preparations, but unfortunately very small amounts had to be used, and there was only enough material for one single run for each preparation.

The values obtained for the composition of DNA were: guanine 0.80, adenine 1.20, cytosine and 5-methyl-cytosine 0.82, and thymine 1.18 (calculated to the sum of four). The second decimal is uncertain. The quotient (A + T)/(G + C) is 1.41 or the same as the mean value in Table III.

The content of ribonucleic acid (RNA) in the precipitates was measured after digestion in 1 *N* NaOH according to SCHMIDT AND THANNHAUSER[12] and according to SCHNEIDER[13]. Ten to fifteen per cent RNA was found counted on total nucleic acid content of extractions No. 3–12 (No. 107–113), The value of A + U/G + C has been found by VOLKIN AND CARTER[14] to be 0.44 for the RNA. This means that the values of (A + T)/(G + C) given in Table III are somewhat too low owing to the amount of coprecipitated RNA.

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TABLE III

PURINE AND PYRIMIDINE CONTENT OF THE PRECIPITATES FROM EARLIER EXTRACTIONS

Preparation *Extraction No.*	*No 112* 3	5	7	*No. 107* 9	*average*	
Guanine	0.87	0.85	0.82	0.87	0.80	0.84	Calculated
Adenine	1.13	1.14	1.18	1.13	1.14	1.15	on 2P
Cytosine + 5-methylcytosine	0.80	0.82	0.89	0.85	0.83	0.84	Calculated
Thymine (+ uracil)	1.20	1.18	1.11	1.15	1.24	1.18	on 2P
Purines/pyrimidines	0.86	0.79	0.79	0.78	0.92	0.83	
$\frac{A+T}{G+C}$	1.32	1.45	1.32	1.47	1.47	1.41	

b. Content of purines and pyrimidines of the residues treated with HCl

To get fully reproducible values for the residues it was found necessary to dissolve the dry hydrolysed material in concentrated HCl. After three hours the acid was diluted and the solution evaporated. The analytical results of the residues treated in this way and of the precipitate from one of the latest extractions are given in Table IV. The quality of the chromatograms was higher than those previously mentioned and the molar ratios have been calculated directly to the sum of four.

From the black-staining residues of one preparation (No. 113) DNA was prepared by the method of GULLAND *et al.* and treated as described above. The three chromatograms given by this substance were very good. The values obtained were: guanine 1.05, adenine 0.97, cytosine and 5-methyl-cytosine 1.04, thymine 0.94 (variation in the three runs $\pm$ 0.02). The quotient (A+T)/(G+C) is 0.91. The RNA content of the residue determined as above, was about 6% counted on total nucleic acid in the residue.

TABLE IV

PURINE AND PYRIMIDINE CONTENT OF THE RESIDUES AFTER TREATMENT WITH CONC. HCl

The ratios are calculated to the sum of 4.

	No 107 residue	*No. 112 residue*			*No. 112 washing of residue*			*Average*
Guanine	0.99	1.01	0.97	0.95	0.96	0.92	0.95	0.97
Adenine	1.01	1.04	0.99	1.01	1.05	1.05	1.08	1.03
Cytosine + 5-methylcytosine	0.98	0.96	1.01	1.00	0.99	0.97	0.95	0.98
Thymine (+ uracil)	1.02	1.00	1.03	1.04	1.01	1.07	1.02	1.02
Purines/pyrimidines	1.00	1.05	0.96	0.96	1.00	0.97	1.03*	1.00
$\frac{A+T}{G+C}$	1.03	1.04	1.02	1.05	1.06	1.13	1.11	1.07 1.03*

* Counting only the value for the residues.

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c. Analysis of precipitates from later extractions and of blackstaining residue without treatment with HCl

The precipitates from extraction Nos. 9, 11, and 17 (No. 112) and the residues were found to give very high absorption values for the adenine and cytosine spots. The substance in the adenine spot gave an absorption curve identical with that found for adenine, as for example given by HOTCHKISS[15], whereas the extract from the cytosine spot gave a high value at the wavelength of 300 mμ compared with that for cytosine or 5-methyl-cytosine.

The values for the four ratios calculated as before—the same molar extinction and to the sum of four—are given in Table IV. For adenine and cytosine this means that the calculation has been made as if the respective spots only contained pure adenine or cytosine.

Let us assume that we are dealing with two new substances (the "adenine type" will be called substance Y and the "cytosine type" substance X). Besides those two substances, we have a DNA with the usual four bases and in which the amount of purines and pyrimidines are in agreement with the Watson and Crick model. If this is the case, the quotient of the absorption "adenine" minus thymine and "cytosine" minus guanine (A — T)/(C — G) multiplied by an unknown constant due to the possible difference in molecular extinction, would give the proportion between the two unknown substances. As seen from Table V the value (A — T)/(C — G) was found to be of the same magnitude in all cases investigated. The values for the residues are the same, whereas the precipitates from preparation No. 112 gave a somewhat higher value. For the latter it must be remembered, however, that the precipitates have been dissolved earlier for other purposes in 0.1 *M* NaOH. In any case the figures seemed to indicate that a closer investigation of the possibility of two unidentified substances would be worth while.

TABLE V

APPARENT PURINE AND PYRIMIDINE CONTENT OF RESIDUES AND PRECIPITATES FROM LATER EXTRACTIONS WITHOUT PREVIOUS TREATMENT WITH CONC. HCl

The ratios are calculated to the sum of 4.

	No. 112 extr. 9	*No. 112 extr. 11*	*No. 107 extr. 17 i.e. washing of residue*	*No. 112 residue*	*No. 107 residue*
Guanine	0.6	0.5	0.3	0.8	0.3
Adenine	1.8	1.8	1.9	1.2	1.9
Cytosine + 5-methylcytosine	1.2	1.2	1.3	1.1	1.5
Thymine	0.5	0.5	0.5	0.8	0.3
$\frac{A-T}{C-G}$	1.6	1.8	1.4	1.3	1.3

SUBSTANCE X

It was found relatively simple to isolate a substance from the hydrolysates of both the precipitates and the residue which have the R_F value of cytosine, but a different U.V. absorption curve.

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Method

A few mg of the hydrolysed substance were dried on the water bath, and the beaker was held in ammonia vapour for a few seconds to make sure that the pH would be above 7 when water was added. Two ml of distilled water were added to the dried substance and this was decanted after a minute. This step had sometimes to be repeated with shorter period of time. To the insoluble residue 4 ml of 0.1 *N* sulphuric acid were added. When all the substance was dissolved 1 ml 0.15 *M* silver nitrate was added, and the purines plus most of the thymine and apparently the Y-substance were precipitated. The precipitate was allowed to stand over night in the cold and then centrifuged down at 2500 r.p.m. The supernatant was neutralised to pH 5.4 with

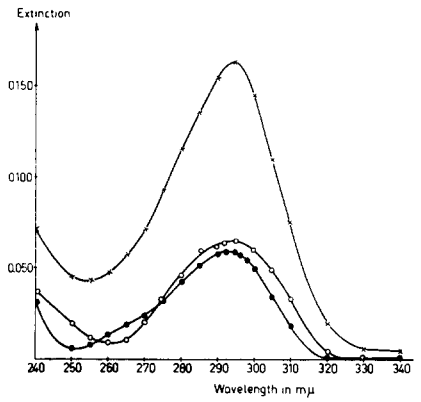

Fig. 4. The ultra-violet absorption of the X substance. Crosses: Original absorption curve in 0.1 *N* HCl. Filled circles: The absorption curve of the same solution at pH 13–14 after 4 hours at that pH. Unfilled circles: The absorption curve of the same solution brought back to low pH (0.5) after 48 hours at the high and 12 hours at the low pH.

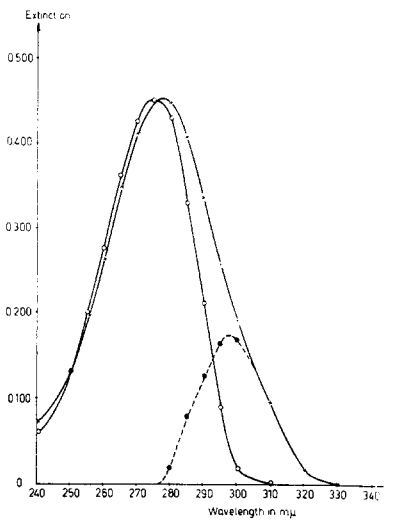

Fig 5. The ultra-violet absorption curve of cytosine according to HOTCHKISS[15] compared with the absorption curve of the extraction from one of the cytosine spots obtained by chromatography of a hydrolysate of nuclear material (preparation No. 112, the residue). Unfilled circles: The absorption curve of pure cytosine according to HOTCHKISS. Crosses: The absorption curve of the "cytosine" spot. Filled circles: Correction curve between the two other curves.

0.1 *N* NaOH. The new supernatant was evaporated to dryness and 2 ml distilled water were allowed to stand in contact with the dry substance for one minute. The water was decanted and acidified by addition of the same volume of 0.2 *N* sulphuric acid.

The solution now gave an absorption in U.V. with maximum at 294–297 as shown by the curve with the highest peak in Fig. 4. Further attempts at purification along the same lines showed no effect.

The curve in Fig. 4 agrees very well with the possible correction curve necessary to change the U.V. absorption curve of the cytosine spot to that of pure cytosine at the same pH (Fig. 5).

From the value at 310 mμ the absorption at 275 mμ of the X substance can be calculated. Cytosine gives no absorption at 310 mμ. The value at this wavelength for the curve given by the cytosine spot can thus be used to calculate a correction to be substracted from the observed maximal value. If this is done the lower value will in all cases give an amount of cytosine which will fit closely to the Watson and Crick model.

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The following is further known of the X-substance.

As could be expected the isolated substance had the same R_F value as cytosine —0.45 under the experimental conditions described above.

The U.V. absorption decreases in alkaline solution. This decrease is partly irreversible (see Fig. 4).

The substance is changed by concentrated HCl, as can be gathered from the experiments to get reproducible values described above.

From the method of preparation it is clear that it is not precipitated with AgCl.

SUBSTANCE Y

The Y substance must apparently have the following characteristics.

It has about the same R_F value as adenine. It is precipitated by Ag. It has an U.V. absorption curve in acid solution which is close to that of adenine.

While this work was in progress LEVY AND SNELLBAKER[16] published a paper wherein they described a substance which they called "fluorescent component". This substance could be isolated from a DNA preparation if the acid was not hydrolysed too strongly. The experiment of LEVY AND SNELLBAKER was partly repeated by the present author, and at present it seems very likely that the Y substance is the same as the "fluorescent component", U.V. data and R_F values being the same.

LIPID AND PROTEIN

The black-staining residue contains lipid. The residue was extracted in Soxhlet-apparatus with ethyl alcohol. The extracted substance was washed with ether. The ether soluble part was found to be slightly yellow with a melting point of 40–50° C. It gave a strong colour reaction with the Liebermann-Burchardt or the Salkowski test. It contained less than 0.3% N and 0.2% P. The lipid must therefore be for the most part of cholesterin type. When the extraction of the nuclear material was performed as described above, the lipid found was 25–30% of the residues. This was measured in three preparations Nos. 107, 110 and 112.

DALY AND MIRSKY[17] (see also LUCY AND BUTLER[17a]) have found a large difference in the lysine and arginine content of two types of nucleohistone. One nucleohistone was rich in lysine and dissociated more readily from the DNA than the other. The lysine to arginine ratio for this fraction was 11.2 and for the other type 1.02.

The amino acid analysis of my preparations was therefore concentrated on the two mentioned amino acids. Run on a column of Dowex-50[18] the precipitate from one of the extractions (No. 112, extraction 8) was found to have nearly the same amount of lysine and arginine. The molecular ratio between lysine and arginine was 1.04. The black-staining residue contained more lysine than arginine. The molecular ratio was 1.42.

COMMENTS

The stabilising effect of weak Mg^{++} solutions compared with NaCl solutions of the same ionic strength is quite striking. This effect can hardly be attributed to the influence of the Mg ions on the nuclear envelope, as crushed nuclei which probably

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form the bulk of the material show the same slow solubility. The effect of Mg ions is therefore probably due to direct combination of Mg^{++} with DNP or to an inactivation of a degrading enzyme system. To the naked eye the difference in appearance of the sediment and supernatant from the two methods of extracting is very noticeable.

As already pointed out the nuclear material in weak Mg^{++} solutions does not show the tendency to form slimy gels, which otherwise is typical for nuclear material. Without pressing the point too hard it may in this connection be remembered that LUCK and co-workers[19] have claimed that DNP in its most native form should not give strongly viscous solutions. The viscous form of DNP should be due to early enzymic activity. The black-staining residue partly dissolved in 1 *M* NaCl (buffered with phosphate buffer pH 6.3) gave a low-viscous solution. The precipitate formed when this solution was diluted with six times distilled water was non-fibrous.

The two fractions of the nuclear material obtained differ in purine and pyrimidine content. The expression (A + T)/(G + C) was found to be 1.4 for the most soluble part, for the residue 1.03, and for the DNA prepared from this fraction 0.91. This may be compared with the values for DNA from thymus calculated from the values given by LUCY AND BUTLER[3] 1.20, 1.22, by WYATT[10] 1.27, by FRICK[7] 1.28, and by CHARGAFF AND LIPSHITZ[20] 1.37. The extreme values for the fractions obtained by CHARGAFF *et al.*[1] are 0.99–1.79, by CRAMPTON[2] *et al.* 1.07–1.61 (from their Table I) or 0.83–1.46 (Table II), and by LUCY AND BUTLER 0.76–1.31. In contrast to my own fractionation, LUCY AND BUTLER with their method get the lowest (A + T)/(G + C) value for the fraction which first goes into solution. CHARGAFF and co-workers get the lowest value of (A + T)/(G + C) at the lowest salt concentration used. The differences between their procedure and the one used here are, however, great.

The aim of the work on the unknown U.V. absorbing substances described in this article has only been to give some figures which may throw light upon their possible interference with measurements of purine and pyrimidine composition. Apparently the comparatively low dissociation of the 85% formic acid has saved them from being destroyed. In future work it will be of interest to see if we here have to deal—as the data hitherto obtained suggest—with substances of purine or of pyrimidine type and also to investigate their relation with the DNA molecule*.

SUMMARY

1. Weak solutions of magnesium ions have a strong stabilising effect on nuclear material.

2. The part of the nuclear material which goes into solution after repeated extractions with 0.0025 *M* $MgCl_2$ contains a different purine pyrimidine content than the residue. The quotient (adenine + thymine)/(guanine + cytosine + 5-methyl-cytosine) was 1.4 compared with 1.03 for the residue. (The values are somewhat too low owing to coprecipitation of RNA.)

3. From the residue a deoxyribonucleic acid was prepared giving the value 0.91 for the last-mentioned ratio.

4. The existence of two substances hitherto not observed in this connection was demonstrated. One of them has an R_F value close to that of adenine and the other close to that of cytosine (chromatograms run according to WYATT[10]).

5. The substance interfering with the cytosine has been isolated. It has an ultra-violet absorption maximum at 294–297 mμ in 0.1 *N* HCl. The absorption is destroyed if the pH is increased to 13–14.

6. The substance interfering with adenine is probably the same as that already described by LEVY AND SNELLBAKER[16].

* After this paper was sent in for publication, a possible explanation why these substances appear has been given by D. B. DUNN (*This journal*, 18 (1955) 317).

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RÉSUMÉ

1. Des solutions diluées d'ions magnésium exercent une puissante action stabilisante sur le matériel nucléaire.

2. La partie du matériel nucléaire qui passe en solution après extractions répétées avec $MgCl_2$ 0.0025 M n'a pas la même teneur en purines et pyrimidines que le résidu. Le rapport (adénine + thymine)/(guanine + cytosine + 5-méthyl-cytosine) est de 1.4, tandis qu'il est de 1.03 pour le résidu. (Ces valeurs sont quelque pue trop faibles du fait de la coprécipitation du RNA.)

3. A partir du résidu, un acide désoxyribonucléique, qui donne une valeur de 0.91 pour le rapport mentionné ci-dessus, a été préparé.

4. L'existence de deux substances jusqu'ici non observées a été démontrée à ce propos. L'une des deux a un R_F voisin de celui de l'adénine, et l'autre, un R_F voisin de celui de la cytosine (Chromatogrammes préparés selon WYATT[10].)

5. La substance qui interfère avec la cytosine a été isolée. Son absorption maximum dans l'ultra-violet se situe à 294–297 mμ, dans HCl 0.1 N. Cette absorption est supprimée si le pH atteint 13–14.

6. La substance qui interfère avec l'adénine est probablement la même que celle déjà décrite par LEVY ET SNELLBAKER[16].

ZUSAMMENFASSUNG

1. Schwache Magnesiumionlösungen üben einen starken stabilisierenden Einfluss auf Zellkernmaterial aus.

2. Derjenige Teil des Zellkernmaterials, welcher sich nach wiederholtem Extrahieren mit 0.0025 M $MgCl_2$ auflöst, enthält einen verschiedenen Purin Pyrimidingehalt als der Rückstand. Der Quotient (Adenin + Thymin)/(Guanin + Cytosin + 5-Methylcytosin) war 1.4, verglichen mit dem für den Ruckstand erhaltenen Wert von 1.03. (Die Werte sind wegen der Kopräzipitation von RNS etwas zu niedrig.)

3. Es wurde aus dem Rückstand eine Desoxyribonukleinsäure mit obengenanntem Quotientwert 0.91 hergestellt.

4. Es wurde die Existenz von zwei, in diesem Zusammenhange bis jetzt noch nicht beobachteten Substanzen bewiesen. Eine derselben weist einen Adenin ähnlichen R_F-Wert auf, während die andere einen Cytosin naheliegenden R_F-Wert hat. (Chromatogramme wurden laut WYATT[10] durchgeführt.)

5. Die mit Cytosin interferierende Substanz ist isoliert worden: ihr Absorptions-maximum in Ultraviolettwellenlänge ist 294–297 mμ in 0.1 N HCl. Die Absorption wird durch Erhöhung des pH-Wertes auf 13–14 zerstört.

6. Die mit Adenin interferierende Substanz ist wahrscheinlich dieselbe wie diejenige, die bereits von LEVY UND SNELLBAKER[16] beschrieben wurde.

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