A Cytochemical Study of Nucleic Acids in Plant Cells V. Nucleic acids of Feulgen negative plants

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It has been reported by several authors that the cells of certain plants are not stained with Feulgen staining method (Shinke and Shigenaga 1933; Hillary 1939; Ueda 1956; Shinke, Ishida and Ueda 1957). It was assumed by Hillary (1939) and Milovidov (1949) that such phenomenon is caused by such several factors as a low concentration of chromatin in cell nuclei and presence of some inhibiting substances. From results of a biochemical study on several lower plants which are not stained with Feulgen's method, Shinke, Ishida and Ueda (1957) have arrived at a conclusion that Feulgen color development is inhibited to a certain extent by presence of certain proteins contained in these plants, though the knowledge about the nucleic acids of Feulgen negative plants is still very meagre.

It is the purpose of the present study to obtain knowledge about the properties and contents of nucleic acids in cells of some Feulgen negative plants by some biochemical procedures.

Materials and methods

Oscillatoria limosa (Cyanophyta) and *Spirogyra* sp. (Conjugatae) were collected from a pond near Kyoto. Materials were washed with showering tap water and distilled water repeatedly to avoid contamination by other microorganisms. Thalli of *Marchantia polymorpha* (Hepaticae) were also used in this study. The nuclei of these materials did not show positive Feulgen staining as shown in Table 1.

Procedures of histochemical test by Feulgen staining, extraction and purification of deoxyribonucleic acid (DNA), and quantitative estimation of nucleic acids and other phosphorus containing substances will be described in appropriate places in the following pages.

Results

1) Histochemical test with Feulgen staining

Cells of plants stated above were fixed with Carnoy's fluid, corrosive sublimate-acetic acid or 95% ethanol followed by washing with distilled water. After washing, materials were hydrolysed as a whole with N-HCl at 60°C for 1, 3, 6, 10, 15, 20, 25 and 30 minutes. After hydrolysis, they

were stained with Schiff's reagent¹ at 15°C for 1 hour, and then washed with SO₂-water repeatedly three times. Part of the materials fixed with Carnoy's fluid or corrosive sublimate-acetic acid, was tested with paraffin sections. Fresh materials were also treated in the same procedure as in the fixed materials. After treatment with Schiff's reagent, the materials were mounted with SO₂-water and observed under a microscope. The results obtained are shown in Table 1.

Table 1. Feulgen staining of the cells of Feulgen negative plants

Materials	Hydrolysis time (min.)	Fresh Material	Fixatives				
			Corrosive sublimate- acetic acid		Carnoy's fluid		95% ethanol
		A	A	B	A	B	A
<i>Oscillatoria limosa*</i>	0	—	—	—	—	—	—
	1	—	—	—	—	—	—
	3	—	—	—	—	—	—
	6	—	—	—	—	—	—
	10	—	—	—	—	—	—
	15	—	—	—	—	—	—
	20	—	—	—	—	—	—
	25	—	—	—	—	—	—
	30	—	—	—	—	—	—
<i>Spirogyra</i> sp.	0	—	—	—	—	—	—
	1	—	—	—	—	—	—
	3	—	—	—	—	—	—
	6	—	—	—	—	—	—
	10	—	—	—	—	—	—
	15	—	—	—	—	—	—
	20	—	—	—	—	—	—
	25	—	—	—	—	—	—
	30	—	—	—	—	—	—
<i>Marchantia polymorpha</i>	0	—	—	—	—	—	—
	1	—	—	—	—	—	—
	3	—	—	—	—	—	—
	6	—	—	—	—	—	—
	10	—	—	—	—	—	—
	15	—	—	—	—	—	—
	20	—	—	—	—	—	—
<i>Tradescantia</i> leaf epidermis**	0	—	—	—	—	—	—
	3	+	+	+	+	+	+
	6	++	++	++	++	++	++
	10	++	++	++	++	++	++
	20	++	++	++	++	++	++
	30	+	+	+	+	+	+

A, wholly; B, in microtome section. —, negative; +, positive reaction. *Central bodies of this alga were stained very faintly in reddish purple occasionally. **Used as a control

It is seen in this table that the nuclei of *Oscillatoria*, *Spirogyra* and *Marchantia* were not stained with Schiff's reagent in all procedures shown in Table 1, while the nuclei of *Tradescantia* showed positive Feulgen stain-

¹ Prepared with *para*-Fuchsin (Glübler) by a procedure of Stowell (1954).

ing in all cases.

2) Ultraviolet (UV)-absorption and some color reactions of DNA from these plant cells *in vitro*

DNAs in cells of *Oscillatoria*, *Spirogyra* and *Marchantia* could not be detected histochemically by the procedure of Feulgen staining, as stated above, that is, presence or absence of DNAs in these plant cells were not confirmed by histochemical procedure. It is necessary, therefore, to determine whether these plant cells contain DNA or not by biochemical method.

Isolation. Isolation of DNA from these plant cells was carried out by modified Schmidt and Thannhauser's method (Schmidt and Thannhauser 1945; Sugiyama, Shinke and Ishida 1954). Firstly, acid soluble substances were removed from the cell homogenate by treatment with cold trichloroacetic acid (TCA), and lipid by heating with ethanol-ether mixture, and then with methanolchloroform mixture.

After these pre-treatments, DNA, RNA and phosphoproteins were extracted from the tissue residues by treatment with N-KOH at 37°C for 15 hours. DNA was then precipitated from the extract by addition of TCA and HCl.

The 'DNA fraction' obtained from each plant material contains a small amount of RNA and fairly large amount of proteins. To remove these substances, following procedures were employed. That is, to remove RNA, distilled water was added to the 'DNA fraction' and pH of the solution was adjusted at 7.8 by an addition of dilute KOH solution. Then, this solution was incubated for 5 hours at 43°C with the mixture of crystalline ribonuclease¹ and MgCl₂ (cf. Lison 1953). After incubation DNA was precipitated by addi-

Fig. 1. UV-absorption curves of DNAs isolated from cells of Feulgen negative plants. O, from *O. limosa*; S, from *Spirogyra* sp.; M, from *M. polymorpha*; T, from calf thymus, used as a control.

¹ Prepared by Worthington Biochemical Laboratory, U.S.A.

tion of suitable amounts of N-HCl and 5% TCA solution. Precipitate (PPT) obtained was redissolved with dilute KOH solution and precipitated again by addition of both acids. This procedure was repeated twice. Then the PPT was washed repeatedly with a small amount of 70% methanol and then with absolute ethanol. This PPT was dried at room temperature. RNA was removed by these procedures.

The dried PPT obtained by procedure stated above, was dissolved in alkaline M.NaCl solution, and deproteinized by shaking with chloroform-amyralcohol (35 : 10). This procedure was repeated until proteins are completely removed. Then aqueous DNA solution obtained was condensed under reduced pressure. Then DNA was precipitated by an addition of dilute HCl and 10% TCA. PPT was, then, washed repeatedly with small amount of 70% methanol, absolute ethanol and finally with ether, followed by drying at room temperature. Biuret test on the DNA samples thus obtained from these Feulgen negative plants, showed negative.

UV-absorption spectra.

DNAs isolated from the cells of *Oscillatoria*, *Spirogyra* and *Marchantia*, and calf thymus were dissolved in 0.1 N-KOH solution. UV-absorption curves of these DNAs were determined by a Beckman's spectrophotometer, and shown in Fig. 1.

It is seen in this figure that the maxima of the absorption curves of these four samples of DNA, locate at $260m\mu$ in all cases, while absorption minima situate at $236m\mu$ in *Oscillatoria*, at $234 m\mu$ in *Spirogyra* and *Marchantia*, and at $232 m\mu$ in calf thymus.

Ogur and Rosen (1950) reported a procedure of extraction of nucleic acids from plant materials. According to them, DNA was extracted from plant cells by heating the materials with 0.5 N-perchloric acid (PCA) at 70°C

Fig. 2. UV-absorption curves of DNAs isolated from cells of Feulgen negative plants with hot 0.5 N-perchloric acid. O, from *O. limosa*; S, from *Spirogyra* sp.; M, from *M. polymorpha*; T, from calf thymus, used as a control.

for 20 minutes after RNA extraction. Ogur and Rosen's method stated above is fundamentally different from Schmidt and Thannhauser's method in their procedure of extraction. It is necessary, therefore, to compare DNA obtained by these two methods. Absorption curves of DNAs isolated from *Oscillatoria*, *Spirogyra* and *Marchantia* cells by Ogur and Rosen's method, are shown in Fig. 2.

In this figure, it is seen that the absorption maxima locate at $268\text{ m}\mu$ in all cases. In the present study, similar shifting is also observed when DNAs from calf thymus and fish sperm are treated with hot PCA. According to Ogur and Rosen, shifting of absorption maximum from $260\text{ m}\mu$ to $268\text{ m}\mu$, is due to the hot PCA treatment (Ogur and Rosen 1950).

Dische's diphenylamine reaction. DNAs isolated by a modified Schmidt and Thannhauser's method from *Oscillatoria*, *Spirogyra* and *Marchantia*, and from calf thymus were dissolved in distilled water. Two volumes of Dische's reagent were added to one volume of the DNA solution. The mixture was heated at 90°C for 15 minutes in a water bath (Ishida 1954, 1956) and blue color was seen develop. After heating, the mixture was cooled in ice water. Absorption curves were shown in Fig. 3.

It is seen in this figure that the wavelength of absorption maxima of the colors developed with the DNAs from the three Feulgen negative plants and calf thymus, situate at $600\text{ m}\mu$ in all cases. Typical absorption curves indicating the presence of deoxypentose sugar, have been obtained from DNAs from these Feulgen negative plants and calf thymus.

Feulgen's reaction in vitro. Detail of the procedure of a modified Feulgen's reaction *in vitro* used in this study was reported in the previous paper (Ishida 1959). Outline of the procedure was; one volume of 0.2 N-HCl was added to each one volume of the solutions of DNAs from *Oscil-*

Fig. 3. Absorption curves of Dische's reaction color with DNAs from Feulgen negative plants. O, from *O. limosa*; S, from *Spirogyra* sp.; M, from *M. polymorpha*; T, from calf thymus.

latoria, *Spirogyra*, *Marchantia* and calf thymus. Each mixture was hydrolysed for 15 minutes at 90°C in a water bath. After hydrolysis, the mixtures were cooled in ice water, and pH of the solutions was adjusted at 2.28 by addition of suitable amount of 0.1 N-glycine—0.1 M-NaCl solution. Then the solutions were kept in a water bath at 10°C. One volume of

Schiff's reagent was added to ten volumes of each hydrolysed solution. Purple color developed gradually. Absorption curves of the Feulgen color with DNAs from these Feulgen negative plants and calf thymus were shown in Fig. 4.

Fig. 4. Absorption curves of Feulgen's reaction color with DNAs from Feulgen negative plants. O, from *O. limosa*; S, from *Spirogyra* sp.; M, from *M. polymorpha*; T, from calf thymus, used as a control.

containing substances from *Oscillatoria*, *Spirogyra* and *Marchantia* cells for quantitative analysis, a modified Schmidt and Thannhauser's method was employed. By this method, acid soluble substances, lipids, DNA, RNA and phosphoproteins were isolated.

Amount of phosphorus in each fraction was determined by phosphovanado-molybdate method (PVM-method, Sugiyama, Shinke and Ishida 1954).

Both DNA and RNA fractions, obtained by the modified Schmidt and

In this figure the absorption maxima of the Feulgen color, developed with DNAs from these Feulgen negative plants and calf thymus *in vitro*, is seen to be 565 mμ at pH 2.28. The results show that while cell nuclei of *Oscillatoria*, *Spirogyra* and *Marchantia* do not show positive Feulgen test in histochemical preparations, DNAs isolated and purified by biochemical procedures from these plants, show positive Feulgen's reaction *in vitro*.

3) Contents of nucleic acids and other phosphorus containing substances

For isolation of nucleic acids and other phosphorus

Thannhauser's method, contained a fairly large amount of proteins as contaminant as stated above, and the development of reaction colors with DNA and RNA by several estimation methods was affected by the presence of proteins. (Ishida 1956, 1959). Presence of proteins also affected the intensity of UV-absorption when nucleic acids were estimated spectrophotometrically. The proteins were, therefore, isolated from both nucleic acid fractions by Ogur and Rosen (1950) procedure in the present study.

DNAs were quantitatively determined by UV-absorption method, Feulgen's and Dische's methods (Ishida 1954, 1956, 1959), after DNAs were isolated with 0.5 N-PCA from each 'DNA fraction' obtained by the modified Schmidt and Thannhauser's method, while RNAs were estimated by the procedures of UV-absorption and orcinol reaction (Mejbaum 1939), after extraction of RNAs from each 'RNA fraction' with N-PCA. Results of quantitative determination of nucleic acids and other phosphorus containing substances in the Feulgen negative plants were shown in Tables 2-5.

Amounts of phosphorus per g of dry weight of materials are shown in

Table 2. Amounts of phosphorus containing substance-P in each fraction from Feulgen negative plants by Schmidt and Thannhauser's method

Fractions	γ per g (dry weight)		
	<i>Oscillatoria limosa</i>	<i>Spirogyra</i> sp.	<i>Marchantia polymorpha</i>
Acid sol.	4124.8(21.4) [%]	4461.4 (65.5) [%]	1445.1 (48.6) [%]
Lipids	2202.2(11.4)	819.6 (12.0)	409.8 (13.8)
D N A	2033.8(10.5)	111.0 (1.7)	93.1 (3.1)
R N A	8630.8(44.8)	1249.9 (18.3)	856.9 (28.8)
P-proteins	1931.0(10.0)	60.8 (0.9)	143.1 (4.0)
'Residue'	357.7(1.9)	109.5 (11.6)	26.5 (0.9)
Total sum of each fraction	19280.5(100.0)	6812.2(100.0)	2974.5(100.0)

'Residue', as mentioned in Tables 2 and 3, is finally obtained, after isolation of the five fractions from tissue homogenates, by Schmidt and Thannhauser's method. It is assumed that the presence of phosphorus in 'Residue' is due to the presence of some contaminants from the five fractions and unknown substances.

Table 2. In brackets of this table, ratios of phosphorus content in each fraction to total sum of phosphorus are expressed in percentage. In this table, it is seen that acid soluble substance-P and RNA-P show high values in all cases of the three plants used; especially the cells of *Spirogyra* and *Marchantia* contain markedly larger amount of acid soluble substance-P than those of *Oscillatoria*. RNA-P content of *Oscillatoria* cells also shows a high value.

The ratio of DNA-P to total phosphorus in the case of *Oscillatoria* is about 10%, while in *Spirogyra* and *Marchantia*, the ratios are in low

percentage. The ratio of phosphoprotein-P is relatively high value in *Oscillatoria*, but the value is negligibly small in *Spirogyra*. The ratios of phospholipids in these three plants are of the same level.

Table 3 shows the contents of nucleic acids and other phosphorus containing substances-P per cell. As seen in this table, RNA-P contents of both cells of *Oscillatoria* and *Spirogyra* are approximately equal. The values of DNA-P and RNA-P per cell in *Marchantia* are much less than the values of above two algae.

Table 3. Amounts of phosphorus containing substance-P in the cell of Feulgen negative plants

Fractions	$\times 10^{-9}$ mg per cell		
	<i>Oscillatoria limosa</i>	<i>Spirogyra sp.</i>	<i>Marchantia polymorpha</i>
Acid sol.	1.62	11.46	0.744
Lipids	0.86	2.11	0.211
DNA	0.80	0.29	0.048
RNA	3.38	3.21	0.441
P-proteins	0.76	0.16	0.074
'Residue'	0.14	0.28	0.014
Total sum of each fraction	7.56	17.51	1.532

As seen also in this table, the value of total sum of phosphorus in *Spirogyra* cell is the highest among these three plants. Such value is due to the high content of phosphorus in 'acid soluble fraction' from this alga. One of the characteristics of *Spirogyra* cells is the presence of relatively large amount of phospholipids.

In Tables 2 and 3, the P-contents of each fraction obtained by the PVM-method are shown. However, in the quantitative determination of nucleic acids, each of the three chemical constituents of nucleic acids must be determined, because the nucleic acid composition is different in the source. The determination of nucleic acids was, therefore, carried out by several estimation methods for the constituents of nucleic acids such as phosphorus, sugars and bases. In Table 4, the results are expressed as the amounts of nucleic acids per unit weight of fresh specimens. Relative values, assuming that values of DNAs and RNAs estimated by PVM-method are 100.0, are shown in brackets.

It is seen in this table that the relative values of nucleic acids estimated by UV-absorption method show the highest values, except *Oscillatoria* RNA, among those by all estimation methods. Generally speaking, the values obtained by Dische's and Feulgen's methods for DNA, are less than those by other estimation methods, as also shown in Table 5, which represents the nucleic acid contents per cell.

RNA values of *Spirogyra* by UV-absorption method are higher about 28% than the value by PVM-method. The values of *Oscillatoria* RNA by UV-absorption and orcinol methods are less about 16% and 28% respectively than the value of the RNA by PVM-method.

Table 4. Nucleic acid contents of some Feulgen negative plants determined by various estimation methods

Estimation methods	γ per g (fresh weight)					
	DNA			RAN		
	<i>O. limosa</i>	<i>Spirogyra</i> sp.	<i>M. polymorpha</i>	<i>O. limosa</i>	<i>Spirogyra</i>	<i>M. polymorpha</i>
PVM	3.656* (100.0)	0.074* (100.0)	0.096* (100.0)	16.14* (100.0)	0.86* (100.0)	0.92* (100.0)
UV	3.843 (104.9)	0.081 (109.4)	0.106 (110.4)	13.51 (83.7)	1.10 (127.9)	0.98 (106.5)
Dische	3.591 (98.1)	0.071 (95.9)	0.091 (94.8)	—	—	—
Feulgen	3.512 (95.9)	0.068 (91.6)	0.088 (91.7)	—	—	—
Orcinol	—	—	—	11.68 (72.1)	0.93 (108.1)	0.87 (94.6)

*Amounts of nucleic acids were calculated from Schneider's factor; DNA-P is converted DNA and RNA-P to RNA by dividing by 0.095 and 0.099 respectively (Schneider 1945). In the estimations of DNAs by UV-absorption, Dische's and Feulgen's methods, calf thymus DNA was used as a standard sample, and also yeast RNA was used as a standard sample for the estimation of RNAs from these plant materials.

Table 5. Amounts of nucleic acids of the cell of some Feulgen negative plants determined by various estimation methods

Estimation methods	$\times 10^{-9}$ mg per cell					
	DNA			RNA		
	<i>O. limosa</i>	<i>Spirogyra</i> sp.	<i>M. polymorpha</i>	<i>O. limosa</i>	<i>Spirogyra</i> sp.	<i>M. polymorpha</i>
PVM	8.1*	2.9*	0.49*	35.5*	33.6*	4.6*
UV	8.5	3.2	0.54	29.8	41.8	4.9
Dische	7.9	2.8	0.46	—	—	—
Feulgen	7.7	2.7	0.44	—	—	—
Orcinol	—	—	—	25.6	36.3	4.4
Mean values	8.1	2.9	0.48	30.3	37.2	4.6

* Amounts of nucleic acids were calculated from Schneider's factor.

Discussion and conclusion

Several investigators have reported on the results of the Feulgen staining of cells of *Oscillatoria*, one species of which was used as materials in

this study, but some of the results obtained by them did not always agree one another. In their histochemical study, Hillary (1939) and Yamaha and Suematsu (1938) showed that central bodies in the cells of *Oscillatoria sp.* were Feulgen positive, while Kiesel and Derukewa (1937) on *Oscillatoria spp.*, and Ueda (1956) and Shinke, Ishida and Ueda (1957) on *Oscillatoria princeps* and *O. limosa* and other algae belonging to this genus had reported that the Feulgen staining was negative in these algae.

The negativity with Feulgen staining of cells of *Spirogyra spp.* was reported by Shinke and Shigenaga (1933), Peter (1933), Hurel-Py (1935), Milovidov (1936), and Shinke, Ishida and Ueda (1957), but Geitler (1935) distinctly observed the Feulgen positive small bodies in the nuclei of this plant.

In the present histochemical observation, it is confirmed that the cells of *Oscillatoria limosa*, *Spirogyra sp.* and *Marchantia polymorpha* do not show positive staining with Feulgen's method, even if fixed with different fixatives and hydrolysed in various periods of hydrolysis time with N-HCl (see Table 1). To put it in other words, DNA has not been detected histochemically in these plants. Then comes the question whether DNA present in the cells of these Feulgen negative plants or not. It is necessary, therefore, to study biochemically these plants to clear this question.

In the present study, extraction of DNAs from these Feulgen negative plants has been, therefore, carried out by a biochemical procedure. DNAs thus obtained show a positive reaction with Feulgen's method and also with Dische's method *in vitro*. The shape and maximum wavelength of absorption curves of the reaction colors with these DNAs with Feulgen's method, are the same with those of calf thymus DNA. Similar results are also obtained on the DNAs from these Feulgen negative plants with Dische's method. Biswas (1956) has shown also that DNA isolated from cells of *Nostoc muscorum*, a Feulgen negative blue-green alga, give a positive test with Dische's reagent *in vitro*.

It is concluded from the results of the present investigation that DNA is contained in the cells of Feulgen negative plants, such as *Oscillatoria limosa*, *Spirogyra sp.* and *Marchantia polymorpha* though the cells of these plants are not stained with Feulgen's method in the histochemical preparations, that is, Feulgen negative staining of cell nuclei, does not always mean absence of DNA.

The nucleic acids are composed with three major constituents; they are sugar, phosphoric acid and nitrogenous bases. Most investigators estimate only one of these constituents to determine total amount of nucleic acids in plant and animal tissues. But, it has been confirmed by Chargaff (1955) that chemical composition of nucleic acids is different in different sources, that is, molar ratios of each base and P-contents of nucleic acids, are different in different organisms. It is not reasonable, therefore, to determine

total amount of nucleic acids by the estimation of only one of these three components. Amount of nucleic acids must, therefore, be determined by estimation of these three components independently.

In the present study, therefore, nucleic acids are determined by estimation of phosphorus (PVM-method), deoxypentose sugar (Dische's and Feulgen's methods) and pentose sugar (orcinol method), and bases (UV-absorption method). The estimated values obtained in the present study by the estimation of these three components, are not always same. For an example, the DNA values per cell of *Oscillatoria limosa*, are 8.1×10^{-9} mg by PVM-method, 8.5×10^{-9} mg by UV-absorption method, 7.9×10^{-9} mg by Dische's method and 7.7×10^{-9} mg by Feulgen's method (Table 5). Generally speaking, the DNA values obtained by the UV-absorption method, are higher than the values obtained by other estimation methods. While the values obtained by Dische's and Feulgen's methods, are lower than those obtained by other methods.

Mean values of nucleic acid content per cell determined by several estimations used in the present study, show that the value of DNA content of *Oscillatoria limosa* is 8.1×10^{-9} mg, and this value is around the same level with those obtained from cells of Feulgen positive animal organs such as pancreas (7.5×10^{-9} mg), thymus (7.6×10^{-9} mg), spleen (6.7×10^{-9} mg) or kidney (6.9×10^{-9} mg) of rat¹ (see review by Leslie 1955). The mean values of DNA per cell of *Spirogyra sp.* and *Marchantia polymorpha* are 2.9^2 and 0.48×10^{-9} mg respectively. These facts show that Feulgen staining of the cells of *Oscillatoria limosa* and *Spirogyra sp.* is negative even though the DNA content per cell of these algae is around the same level with those of other Feulgen positive cells. Similar result was reported by Shinke, Ishida and Ueda (1957) in *Oscillatoria princeps* that the content of DNA per cell of this alga is 9.2×10^{-9} mg, while this algal cells are not, or only very faintly, stained with Feulgen's method.

The cause of the Feulgen negativity of DNA in *Oscillatoria*, *Spirogyra* and *Marchantia* in the histochemical preparations, is not cleared in the present study, but it is assumed that some substances contained in the cells inhibit the Feulgen staining in the histochemical preparations. In a preliminary experiments, it has been reported that some proteins isolated from *Oscillatoria princeps* and *Spirogyra sp.* inhibit the development of the Feulgen reaction color *in vitro*.

The content of DNA per cell of *Marchantia polymorpha*, on the other hand, is much less than that of other plants used. It is not improbable to assume that one of the causes on Feulgen negativity of this plant, is ex-

¹ These values obtained on rat organs were calculated from Schneider's factor by the present author (Schneider 1945).

² Cell nuclei of fowl liver which contain 2.4×10^{-9} mg per cell (Leslie 1955) are Feulgen positive.

tremely low DNA content of the cells.

Several factors, which are regarded as the cause of the Feulgen negativity other than those stated above, have been found in the biochemical and histochemical investigations carried out by the present author. Details of which will be reported in a subsequent paper.

Summary

1. Some findings on the nucleic acids of Feulgen negative plants, *Oscillatoria limosa*, *Spirogyra sp.* and *Marchantia polymorpha*, studied by the Feulgen's staining method and biochemical procedures, are reported.

2. Cells of these plants were not stained with Feulgen's method under various conditions of fixations and acid hydrolysis, but certain amounts of DNA were isolated biochemically from cells of these plants. Isolated DNA gives positive reaction with Feulgen's and Dische's tests *in vitro*. Absorption maximum of the Feulgen's color at pH 2.28 with DNA from these plants, locates at 565 m μ .

3. Maximum of UV-absorption curve of DNA, isolated by Schmidt and Thannhauser's method from above plants, situates at 260 m μ in all cases, and no significant difference among UV-absorption curves of DNA from these plants and calf thymus, has been found. UV-absorption maximum of DNA, isolated by the procedure of Ogur and Rosen using hot PCA, however, locates at 268 m μ . Similar results are obtained when calf thymus and fish sperm-DNAs have been heated with hot PCA.

4. Nucleic acids and other P-containing substances in cells of these Feulgen negative plants are determined quantitatively. Especially, DNA and RNA have been determined by several methods for the estimation of three major constituents of nucleic acids. Mean values of DNA content per cell in *Oscillatoria limosa*, *Spirogyra sp.* and *Marchantia polymorpha* are 8.1, 2.9 and 0.48×10^{-9} mg respectively.

Causal analyses of Feulgen negativity in histochemical preparations will be discussed in another paper.

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