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A Cytochemical Study of Nucleic Acids in Plant Cells
II. Effects of proteins on the Dische reaction.*

By

Masahiro Ruè ISHIDA
Cytological Laboratory
Botanical Institute, College of Science, University of Kyoto
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Dische reaction has frequently been employed for quantitative estimations of DNA (Dische, 1931; Seibert, 1940; Schneider, 1945).

Results of the experiments reported in the previous paper show that the color produced by the Dische's original method is not stable and that the color produced by a method modified by the present author is more stable than that produced by the original method (Ishida, 1954).

It has been pointed out by several workers, however, that when some proteins are contained in DNA solutions, the intensity of the color produced by the reaction is increased, and that some substantial errors rise in colorimetry by the presence of proteins (cf. Overend, 1951; Dische, 1955).

It is intended in the present paper to report a result of the experiments to see effects of proteins on the Dische reaction by the modified method stated above.

Method

The procedure used for the determination of DNA[1] is as follows: Two volumes of Dische reagent[2] were added to one volume of sample solution and heated in water bath at 90°C for 20 minutes. The mixture became blue after heating. Then the mixture was cooled in ice water. The intensity of the color developed by the reaction, then, was determined with a Beckman spectrophotometer.

Experiments

Experiment 1. Effect of proteins on the absorption curve of the reaction color. One volume of 0.04% histone·sulfate was added to one volume of 0.1% Na·DNA solution. After addition of the Dische reagent, the mixture was heated in a water

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1) Sample of DNA used in the present experiments is Na·DNA prepared from calf thymus.

2) Preparation of the reagent: One volume of conc. H_2SO_4 is added to 39 volumes of 2% diphenylamine solution in glacial acetic acid.

bath. Similar experiments were carried out with protamine·sulfate, casein and gelatin. The Na·DNA solution was used as a control. The results obtained in the above experiments are shown in Figure 1.

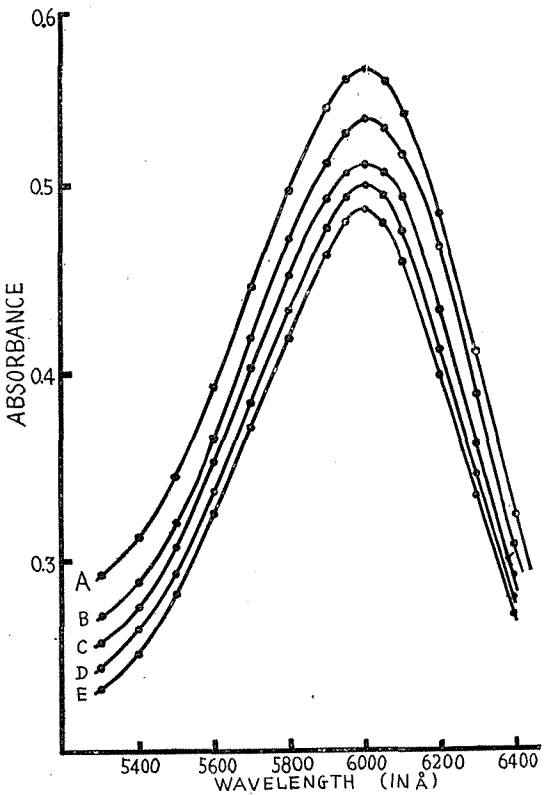

Fig. 1. Absorption curves of the color developed with Dische reaction.
A: Na·DNA+casein, B: Na·DNA+gelatin, C: Na·DNA+protamine·sulfate, D: Na·DNA+histone·sulfate, E: Na·DNA alone.

In this figure, it is seen that the forms of the absorption curves show no significant difference with that of Na·DNA alone. Each curve has a sharp maximum at 6000Å.

Experiment 2. Effect of proteins on the intensity of the reaction color. Solutions of various concentrations of histone·sulfate, protamine·sulfate, gelatin and yeast·protein[1] were prepared in separate test tubes. One volume of 0.04% Na·DNA solution was added to one volume of each protein solution. After addition of the

1) Yeast·protein was prepared from N-KOH extracts of Fleischman yeast with Schmidt and Thannhauser method (cf. J. Biol. Chem. 161, 1945).

Dische reagent, the mixture was heated. The results of the experiments are shown in Figures 2, 3 and Table 1.

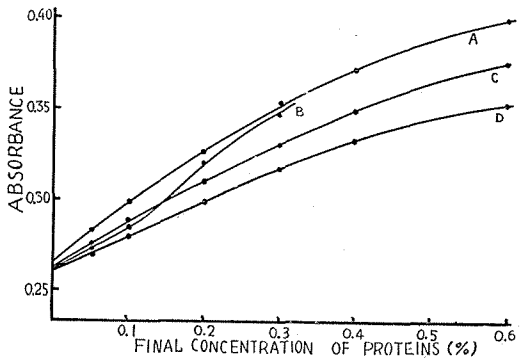

Fig. 2. Effect of the proteins on the development of the reaction color.
A: Gelatin, B: Yeast·protein, C: Protamine·sulfate, D: Histone·sulfate

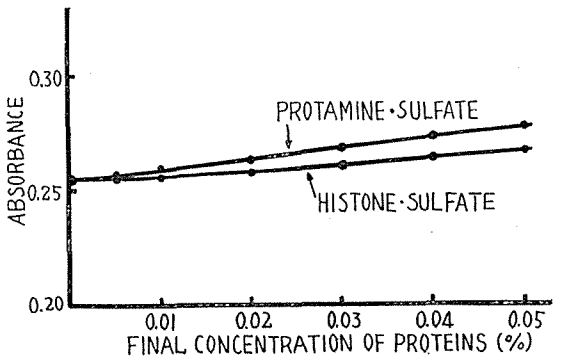

Fig. 3. Effect of histone·sulfate and protamine·sulfate on the development of the reaction color.

Table 1. Ratio (%) of absorbances of the color produced in Na·DNA-protein mixture against Na·DNA alone.*

Proteins added	Final concentration of the proteins in Na·DNA-protein mixtures.	
	0.04 %	0.2 %
Histone·sulfate	103.9	116.9
Protamine·sulfate	107.5	121.7
Yeast·protein	104.6	129.0
Gelatin	107.5	130.3
Na·DNA alone (control)	100.0	100.0

* Final concentration of Na·DNA in all mixtures is 0.02 %.

In these figures, it is seen that the higher the concentration of the proteins in the mixtures, the more increase of the absorbance of the color developed. As shown in Table 1, when the proteins present in amounts corresponding to twice that of Na·DNA, the intensities of the color developed are more increased than that of control, but are less than 110% against 100% of the control. In the case of 10 times the intensities of the colors are strongly increased more than 110%.

Moreover, effects of different proteins on the Dische reaction are different (Table 1). That is, the absorbance of the color produced by the presence of histone·sulfate is less than that of protamine·sulfate when the concentration of both proteins is same.

Experiment 3. DNA determination after deproteinization. It has been shown in the above experiments that the intensity of the color developed is strongly affected by the presence of proteins in the DNA solution. Hence it must be necessary to remove proteins before applying the colorimetric tests for the estimation of DNA from tissues. In this experiment, Schneider's method was employed for removal of the proteins from the DNA-protein mixture (Schneider, 1945).

Solution of histone·sulfate was added to Na·DNA solution. The final concentrations of protein and Na·DNA were 0.8% and 0.04% respectively. One volume of 20% trichloroacetic acid (TCA) solution was added to one volume of the above mixture, and heated for 10, 20 and 30 minutes at 90°C.

Same experiment was carried out with a mixture of yeast·protein and Na·DNA solutions in the same concentration with the above case. Na·DNA solution was used as a control. After heating at 90°C, the mixtures were cooled in ice water, then proteins precipitated was removed by centrifugation. To the supernatant added the Dische reagent and heated. Results of the experiment obtained are shown in Table 2.

Table 2. Absorbances of the color developed after removal of proteins.

Proteins added	Time of heating with TCA (minutes)	Absorbances				Ratio (%)
		Na·DNA alone		Na·DNA–protein mixture		
		With TCA	Without TCA	After deproteinization	Without deproteinization	
Histone·sulfate	10	0.254	—	0.269	—	105.9
	20	0.231	—	0.246	—	106.5
	30	0.218	—	0.238	—	109.2
	—	—	0.236	—	0.315 *	133 5
Yeast·protein	10	0.259	—	0.241	—	93 1
	20	0.238	—	0.248	—	104.2
	30	0.224	—	0.223	—	99.6
	—	—	0.234	—	0.421 *	179.9

* TCA was not added to the mixtures.

In this Table, it is seen that the absorbances of the color developed as a result of Dische reaction with DNA-protein mixtures without deproteinization, is stronger than that of control solution. When the protein is removed from the mixture with TCA, the absorbances of the deproteinized solution are approximate to that of control solution.

Experiment 4. Effects of proteins on the stability of the color developed. One volume of 0.06, 0.12 or 0.60% histone·sulfate solution was added to the equal volume of 0.06% Na·DNA solution. Then, two volumes of the Dische reagent were added to one volume of each mixture followed by heating. Same experiments were carried out with the Na·DNA-protamine·sulfate and the Na·DNA-gelatin mixture. The results of the experiments are presented in Figures 4, 5 and 6.

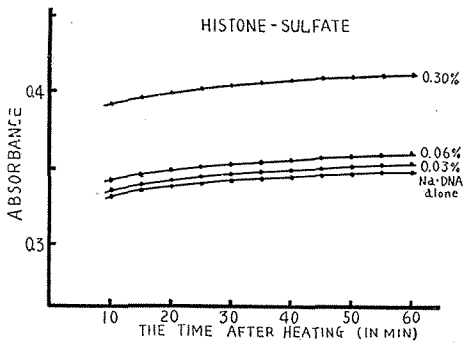

Fig. 4. Relation between changes of absorbance of the developed color after heating and concentration of histone·sulfate. The final concentration of histone·sulfate is noted in each curve.

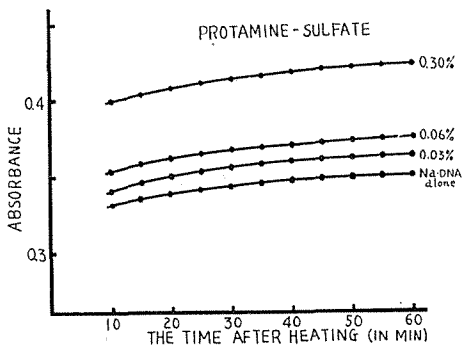

Fig. 5. Relation between changes of absorbance of the developed color after heating and concentration of protamine·sulfate. The final concentration of protamine·sulfate is noted in each curve.

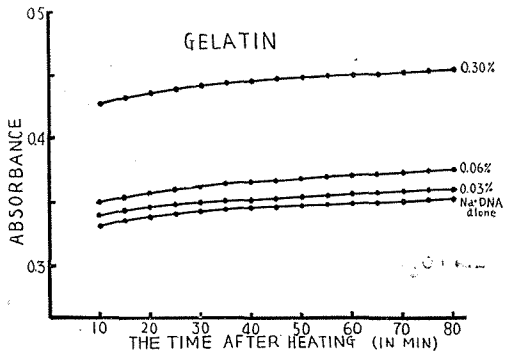

Fig. 6. Relation between changes of absorbance of the developed color after heating and concentration of gelatin. The final concentration of gelatin is noted in each curve.

It is seen in these figures that the increase of color intensity after heating is slow and that the presence of proteins did not affect the stability of the reaction color.

Discussion and Conclusion

Absorption curves of the reaction color developed by Dische reaction of DNA show sharp maxima at 6000 Å whether the proteins are present in DNA solution or not, that is, the presence of proteins with DNA solution did not shift the maximum of the abosrption curve.

In respect to the effects of the proteins on the development of the reaction color, Euler and Hahn (1946)[1] reported that the presence of certain proteins increases the intensity of the diphenylamine reaction. While, Bergold (1948) was not able to confirm the results obtained by them (cf. Dische, 1955).

In the present experiments, it is shown that when proteins are contained in DNA solution, the intensity of the color developed with the Dische test is higher than that of pure DNA.

The effect of histone·sulfate was greater than that of protamine·sulfate in this experiment. This result is quite in accordance with that obtained by Overend (1951). It is concluded, therefore, that the presence of certain proteins causes some error in the estimation of DNA with colorimetric method.

After removal of the proteins, however, the error caused by the presence of proteins is less than that of the case without deproteinization.

From the results of Experiment 4, it is concluded that the stability of the color developed by the Dische reaction is not affected by the presence of proteins.

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[1] cited from Dische (1955).

Summing up the results of the previous and the present investigations, we arrived at the following conclusion: The Dische reaction is an excellent method for a quantitative determination of DNA, but the DNA fraction separated from tissues must be heated for 20 minutes at 90°C with Dische's reagent after deproteinization to avoid the error caused by the presence of proteins.

Summary

1) Effects of proteins to the Dische reaction were investigated.

2) Absorption maximum of the reaction color is not shifted by the presence of certain proteins with DNA.

3) Intensity of the reaction color produced is stronger than that of DNA alone when proteins present in DNA solution.

4) After deproteinization of the DNA-protein mixture with Schneider's method, the intensity of the color developed is approximate to that of DNA alone, though the intensity of the former was not same to that of the latter.

5) The presence of different proteins in different concentrations does not effect on the stability of the color produced.

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