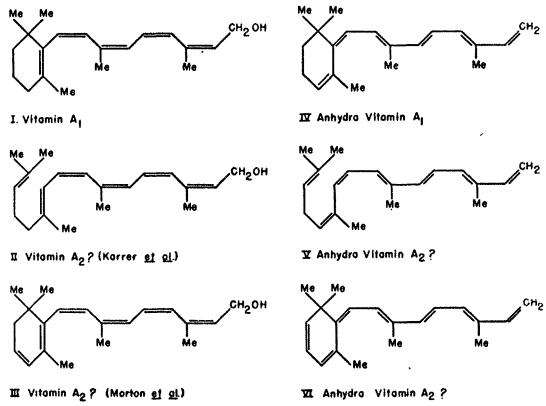
different blue colors has been disregarded by most investigators, although an account of this seeming anomaly was first published in 1940 (*2*). Compounds IV and VI could hardly have the same ultraviolet spectra, but com-



pounds IV and V might be very similar. The surprisingly large difference in the blue colors could then be accounted for by assuming that antimony trichloride brings the isolated double bond of compound V into play. On the other hand, the shift in ultraviolet absorption maximum of compound II to 351 mμ from that of vitamin A_1 (I) at 326 mμ is rather large to be accounted for by mere opening of the ring. In the author's opinion, the evidence slightly favors structure II for vitamin A_2, if a choice must be made between these two proposals.

Biological tests indicate that the vitamin A_2 alcohol described above has a potency of approximately 1,300,000 U.S.P. units/gm, or about 40% of the activity of crystalline vitamin A_1. The biological experiments will be reported in greater detail elsewhere.

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Some Quantitative Aspects of the Feulgen Reaction for Desoxyribose Nucleic Acid

M. A. Lessler

Department of Biology, New York University

The Feulgen reaction, originally described by Feulgen and Rossenbeck (*2*), has been used for the demonstration of desoxyribose nucleic acid (or thymonucleic acid) in tissue sections. The reaction involves the hydrolysis of sectioned material in 1N HCl at 50–60° C for about 10 min, followed by the application of the leuco-fuchsin (Schiff's) reagent for 1–2 hrs. Stowell (*5*) and Dodson (*1*) presented strong evidence that the reaction is specific for thymonucleic acid. The Feulgen technique has been applied in various ways for the quantitative estimation of the desoxyribose nucleic acid (DNA) in the cell. In order to establish its possible value as a quantitative method, the Feulgen reaction was tested on purified preparations of DNA.

It was impossible to separate the components of the reaction in liquid medium; therefore, it was necessary to find a solid medium into which the DNA could be incorporated. Weighed amounts of DNA were dissolved in hot 5% agar as previously described by Hillary (*3*). Eight different samples of agar without DNA, however, gave a positive Feulgen reaction following the usual hydrolysis. The fixatives used by Hillary were tried, but none was able to inhibit the Feulgen reaction with hydrolyzed agar. It became evident that Hillary's methods involving agar were unsatisfactory and that his conclusions on the effect of fixatives on the Feulgen reaction were open to question.

The problem was to find a medium that would gelate or solate at appropriate temperatures and which, after fixation and hydrolysis, would be negative to the Feulgen reaction. Eastman Kodak ash-free and Difco-Bacto gelatin were satisfactory. After fixation of a 20% gel in 6% formalin for 1½ hrs, there was no appreciable hydrolysis of the gelatin by the HCl. The gelatin was Feulgen negative after long periods of hydrolysis provided the formalin was thoroughly washed out. Formalin itself gives an intense Feulgen reaction even at very low concentration. Sodium DNA, at the concentrations used, readily dissolved in the 20% gelatin sol and was not appreciably lost from the gel during fixation, washing, hydrolysis, and the subsequent reaction with the leuco-fuchsin reagent (prepared by the Rafalko technique, *4*).

The procedure was to add 5 cc of the 20% gelatin sol, at 32–35° C, to small test tubes containing weighed amounts of sodium DNA. Careful agitation produced a uniform mixture of the gelatin sol with the DNA. Drops of the warm gelatin-DNA preparations, approximately 0.1 ml in volume, were then placed on 1″ × 3″ glass slides (previously coated with albumin fixative and heat dried) and allowed to gel. The gelatin drops were hardened in 6% formalin for 1½ hrs and washed in running tap water for 2–3 hrs to remove the formalin. The

preparations were considered to be formalin free if, after a 30-min period in the leuco-fuchsin reagent, the test slide remained completely colorless.

With this procedure, gelatin preparations containing DNA at 0.2, 0.5, 0.8, 1.0, 1.5, 2.0, and 3.0 mg/cc were tested. One drop of each preparation together with two or more DNA-free control drops were placed on a slide. These slides, each representing a test series, were hydrolyzed in 1N HCl at 55° C for from 3 to 39 min and then immersed for 1½ hrs in the leuco-fuchsin reagent. Color estimations were made after a quick rinse in distilled water and then two 10-min changes of water saturated with SO_2. The data from several test series are summarized in Table 1.

TABLE 1

Conc. DNA (mg/cc)	Hydrolysis in 1N HCl at 55° C (min) 0	3	5	7	9	11	13	15	17	19	22	27	34	39
0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0	0	0	0	1	0	0	0
0.8	0	0	0	0	1	2	2	1	2	3	2	1	1	2
1.0	0	0	0	1	1	2	2	1	1	2	3	3	3	3
1.5	0	1	1	2	2	2	3	4	4	4	4	4	4	
2.0	0	1	1	2	2	3	3	4	4	4	4	4	4	
3.0	0	1	1	2	2	3	3	4	4	4	4	4	4	

The values indicate the relative intensity of color developed in the gelatin drop: 0 = absence of color or, at most, a faint tinge, 1 = light lavender, 2 = lavender, 3 = light purple, 4 = deep purple.

No appreciable color was developed with small amounts of DNA, while within the middle range there was a gradual increase of color until a maximum intensity was reached. It should be pointed out that there was no further development of color with increasingly greater amounts of DNA. The intensification of the color of the drops that develops with increasingly longer periods of hydrolysis indicates that DNA was not lost in the process. With higher concentrations of DNA there was no increase or decrease in color intensity following periods of hydrolysis beyond those required for maximum color development. These observations indicate the necessity for a critical control of hydrolytic time.

The Feulgen reaction is quantitative only over a short range of DNA concentrations. At least 0.8 mg/cc of DNA must be present in order to produce an appreciable color. Beyond a concentration of 1.5 mg/cc no further intensification of color may be visually detected after an hydrolysis of 15 min or longer. Experiments are in progress to study the reaction by photometric methods.

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Photomicrography at Your Convenience[1]

R. B. Stevens and J. Herbert Taylor

Department of Botany,
The University of Tennessee

There can be no question but that photomicrography, using standard equipment and methods of the biological laboratory, is a proven and acceptable procedure. We should like to report an alternative which, after some use, appears particularly suited to our needs and which combines features not found in the more widely used methods. In such measure as the reader's problem parallels our own, we believe these suggestions will be of value.

Interests so diverse as plant pathology and cytogenetics necessitated the development of photographic equipment which would allow reasonably rapid change from one technique to another. It was desirable to take pictures not only at high magnifications through the compound microscope, but through the dissecting microscope, and at low magnifications by use of extension tubes.

One camera, the Kine Exakta 35-mm single lens reflex seems ideally constructed for the purpose. Especially valuable features of this machine are: attached magnifier for use with ground-glass focusing, shutter speeds from .001 to 12 sec, bayonet lens mount, focal plane shutter, and provision for removal of short lengths of exposed film. Except for the added light intensity obtainable for focusing, there is little advantage of the f 2.8 over the f 3.5 lens. This camera, if the tripod socket is fitted with the proper reducing insert, can be attached to the horizontal arm of any enlarger stand, either by the bolt used to hold the enlarger unit itself or by an ordinary ¼″ bolt of the proper length and of standard thread. For maximum speed and accuracy it is well to calibrate the vertical post of the enlarger stand in convenient units and to fix the height of the camera at will by use of a radiator hose coupling about the enlarger post. This hose coupling, if selected of the proper diameter, can readily be moved from one position to another, allowing the arm to swing freely in the horizontal plane (Fig. 1).

Probably the most effective way of joining the camera to the barrel of the microscope is by use of a 6″–8″ length of automobile radiator hose, which may be obtained in a size which snugly fits the extension tube. By slipping the rubber sleeve up over the metal tube, the eyepiece of the microscope is cleared, and the camera can be swung to one side. By slipping it down over the barrel of the microscope, an essentially lightproof seal is secured. The manipulations are completed by fixing the enlarger stand firmly to the working desk and marking the position of the base of the microscope and lamp.

Exposure time, inevitably a matter of trial and error, when once established, will be constant. The complexity of the lamp and optical equipment available will deter-

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