BIOCHEMISTRY

Feulgen–Naphthoic Acid Hydrazide Reaction applied to Blood Smears

HÆMATOLOGICAL use of Schiff's reagent for the Feulgen reaction[1] has been described by Gardikas and Israels[2]. This technique, or minor variations of it, has been used extensively to identify the number of nucleoli in primitive normal or pathological leucocytes. There is a perinucleolar concentration of nuclear protein, but the nucleoli themselves are free of deoxyribonucleic acid.

The method has one disadvantage in that deep staining cannot be obtained and smears must usually be examined for the specific red coloration with the aid of a green sub-stage filter. The purpose of this communication is to direct attention to the naphthoic acid hydrazide reaction described by Pearse[3,4]. A modification of Pearse's method has been found to give excellent results with smears of blood or bone marrow from a number of species (man, horse and dog). The intensity of staining available with fast blue *B* is considerably greater than that available with Schiff's reagent. The method is as follows.

Fresh, air-dried smears are fixed for 30 sec. on the staining bridge with a solution consisting of 10 ml. of 40 per cent formaldehyde and 90 ml. of absolute methanol kept in the refrigerator at 0° C. The smears are rinsed in distilled water and then in *N* hydrochloric acid. Hydrolysis is performed at 60° C. for 10 min. in *N* hydrochloric acid. After this the smears are again rinsed in *N* hydrochloric acid, in distilled water, and in 50 per cent ethyl alcohol. They are incubated for 5 hr. in naphthoic acid hydrazide at 20–22° C. made up as follows: 0·1 gm. 2-hydroxy-3-naphthoic acid hydrazide (L. Light and Co.) is dissolved in 50 ml. absolute ethanol. 45 ml. distilled water and 5 ml. glacial acetic acid are then added. After incubation the slides are immersed in three changes of 50 per cent ethyl alcohol for 10 min. each, rinsed in distilled water, and stained in freshly prepared fast blue *B* at 0° C. for 3 min. prepared as follows. Fast blue *B* (G. T. Gurr) 5 mgm. dissolved in 0·1 *M* veronal buffer *p*H 7·4, 5 ml. The buffer is kept in the refrigerator in small jars of 5 ml. capacity to which the fast blue *B* is added just before staining. The latter is performed in a Petri dish in the refrigerator. The slides are then washed in distilled water and blotted dry.

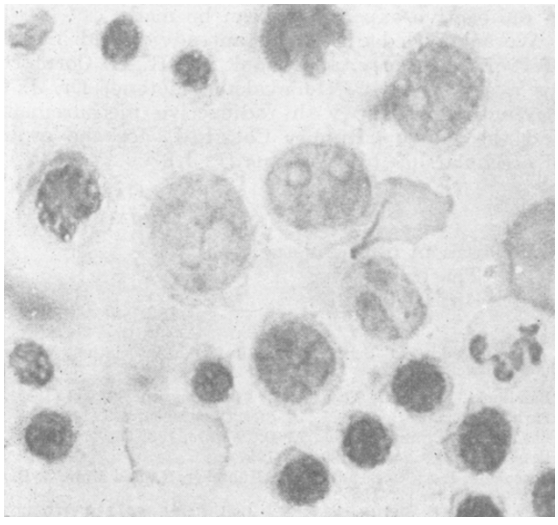

Fig. 1. The Feulgen–naphthoic acid hydrazide reaction applied to a smear of horse bone marrow. Deoxyribonucleic acid stains bluish-purple. Cytoplasm stains faint pink

Using this technique Fig. 1 illustrates a typical group of cells from a horse bone marrow specimen. Deoxyribonucleic acid stains bluish-purple while the cytoplasm of the cells stains a faint pink. The smears are easily seen microscopically without light filtration and the distribution of the bluish-purple fast blue *B* stain is identical with that of the more usual Schiff reagent in the Feulgen reaction.

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[2] Gardikas, C., and Israels, M. C. G., *J. Clin. Path.*, 1, 226 (1948).
[3] Pearse, A. G. E., *J. Clin. Path.*, 4, 1 (1951).
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Destruction of Cortisone and Related Steroids by Traces of Copper during Purification Procedures

WE wish to report the destruction of μgm. quantities of cortisone and related steroids by traces of cupric ion and the prevention of such destruction by addition of ethylenediamine tetraacetic acid.

In the course of developing paper chromatographic procedures for the purification and estimation of adrenal steroids in urine, poor recoveries of cortisone and cortisol were obtained repeatedly. In addition, the simultaneous formation of several transformation products was observed.

The cause of the destruction was traced ultimately to the glassware, which routinely had been washed in hot detergent solution ('Diflex'), rinsed with distilled water, and dried in an oven. Analysis of the distilled water revealed the presence of approximately 1 mgm. of copper/l. Two 125-ml. Erlenmeyer flasks received from the washroom on separate days contained 2·7 and 3·0 μgm. copper. Special treatment of test-tubes with sulphuric acid–dichromate, followed by repeated rinsing with copper-free water obtained from a de-ionizer, greatly reduced the loss of added steroid but did not prevent it completely. However, the prior addition of a small amount of ethylenediamine tetraacetic acid, even to tubes not especially pretreated, resulted in quantitative recoveries of added cortisone.

In a typical experiment 4 μgm. of cortisone in 10 μl. of methanol was added to each of three 'Pyrex' test-tubes, size 100 mm. × 10 mm., only the first of which had been washed in sulphuric acid–dichromate and rinsed with copper-free water. The second tube served as a control, and 50 μgm. of disodium ethylenediamine tetraacetic acid in 0·5 ml. of methanol was added to the third tube. 1 ml. of methanol was added around the sides of each tube, and then evaporated in a stream of carbon dioxide. After being allowed to stand for 3 hr. at room temperature, the residues were transferred individually with two 25-μl. portions of methanol to spots on a 7-in. sheet of unwashed Whatman No. 1 paper. Cortisone standards of 0·5–4·0 μgm. were added in spots along the same line. Chromatography was performed by Bush's[1] system *C* after an overnight equilibration. By a procedure similar to that of Neher and Wettstein[2], quantitation was done on the bases of blue