It should be pointed out that in so-called physiological conditions of pH and concentration, the NADH signal is much larger than that produced by the Schiff-base. Indeed, NADH 10^{-5} *M* in *Tris* buffer 20 m*M* pH 7.4 gives a fluorescence signal of 137% of that of the standard quinine sulfate solution, while the emission of a mixture of P-5-P 3 μg/ml and glutamate 3.75 m*M* is only 19.5% of the emission of the reference solution. Nevertheless, not only glutamate but also the other amino acids can participate in the formation of Schiff-bases, and care should be taken in the interpretation of the results of experiments which are not inducing changes in tissue fluorescence emission large enough to be safely attributed to a modification in the redox state of the pyridine nucleotides.

In the course of our experiments, a good correlation between the emission intensity at 468 nm and the concentration of P-5-P in the concentration range between 0.01 μg to 1 μg/ml has been observed in a reaction medium heated at 50 °C for 5 min and containing glutamate 5 m*M* and $AlCl_3$ 2 m*M* in acetate buffer 20 m*M* at pH 5. This indicates the possibility of the use of the fluorescence characteristics of the Schiff-base for the development of a specific technique of determination of free pyridoxal phosphate concentration in biological material.

Résumé. La base de Schiff formée à partir de pyridoxalphosphate et d'un acide aminé présente une fluorescence dont les caractéristiques spectrales sont similaires à celles du NADH. Les possibilités éventuelles de faire la distinction entre le signal émis par la base de Schiff et le NADH au cours de travaux réalisés avec les microfluorimètres utilisés pour étudier les modifications du niveau d'oxydoréduction des nucléotides de la pyridine sont discutées.

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A Simple Technique for Demonstrating Heterochromatin in *Nigella*

This is to report a new technique which enabled us to document the presence of heterochromatin in *Nigella*, a species in which a lack of demonstratable heterochromatin was reported previously[1]. The technique is a modification of the cytological procedures used for the demonstration of heterochromatin in rye chromosomes[2].

Actively growing primary root tips of *Nigella damascena* var Miss. Jekyl, were pretreated in monobromonaphtalene solution for 3 h at room temperature and fixed in glacial acetic acid overnight (22–24 h). After fixation, the root tips were softened by immersing in an enzyme solution[3] for ½ h at room temperature. The root tips were then squashed. The cover slips were separated from the slides by CO_2 freezing, dehydrated in absolute ethanol for 30 min and dried by blowing air. The chromosome preparations, were then immersed in a freshly prepared saturated barium hydroxide solution for 5 min at room temperature. After through rinsing in several changes of distilled water and air drying, the slides were incubated in a 2X SSC solution[4] for 1 h at 60 °C, washed thoroughly in distilled water, air dried, stained in a Giemsa staining solution[5] for 2 min, rinsed and air dried. The stained slides were left in Xylene overnight and mounted in Canada balsam.

All the chromosomes of *Nigella damascana* var. Miss. Jekyl (5 pairs metacentric and 1 pair acrocentric) showed the presence of heterochromatin (C-bands) in the centromeric area (Figure). Another characteristic feature of the heterochromatin: chromocenter formation in interphase nuclei, were also seen. Their number varied from 3 to 5 per nucleus (Figure).

Zusammenfassung. Feststellung von C-Banden in *Nigella damascena* var. Miss Jekyl mittels eines neuen zytologischen Verfahrens. Es waren 3–5 Chromozentren pro Nukleus zu erkennen.

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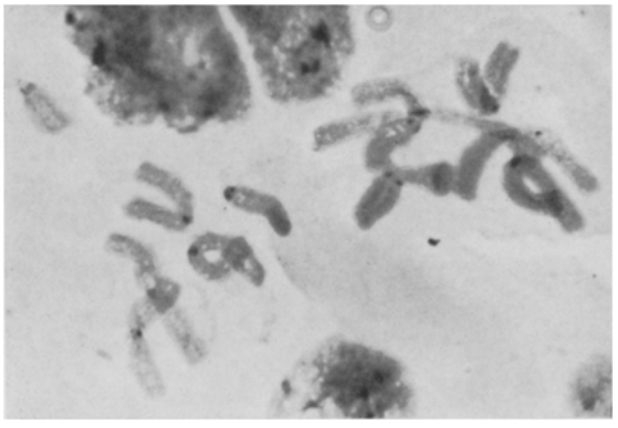

Metaphase spread showing the centromeric localization of heterochromatin. Chromocenters are visible in the interphase nuclei.

[1] A. T. Natarajan and G. Ahnstrom, Chromosoma *28*, 48 (1969).

[2] B. S. Gill, G. Kimber, Proc. natn. Acad. Sci., USA, in press.

[3] The enzyme mixture is prepared by mixing equal parts of 5% pectinase and cellulase solutions to which 2–3 drops of 1*N* HCl were added for each 5 ml of the mixture.

[4] The 2X-SSC solution is made with 0.887 g of NACl and 0.411 g of trisodium citrate in 100 ml of distilled water.

[5] The stock Giemsa solution is made by mixing 1 g Giemsa powder (Fisher) in 66 ml of glycerine and heating it for 2 h in a 60 °C oven with frequent agitation. Then 66 ml of absolute methanol is added and this mixture is cooked for 2 days in 60 °C oven with frequent agitation. The working staining solution is made by mixing 5 ml of stock Giemsa solution, 1.5 ml MacIlvaine buffer (pH 7.0), 1.5 ml absolute methanol and 50 ml of distilled water. This staining mixture is filtered and used.