MODIFIED METHOD OF C BANDING USING BARIUM HYDROXIDE *

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A modified centromeric heterochromatin banding technique using barium hydroxide octahydrate is described. The relationship between slide maturity and time of denaturation by barium-hydroxide is discussed.

The original method of Pardue and Gall (1970) for the visualisation of centromeric heterochromatin was modified by Arrighi and Hsu (1971). They avoided the treatment of chromosomal preparations by radioactive complementary RNA. Sumner et al. (1971) and Sumner (1972) observed that sodium-hydroxide was a very strong denaturing agent and, unless treatment time is controlled carefully, damaged and swollen chromosomes may result. As a result of this observation, they suggested the use of barium hydroxide octahydrate — a weak alkali — as the denaturing agent, so that the treatment period can be controlled easily. We have observed in our laboratory that there exists a lot of variation in $Ba(OH)_2 \cdot 8H_2O$ treatment time also for denaturation, depending upon the age of the slides. Fresh slides need much less time for denaturation compared to older slides.

Chromosome preparations from lymphocyte cultures were obtained by conventional techniques. Air-dried preparations were treated with 0.2 N HCl for 1 hour. The slides were then washed in distilled water and dried. They were then treated with 5% solution of barium hydroxide octahydrate for a specific time period, i.e., from 5 to 30 minutes at 50 °C for slides which are 3 days to 3 months old and 15 to 30 minutes at 60 °C for slides which are more than 3 months old. After thoroughly rinsing in distilled water, they were incubated in 2 × 55C at 60 °C, for 1 hour, rinsed in distilled water and dried. They were then stained for 90 minutes in Giemsa (3 ml of 0.1 M citric-acid, 3 ml of methyl alcohol, 10 ml of Giemsa stock solution, and 100 ml of distilled water), dried and made permanent with Eukitt.

It has been observed that the period between slide making and exposure to denaturing agent is a crucial factor in C-banding. As a result of experimentation in our laboratory, the following treatment times, as detailed in Table 1, are recommended for denaturation of chromosomal material by barium hydroxide octahydrate.

If the slides are more than 6 months old, it is very difficult to denature them by barium hydroxide octahydrate and as such the use of NaOH is preferred for this kind of slides. Treatment of the slides at 60 °C in barium hydroxide is not advisable for more than 30 minutes. Prolonged treatment at 60°C may lead to loss of cell material from the surface of the slides.

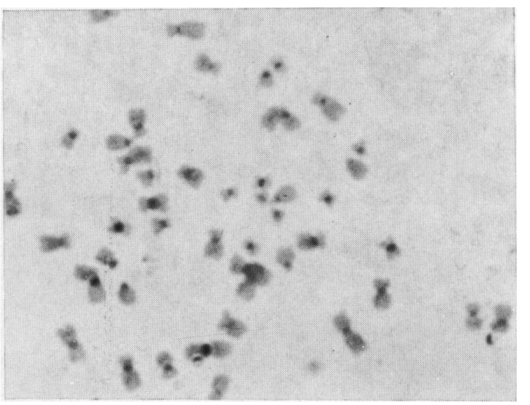

Fig. 1. Metaphase showing C-bands revealed by $Ba(OH)_2$ treatment.

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Table 1. *Optimum denaturation time of the slides of different ages*

Age of the slides (in days)	Treatment time (in minutes)	Temperature preferred (°C)
3-7	5	50
8-20	10	50
21-45	15	50
45-60	20	50
60-90	30	50
90-120	15	60
120-150	25	60
150-180	30	60

Fresh chromosomal material as a result of fixation in Caronoy's fixative are in a denatured stage. Gradually with time the DNA/protein complex gets hardened and it becomes difficult to denature them. That is why fresh preparations need very less time for denaturation as compared to older slides which have to be treated for a prolonged period of time.

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