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BRIEF NOTE

INFLUENCES OF THE TEMPERATURE ON THE DETECTION OF Y-CHROMOSOMES IN BLOOD SMEARS

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In 1968 Caspersson et al.[1] reported the use of quinacrine mustard to the visualization of chromosomes in the metaphase nucleus. Later, Pearson et al.[2] reported that the Y-chromosomes in human interphase nuclei were detectable by using quinacrine dihydrochloride. In the field of legal medicine, this method has been developed for determining the sex of the donor of the various materials such as bloods, bloodstains, hairs and tissues. Ishizu et al.[3-5] applied this method to many materials in legal medicine and concluded that it was possible to identify the sex of the donor by detecting the fluorescence of Y-chromosome (F-body). The detection seemed to be dependent on various factors and in the case of bloodstains, the factors influencing the results were discussed by many workers. In this preliminary report, we would like to clarify the influence of the temperature conditions in which the bloods are stored.

Bloods examined were placed in test tubes containing anticoagulant and kept at 4°C and 37°C. At various day intervals, blood smears were made from the bloods and allowed to dry at room temperature before fixation in absolute methanol. The fixed samples were then stained with 0.5% quinacrine dihydrochrolide solution for 30 min. They were then washed in running tap-water, in buffer (pH 5.5) and mounted in buffered glycerol. The F-body count was determined under incident-light fluorescence microscope in the dark field.

Time-lapse changes of the percentage of nuclei with F-body were shown in Fig. 1 and Fig. 2. When the test tubes were stored at 4°C, on and after the second the decrease of positive nuclei in male blood smears was observed gradually (Fig. 1). In spite of increasing of karyolysis, clear F-body was observed in male smears for a long period, and it seemed that the identification of the sex of the blood samples could be possible at least on the 7th to the 10th. On the contrary, the blood that was stored at 37°C showed a very rapid decline in F-body count on and after the first day (Fig. 2). There was observed karyolysis in most nuclei and after the 4th, karyopycnosis in addition to karyolysis was observed. Karyopicnosis as well as degeneration of components of blood resulted in false positive counts in female blood smears. On the

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second day, the percentage of F-body observed in male blood smears was 15.3% on the average and after that time there was overlapping between male and female counts. The method of F-body detection is very useful in legal medicine, and it seems to be influenced by various factors but it should be considered to achieve the best results. As one of these factors, the significance of the temperature at which the blood was preserved was discussed.

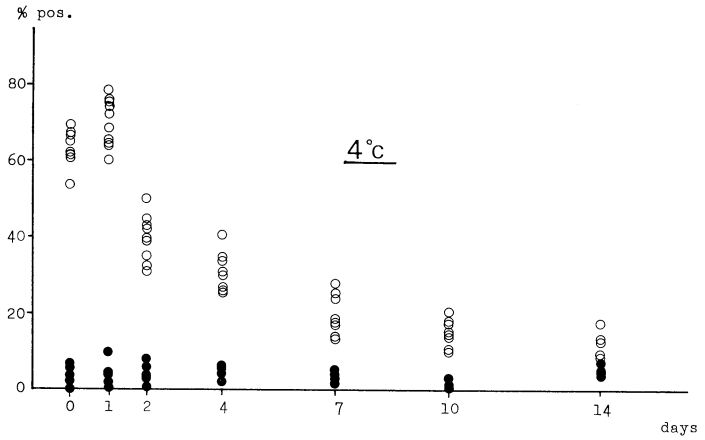

Fig. 1 The percentage of nuclei with F-body in the blood smears.
○ ; male ● ; female

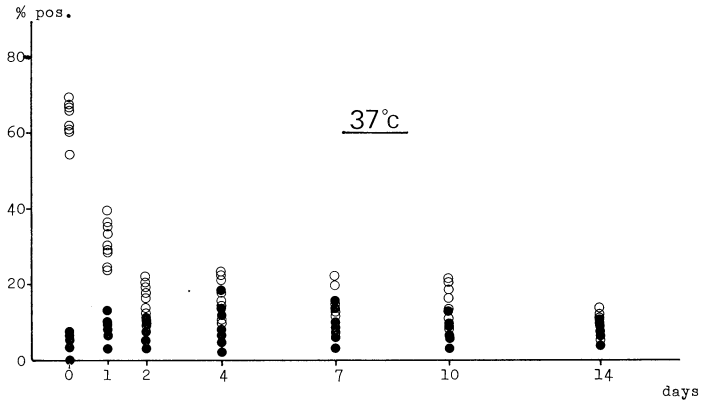

Fig. 2 The percentage of nuclei with F-body in the blood smears.
○ ; male, ● ; female

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