The fluorescent pattern of normal chromosomes in biopsies of malignant lymphomas, and its computer display

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The purpose of the present work was to investigate whether the fluorescence pattern of apparently normal chromosomes from a biopsy material of human malignant lymphomas agrees with that of normal lymphocytes, or if differences exist. As normal reference material the data on the fluorescence pattern of normal human chromosomes published by CASPERSSON et al. (1971) and MANOLOV et al. (1971 a) were utilized. We also wished to display the normal fluorescence patterns of human chromosomes with the aid of the computer technique earlier applied in the case of a characteristic marker chromosome (FLEISCHMANN et al. 1971).

Material and methods

The material consisted of tumorous lymph nodes from 7 patients with the histologic diagnoses of Hodgkin’s disease, lymphosarcoma, reticulum-cell sarcoma and malignant lymphoma of uncertain type. Tumorous lymph nodes were extirpated from the neck, from the axilla and from the inguinal regions. From the fresh biopsy material short-term cultures were made. The time of cultivation was 16–48 hours and the culture medium was Earle-Eagle with 20 % fetal calf serum.

The chromosomes were processed according to the technique of MOORHEAD et al. (1960) with some modifications. At first, the staining of the chromosomes with fluorochrome was performed according to the method of CASPERSSON et al. (1970). The results, however, were not satisfactory in our material, even though in some cases the pattern of fluorescence was clear enough for identification purposes. After considerable amount of experimentation the following technique was adopted and is now in use routinely, consistently yielding good results with our material. This technique is essentially an ordinary air drying technique, but with significant modifications: Thus, a definite improvement was achieved by substituting colcemid with vinblastine sulfate (Velbe, Lilly) in concentrations of 0.0075–0.015 μg/ml. The duration of the vinblastine treatment was 1 hour, and after that the slides were allowed to dry in the air without heating. When completely dry, the slides were stained in a 0.5 % solution of atebrin (Gurr) for 5–10 minutes. In our material atebrin was by far superior to quinacrine mustard. The staining was followed by rinsing

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Fig. 1. Fluorescence patterns of human chromosomes 1 to 7; the chromosomes underlined in white, submitted to computer analysis.
Fig. 2. Fluorescence patterns of human chromosomes 8 to 18.
in 3 changes of isotonic salt solution, 2 minutes in each. After that the coverslip was applied and the slides were ready for microscopy and photography, performed according to ordinary techniques.

Computer processing (Gustafsson and Todd-Pokropek 1972) and display of chromosome pictures digitized with a scanning technique were carried out according to the method recently described by Fleischmann et al. (1971), using a densitometer constructed by Gustafsson and Nordberg (unpubl.).

Results and conclusions

The present observations and experiments in computer display were made on normal-looking chromosomes selected from 150 metaphases of the 7 tumors. All chromosomes with abnormal morphology were disregarded in the present connection but will be dealt with in a separate publication. Our results are presented as photomicrographs of 10 chromosomes of each of the 24 human chromosome types (Fig. 1–3) and as computer diagrams of 1 chromosome of each type (Fig. 4–11).

The photomicrographs showed the most characteristic features of the normal fluorescence pattern, even though all the fine details were not visible in every chromosome. In 10% of the cells photographed, the fluorescence pattern was insufficiently clear to permit a complete analysis. The conclusion was drawn from our observations that good agreement existed between the fluorescence pattern of "normal" tumor chromosomes and that of normal leukocyte chromosomes published in the literature. In this respect our results concord with those of Manolov et al. (1971 b) in "normal" chromosomes of Burkitt tumors. The only deviation from normality in our material was a more pronounced variation in size sometimes noticed both among the different pairs and between the homologues of each pair. This phenomenon will be submitted to detailed analysis in a coming publication.

Those chromosomes, one of each type, which

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Fig. 4. Chromosome 1, contour map of isobrightness curves (upper picture); three-axis diagram (lower picture).

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Fig. 5. Chromosomes 2 and 3.
Fig. 6. Chromosomes 4 and 5.
Fig. 7. Chromosomes 6 to 8.
Fig. 8. Chromosomes 9 to 12.
Fig. 9. Chromosomes 13 to 18.
Fig. 10. Chromosomes 19 to 22.
Fig. 11. The X and Y chromosomes.
were underlined in white in Fig. 1–3, were the ones selected for computer treatment. In Fig. 4–11, 2 aspects of each chromosome are presented: contour maps of isobrightness curves and three-axis diagrams. These diagrams give objective representations of the variations in brightness in all parts of the chromosomes, and the characteristic features of their fluorescence pattern are clearly displayed.

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