Chromocentres in polymorphs as interphase markers for chromosomes having increased constitutive heterochromatin

N. B. ATKIN and MARION C. BAKER

Department of Cancer Research, Mount Vernon Hospital, Northwood, Middlesex HA6 2RN

Summary. After staining with the BSG technique for constitutive heterochromatin, human polymorphs exhibit chromocentres which are presumably derived from these chromosomal regions. In five individuals having an extended centromeric heterochromatin region on one No. 1 chromosome, a particularly large chromocentre was seen in the majority of polymorphs. Thus, prominent chromocentres in polymorphs may indicate the presence of constitutive heterochromatin variants.

In man, variation in the form of an increased amount of constitutive heterochromatin in chromosome Nos. 1, 9, and 16 and the Y chromosome appears to be common (Chen and Ruddle, 1971; Craig-Holmes and Shaw, 1971; Lubs and Ruddle, 1971; Aula and Saksela, 1972; Alfi and Menon, 1973). The significance of this polymorphism is uncertain but in general it appears to be without phenotypic effect.

We have noted that in preparations subjected to the BSG technique for the demonstration of centromeric heterochromatin (Sumner, 1972) interphase nuclei commonly present a number of discrete chromocentres of varying size on a fairly homogeneous background. Whereas their incidence is somewhat variable in lymphocytes transformed by phytohaemagglutinin and in cancer cells they are more constant in their appearance in polymorphs. Four to eight chromocentres are commonly present; two of these are often slightly larger than the rest and may be derived from the No. 1 chromosomes which usually have the largest centromeric heterochromatin blocks (Fig. 1). In support of the view that these chromocentres correspond to the darkly-staining, juxta-centromeric regions seen in metaphase chromosomes with the same technique, we examined polymorphs in blood cultures from a patient with Hashimoto's disease known to have a

markedly extended heterochromatic region on one No. 1 chromosome: a particularly large chromocentre was seen (Fig. 2). Likewise, having noted the presence of a large chromocentre in polymorphs in smears made from ovarian carcinoma tissue, we were able to confirm that one No. 1 chromosome in cultured lymphocytes from this patient presented an extended heterochromatic region (Fig. 3). We have made similar observations on three further individuals. The large chromocentres were observed in over 50% of suitable polymorphs (ie, those that showed any chromocentres).

The chromocentres always appear to be situated in contact with the nuclear membrane. Drumsticks in the female, in keeping with their origin from the whole or greater part of an X chromosome, are generally lightly stained but may present a small peripheral chromocentre (Fig. 4). Smaller nuclear protrusions in both sexes (the 'small clubs' of Davidson and Robertson Smith, 1954) are, however, usually darkly stained and presumably encompass the constitutive heterochromatin regions of chromosomes.

Geraedts and Pearson (1973) noted the presence of large chromocentres due to variant No. 1 chromosomes in spermatozoa and lymphocytes, using a staining technique which stains especially intensely the centromeric heterochromatin of this chromosome. The technique we have used cannot distinguish between No. 1 and other chromosomes

Fig. 1. Normal female: (a) No. 1 chromosomes ($\times 4610$) and (b) polymorphs ($\times 2420$).

Fig. 2. Female with Hashimoto's disease: (a) No. 1 chromosomes ($\times 4610$), (b) and (c) polymorphs ($\times 2420$).

Fig. 3. Patient with carcinoma of ovary: (a) No. 1 chromosomes ($\times 4610$), (b) and (c) polymorphs ($\times 2420$).

Arrows indicate chromocentres possibly derived from the No. 1 chromosomes (Fig. 1) and the large chromocentre presumed to be derived from the No. 1 chromosome with an extended heterochromatic region (Figs. 2 and 3). All the chromosomes—and the polymorphs in Figs. 1b, 2b, and 2c—were from lymphocyte cultures. The polymorph in Fig. 3b was from an air-dried preparation of carcinoma tissue fixed in methanol-acetic acid after hypotonic pretreatment, and that in Fig. 3c was from a blood smear fixed in methanol after air drying.

which may similarly have excessive heterochromatin; to determine which chromosome is involved, chromosome analysis would in general be necessary, although interphase chromocentres due to accentuated heterochromatin regions on No. 9 chromosomes may be specifically revealed by the 'Giemsa-11' technique (Bobrow, Madan, and Pearson, 1972).

In the mouse, three or four heterochromatin blocks are present in polymorphs which are larger than those seen in man; this difference may be due to the 'aggregation of centromeres' of the telocentric

chromosomes in the former species (Hsu *et al*, 1971) with the consequent tendency to fusion of the associated heterochromatic regions.

We conclude that examination of chromocentres in polymorphs in blood smears, following staining by a relatively simple C-banding technique, may provide a useful screening procedure for the detection of constitutive heterochromatin variants. To some degree, the extent of the increase in heterochromatin can be assessed; thus, the approximately three-fold increase in one No. 1 chromosome of the

FIG. 4. Polymorph showing drumstick with small chromocentre (arrow) from normal female. ($\times 1860$.) Blood smear fixed in methanol after air drying.

individual shown in Fig. 2 is associated with the presence of a somewhat larger chromocentre than is seen in Fig. 3, where the increase is two-fold or less.

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