In the area of hybridization between Oxford and Hermitage races of common shrew, hybrids which are simple Robertsonian heterozygotes were collected over a larger area and at a higher frequency than double Robertsonian heterozygotes with monobrachial homology, despite the fact that the frequency of the metacentric morph of arm combination *pr* is not higher than that of *no* or *kq* away from the area of hybridization[3]. This suggests that there is stronger selection against double Robertsonian heterozygotes with monobrachial homology than simple Robertsonian heterozygotes, presumably due to higher rates of nondisjunction and perhaps male-sterility. Clearly a large series of hybrids needs to be screened to test this hypothesis. The one adult male double Robertsonian heterozygote with monobrachial homology so far examined was apparently fertile and revealed no prophase I-metaphase I abnormalities.

1 I thank Dr C.E. Ford F.R.S.., Prof. F.W. Robertson and Dr A.E. Douglas for reading earlier drafts of this paper, Prof. F.W. Robertson, Dr J.R.K. Savage and Dr J.R. Clarke for laboratory facilities, Mrs J.E. Evans for technical assistance and S.E.R.C. for financial support.
2 Present address: Department of Agricultural Science, University of Oxford, Parks Road, Oxford OX1 3PF (England).
3 Searle, J.B., Syst. Zool., in press.
4 Fredga, K., and Narwin, J., Chromosomes today *6* (1977) 153.
5 Volobouev, V.T., personal communication.
6 Searle, J.B., Lab. Animals, in press.
7 Evans, E.P., personal communication.
8 Sumner, A.T., Evans, H.J., and Buckland, R.A., Nature New Biol. *232* (1971) 31.
9 Seabright, M., Lancet *2* (1971) 971.
10 Evans, E.P., Breckon, G., and Ford, C.E., Cytogenetics *3* (1964) 289.
11 Wahrman, J., and Goitein, R., Chromosomes today *3* (1972) 228.
12 Hall, W.P., and Selander, R.K., Evolution *27* (1973) 226.
13 Hewitt, G.M., and Barton, N.H., Symp. R. ent. Soc. Lond. *10* (1980) 149.
14 Lécher, P., Archs Zool. exp. Génet. *108* (1967) 633.
15 Smith, S.G., and Virkki, N., Animal Cytogenetics, vol.3, Insecta, chapter 5, Coleoptera. Borntraeger, Berlin 1978.
16 Gropp, A., Winking, H., Redi, C., Capanna, E., Britton-Davidian, J., and Noack, G., Cytogenet. Cell Genet. *34* (1982) 67.
17 Gropp, A., and Winking, H., Symp. zool. Soc. Lond. *47* (1981) 141.
18 White, B.J., Crandall, C., Raveché, E.S., and Tjio, J.-H., Cytogenet. Cell Genet. *21* (1978) 113.
19 Gropp, A., Kolbus, U., and Giers, D., Cytogenet. Cell Genet. *14* (1975) 42.

0014-4754/84/080876-03$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1984

Chromatin of *h* regions of human chromosomes at high resolution

R.S. Verma*, J. Rodriguez and H. Dosik

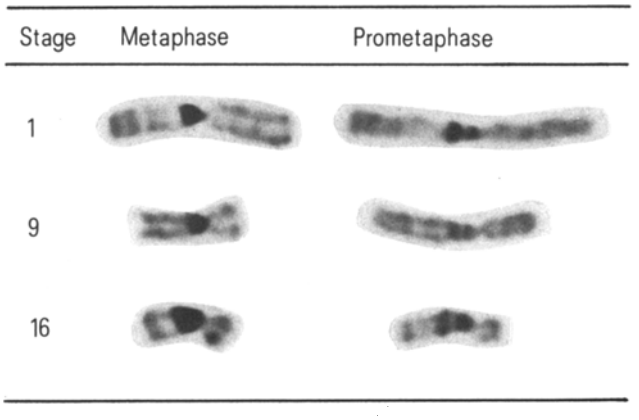
Departments of Laboratories and Medicine, The Jewish Hospital and Medical Center of Brooklyn and The State University of New York Downstate Medical Center, Brooklyn (New York 11238, USA), 29 June 1983

Summary. Segmentation of the secondary constriction region (*h*) of human chromosomes 1, 9 and 16 is demonstrated by a high resolution banding technique. Based on these staining properties, it is suggested that the composition of the *h* region in human chromosomes is heterochromatic as well as euchromatic.

Many structural and functional aspects of human chromosomes have been studied by various banding techniques. The CBG-technique has been used to reveal constitutive heterochromatic material[1,2]. This material is present in the centromere, the secondary constriction regions (*h*) of chromosomes 1, 9 and 16 and the distal portion of the long arm of the Y chromosome. The relative amount of satellite DNA in the C-bands of chromosomes 1, 9 and 16 shows no correlation with the size of the bands[3]. Instead there is a considerable amount of repetitive DNA[4]. It has been shown by the CBG technique that the h regions of chromosomes 1, 9 and 16 are most variable, yet are stable and inherited[5].

Recently, an international system for nomenclature of bands at high resolution has been established[6]. In this system the *h* region has been shown to vary only in size and it is represented by only 1 band. We have used a high-resolution banding technique to demonstrate the further segmentation of *h* regions not noted by ISCN[6]. Further we report that the *h* region is not only heterochromatic (dark staining) but euchromatic (light staining) as well when examined by a high resolution banding technique.

Materials and methods. Chromosome preparations from several individuals were made from cultured peripheral blood lymphocytes that were harvested as usual[7]. First, the CBG technique was carried out as described by Sumner[2] with a few modifications[8] using metaphase chromosomes. There were 3 individuals who had enlarged secondary constriction regions *h* on chromosomes 1, 9 and 16. Therefore the peripheral blood lymphocytes of these individuals were recultured for high resolution banding as follows: Immediately after completion of 68–72 h of incubation, cultures were treated with 5-bromo-2-deoxyuridine (200 µg/ml; Sigma) and 0.3 µg/ml of thymidine (Sigma) for exactly 4.5 h. Prior to harvest, cultures were



Demonstration of segmentation of the regions in chromosomes 1,9 and 16 at prometaphase by the CBG technique; such segmentation is not seen when chromosomes are at metaphase. There is a considerable amount of information lost during photography and printing. However, differentiation of the *h* region is clear.

treated for an additional half hour with colcemid (0.03 µg/ml; Gibco)[9]. The rest of the technique for harvesting of the cells is standard. The CBG technique was performed on prometaphases and the cells were photographed on Kodak technical Pan film No. 2415 using a Zeiss Photomicroscope II.

Results and discussion. Differentiation of secondary constriction regions *h* by the CBG-technique in chromosomes 1, 9 and 16 is shown in the figure. When chromosomes were C-banded at metaphase such differentiation was never seen. However, at prometaphase or prophase the *h* regions differentiate into light and dark bands. These observations suggest that the *h* regions are composed of euchromatin (light staining areas) as well as heterochromatin (dark staining areas) which suggests the presence of different satellite DNAs in these regions. Some understanding about the properties of the *h* regions has been obtained recently by the use of BrdU in conjunction with fluorescent dyes such as DAPI[10]. It is believed that the heterochromatin of the region is late replicating[10]. However, the present observations suggests that the whole region does not replicate at the same time as it differentiates into light and dark bands. Furthermore, the definition of constitutive heterochromatin in man by C-banding needs further investigation. Although a possible clinical significance of the heteromorphisms of *h* regions has been suggested, no serious attempt has been made to conduct the study in a systematic fashion. Perhaps the present approach might lead to some conclusion regarding variability of staining properties of the *h* region with respect to clinical consequences.

* Reprint requests to R.S. Verma, Division of Cytogenetics, Interfaith Medical Center, 555 Prospect Place, Brooklyn, N.Y. 11238, USA.

1 Pardue, M.L., and Gall, J.G., Science *168* (1970) 1356.
2 Arrighi, F.E., and Hsu, T.C., Cytogenetics *10* (1971) 81.
3 Sumner, A., Expl Cell. Res. *75* (1972) 304.
4 Marx, K.A., Allen, J.R., and Hearst, J.E., Biochim. biophys. Acta *425* (1976) 129.
5 Verma, R.S., Ved Brat, S., and Dosik, H., J. Hered. *73* (1982) 74.
6 ISCN, An International System for Human Cytogenetic Nomenclature, Birth Defects. Original Article Series, vol. XIV, No. 8. The National Foundation, New York 1978.
7 Verma, R.S., Rubenstein, C., and Dosik, H., In Vitro *13* (1977) 806.
8 Rubenstein, C.T., Verma, R.S., and Dosik, H., Hum. Genet. *40* (1978) 279.
9 Shah, J.V., Verma, R.S., Rodriguez, J., and Dosik, H., J. med. Genet. *20* (1983) 452.
10 Lin, M.S., and Alfi, O.S., Cytogenet. Cell Genet. *21* (1978) 243.

0014-4754/84/080878-02$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1984

Teratogenic effects of methylnitrosourea on pregnant mice before implantation

I.K. Takeuchi

Department of Embryology, Institute for Developmental Research, Aichi Prefectural Colony, Kasugai, Aichi 480-03 (Japan),
11 October 1983

Summary. Methylnitrosourea at a dose of 10 mg/kg is teratogenic when applied to pregnant mice on gestational days 2.5, 3.5 or 4.5, but has no such effects on gestational days 0.5 and 1.5.

In teratological studies, it has generally been considered that treatment of pregnant animals with teratogenic agents during their preimplantation period results either in embryonic death or intact live fetuses, but no specific malformations[1,2]. However, some agents such as X-rays[3,4] cyclophosphamide, nitrogen mustard, thalidomide[5], triparanol[6], leucine[7], actinomycin D[8], aminoacetonitril[9], and a synthetic analog of 3β-hydroxysteroid dehydrogenase[10] have been shown to yield malformed fetuses when they are given to pregnant animals before implantation. Napalkov and Alexandrov[11], and also Tamaki et al.[12], have briefly described a few fetuses malformed by application of methylnitrosourea (MNU) or ethylnitrosourea (ENU) to pregnant rats in the preimplantation period. The teratogenic effects of MNU treatment of pregnant mice during their gestational days before implantation are investigated in this study.

Materials and methods. Virgin *slc:* ICR mice were housed with males of the same strain overnight, and the presence of a vaginal plug following mating was considered as marking day 0 of gestation. MNU (Nakarai) was dissolved in distilled water just before injection, and applied i.p. to mice at a concentration of 10 mg/kg from 13.00 to 13.30 h on gestational days 0 (day 0.5), 1 (day 1.5), 2 (day 2.5), 3 (3.5) and 4 (day 4.5), or 5 mg/kg and 1 mg/kg on gestational days 2.5, 3.5 and 4.5. Each mouse was injected with 0.05 ml/g of MNU solution. Mice given 0.05 ml/g distilled water and untreated mice were used for the controls.

On the morning of gestational day 18, the animals were killed by over-anesthesia, and the number and position of live and dead fetuses were noted. Dead fetuses were subdivided into early deaths (fetuses completely resorbed) and late deaths (fetuses remain). Live fetuses were sexed, weighed, and examined for external malformations. For the statistical analysis, the litter rather than the fetus was taken as the experimental unit, according to the recommendation of Haseman and Hogan[13]. The fetal responses were presented as the average for each litter within each group in the accompanying table 1. Except for mean fetal body weight, the fetal responses in each group were examined using Willcoxon's rank sum test. Student's t-test was used for the analysis of mean fetal body weight.

Results and discussion. No significant differences were observed in the number of implantation sites between all the MNU-trated groups and the control groups (table 1), indicating that the preimplantation mouse embryos in the MNU-treated mothers can survive at least until implantation. Spielmann and Eibs[14] also reported that treatment of pregnant rats with cyclophosphamide during the preimplantation period did not influence the number of implantation sites. Iannaccone et al.[15] reported that the mouse blastocysts which had been treated in vitro with MNU could implant to the same degree as the untreated blastocysts after transferring into the uteri of pseudopregnant mice.

The mean fetal death rates were significantly increased in the groups treated with 10 mg/kg MNU on gestational days 0.5, 2.5, 3.5 or 4.5, and with 5 mg/kg MNU on gestational days 2.5, 3.5 or 4.5, but in the 1 mg/kg MNU-treated groups only that in the group treated on gestational day 4.5 was signi-