The analogy reported between the diabetes of BB rats and type I human diabetes allows one to predict that similar perturbations might exist in human diabetic livers.

Acknowledgments. The authors wish to thank Prof. Assan and C. Laborit for having provided BB rats. This work was supported by a grant from CNRS URA 022.

* To whom all correspondence should be addressed.

1 Schwartz, A. L., CRC Crit. Rev. Biochem. *16* (1984) 207.
2 Dodeur, M., Durand, D., Dumont, J. P., Durand, G., Feger, J., and Agneray, J., Eur. J. Biochem. *123* (1982) 383.
3 Dodeur, M., Coumoul, S., Durand, D., Durand, G., Feger, J., and Agneray, J., Biochem. biophys. Res. Commun. *115* (1983) 86.
4 Nakhooda, A. F., Like, A. A., Chappel, C. I., Murray, F. T., and Marliss, E. B., Diabetes *26* (1977) 100.
5 Marliss, E. B., Nakhooda, A. F., Poussier, P., and Sima, A. A. F., Diabetologia *22* (1982) 225.
6 Strom, S. C., Jirtle, R. L., Jones, R. S., Novicki, D. L., Rosenberg, M. R., Novotny, A., Irons, G., McLain, J. R., and Michalopoulos, G., JNCI *68* (1982) 771.
7 Scarmato, P., Feger, J., Dodeur, M., Durand, G., and Agneray, J., Biochim. biophys. Acta *843* (1985) 8.
8 Munson, J., and Rodbard, D., Analyt. Chem. *107* (1980) 220.
9 Nassar, K., Cheng, S., and Levy, D., Exp. Cell Res. *132* (1981) 99.
10 Jefferson, L. S., Diabetes *29* (1980) 487.

0014-4754/89/050480-03$1.50 + 0.20/0

Colcemid effects on homologue pairing and crossing over during fetal mouse oogenesis

G. Jagiello, W. K. Sung, J.-S. Fang and M. B. Ducayen

Departments of Obstetrics and Gynecology, Human Genetics and Development, and Center for Reproductive Sciences of the International Institute for the Study of Human Reproduction, College of Physicians and Surgeons of Columbia University, 630 West 168th Street, New York (New York 10032, USA)
Received 29 November 1988; accepted 2 February 1989

Summary. Colcemid was administered to gestational day 13 female mice to test effects on homologue pairing, synapsis and recombination of fetal oogenesis. Pairing abnormalities were detected in pachytene oocytes by light and electron microscopy examination of bivalents and synaptonemal complexes. Reduction of total chiasmata per treated diplotene oocyte (22.74) compared to controls (31.07) was found.
Key words. Colcemid; meiosis; recombination; oogenesis; nondisjunction.

The organization of events responsible for the accurate pairing of homologous chromosomes and the molecular mechanisms of genetic recombination, particularly in mammals, is not fully understood[1]. Levan in 1939[2], reported that colchicine disrupted prophase in *Allium* and affected crossing over by mechanisms which appeared to be distinct from the well established microtubule inhibitory effects. Subsequent studies attempted to separate out effects on spatial pairing, synapsis and recombination[3,4]. Loidl[5] has recently reported delay or prevention of synapsis at leptotene and defective pairing initiation at zygotene in colchicine-treated *Allium ursinium*. Studies with the pachytene synaptonemal complexes of mouse oocytes and spermatocytes from inversion heterozygotes have suggested that vinblastine and nocodazole, two colchicine-like compounds, revealed specific autosomal and sex bivalent pairing abnormalities[6]. Khawaja and Ellis[7] have also described a colchicine-induced heritable double recessive desynaptic mutation of *Lathyrus*. The present experiments examined effects of demecolcine on pachytene chromomere maps and synaptonemal complexes and diplotene chiasmata frequency in mouse oocytes as measures of perturbation of pairing and recombination during early oogenesis.

Materials and methods. Dated gestations were obtained in female Swiss mice (CAMM) by mating proestrous 2–4-month-old females with 4-month-old males of known fertility. Day of vaginal plug was designated day 0 of gestation. Demecolcine (Colcemid, Sigma) was injected as a single dose of 0.2 µg/g of b. wt i.p. to groups of 3 mothers on gestational day 13 when preleptotene oocytes are maximal[8]. Animals were sacrificed on day 17 for examination of pachytene and diplotene oocytes. Fifteen controls were injected with an equal volume of diluent. Sixteen treated pregnant females provided 89 female feti and 178 fetal ovaries were immediately dissected into balanced salt solution. Control females yielded 74 feti and 148 ovaries by the same method. Each ovary was divided into three portions for preparation of oocytes for cytogenetic examination and microscopy of synaptonemal complexes. Pachytene oocyte chromomere maps were prepared by the method of Jagiello and Fang[9], and diplotene oocyte chromomere patterns by the method of Jagiello and Fang[10]. Final identification of pachytene and diplotene bivalents was made by visualizing each bivalent of each cell with a 100X Planapo light and phase objective and simultaneously observing the drawings and photomicrographs (fig. 1 a, b). Chiasmata were identified

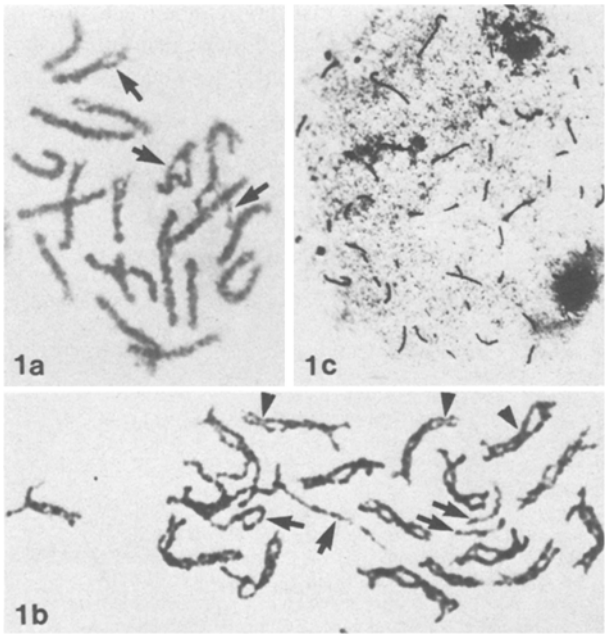

Figure 1. Cytogenetic preparations of treated oocytes: *a* Pachytene oocyte with multiple unpaired regions (arrows) ×1100; *b* Diplotene oocyte with four univalents (arrows), chiasmata (arrow heads) ×1200. *c* Synaptonemal complexes (light microscopy): Multiple unpaired regions ×1100.

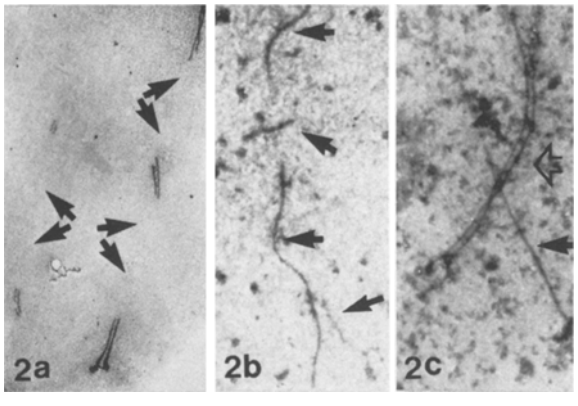

Figure 2. Synaptonemal complexes (electron microscopy) of treated oocytes: *a* Multiple pairing failure (arrows) ×2600; *b* Four unpaired single elements (arrows) ×1400; *c* Normal (open arrow) and single element (arrow) ×1000.

on diplotene bivalents of oocytes using a 100X Planapo lens and a Zeiss VG-9 green or an interference green filter (fig. 1 b). The method of Sung and Jagiello [11] was used to prepare pachytene oocytes for light (fig. 1 c) and electron microscopy of synaptonemal complexes (fig. 2 a–c). Modification of the method of Baker and Franchi [12] for obtaining thin section standard electron micrographs of meiotic oocytes was used and nuclear stages were identified using the criteria of Hertig and Barton [13]. A second series of experiments was done to test for a dose response effect on diplotene chiasmata number using a concentration of 0.2–1.0 µg of Colcemid/g maternal body weight in the protocol noted above. Scoring of coded preparations for all experiments was done by two observers.

Results and discussion. The litter size (12–14) and sex ratio (1:1.5) of treated animals was not different from controls. No gross phenotypic abnormalities were seen in treated feti.

202 complete pachytene oocyte chromomere maps from control ovaries were identical with previously published data [9]. 252 complete pachytene oocytes from treated feti were analyzed for chromomere patterns and pairing of bivalents. The major and minor chromomere patterns were not altered as compared with the control pachytene map. Of the treated oocytes 44% showed abnormal bivalent pairing patterns which varied from a single unpaired region on one or many bivalents to multiple unpaired regions on many bivalents (fig. 1 a). 56% were normal. Total failure of pairing was observed in an occasional oocyte.

Light microscopic examination of whole mount spreads of synaptonemal complexes of 209 treated cells revealed 48% normal oocytes, 14% with interstitial unpaired lateral elements, 7% with terminal unpaired lateral elements, and 31% with multiple pairing failures in register (fig. 1 c). Synaptonemal complexes from 107 control cells prepared by the identical method contained these alterations in $< 1\%$ as seen in a previously published study [11].

Electron microscopic examination of whole mount synaptonemal complexes of 122 treated oocytes showed multiple pairing failures in several bivalents of a nucleus (fig. 2 a), fragmented complexes without visible elemental structures and cells with unpaired single synaptonemal complex elements (fig. 2 b, c). These findings were seen in 4 of 82 untreated oocytes at day 17 of gestation, a figure similar to previous data [11]. Transmission electron microscopy of thin sections from 12 oocytes demonstrated absence of the central element in unpaired treated oocytes, but normal attachment plaques were found at the nuclear membrane (data not shown).

Analysis of the total chiasma number in diplotene oocytes indicated that the disruption of pairing and synaptonemal complex formation noted at pachytene had apparently interfered with recombination as represented by diplotene chiasma frequencies. In 109 treated diplotene oocytes, all with normal chromomere maps, an average of 22.74 chiasmata per genome were found as compared with 31.07 per genome in the control oocytes. This difference was significant at the $p < 0.001$ level

Dose (µg/g b. wt)	Diplotene oocytes (n)	Chiasmata (mean/cell)
0.0	57	29.75
0.2	73	24.70
0.4	54	25.39
0.6	123	24.97
0.8	59	22.80

(t-test). In addition, occasional achiasmate bivalents and univalents were found at diplotene (fig. 1 b).

The table presents the diplotene chiasma counts obtained from the second series in which the pregnant mouse was treated with 0–0.8 μg/g b.wt of Colcemid. It can be seen that while a significant reduction of chiasmata ($p < 0.001$) was induced in fetal diplotene oocytes at all doses tested, no dose response was achieved in the range 0.2–0.8. A dose of 1 μg/g b.wt was toxic to oogenesis.

The present data obtained by treating gestational day 13 mouse fetal ovaries address possible sequelae from affected preleptotene oocytes. The results closely parallel findings of Shepard in colchicine-treated *L. speciosum*[14] of pachytene univalents, pairing gaps, and synaptonemal complexes with lateral elements and absent central elements. These authors reported a reduced diplotene chiasma frequency as did Puertas et al.[15] who studied premeiotically-treated *Secale cereale*. The chiasmata response in mouse oocytes to increments of Colcemid was consonant with a threshold effect on multiple recombinant sites rather than a true dose response of a population of susceptible sites. Identification of treated bivalents which are consistently unpaired in a non-random fashion may shed some light on the question of mechanisms of Colcemid action on chiasma formation and be found to relate to the findings of Toledo[4] that larger chromosomes resist total pairing disruption. The mechanism of Colcemid action on pairing and sequelae in mouse oogenesis and other species remains unknown and provocative.

Acknowledgments. This work was supported by National Institutes of Child Health and Human Development Grant No. HD 10037. The authors are grateful to Dr Amy Jonassen for technical assistance. Portions of these data were presented in Abstract 1454, American Cell Biology Meetings, Atlanta, Georgia, 1985.

1 Holliday, R., Phil. Trans. Roy. Soc. Lond. *B277* (1977) 359.
2 Levan, A., Hereditas *25* (1939) 9.
3 Bennett, M. D., Toledo, L. A., and Stern, H., Chromosoma *72* (1979) 175.
4 Toledo, L. A., Bennett, M. D., and Stern, H., Chromosoma *72* (1979) 157.
5 Loidl, J., Exp. Cell Res. *178* (1988) 93.
6 Gibson, J. B., and Moses, M. J., Genetics suppl. *113* (1986) s66.
7 Khawaja, H. I. T., and Ellis, J. R., Genome *29* (1987) 859.
8 Nogawa, T., Sung, W. K., Jagiello, G., and Bowne, W., J. Morph. *195* (1988) 225.
9 Jagiello, G., and Fang, J. S., Chromosoma *77* (1980) 113.
10 Jagiello, G., and Fang, J. S., Cytologia *52* (1987) 125.
11 Sung, W. K., and Jagiello, G. M., Can. J. genet. Cytol. *24* (1982) 675.
12 Baker, T. G., and Franchi, L. L., J. Cell Sci. *2* (1967) 213.
13 Hertig, A. T., and Barton, B. R., Female reproductive system, part I, in: Handbook of Physiology, Section 7, Endocrinology, vol. II, pp. 317–348. Williams and Wilkins, Baltimore, Maryland 1973.
14 Shepard, J., Boothroyd, R., and Stern, H., Chromosoma *74* (1974) 423.
15 Puertas, M. J., de la Pena Estades, B., and Merino, F., Chromosoma *89* (1984) 121.

0014-4754/89/050482-03$1.50 + 0.20/0

Effects of ethanol and acetaldehyde on cultured pre-implantation mouse embryos

G. W. Kalmus and C. C. Buckenmaier III

Department of Biology, East Carolina University, Greenville (North Carolina 27858, USA)
Received 24 August 1988; accepted 16 February 1989

Summary. Pre-implantation 2-cell stage mouse embryos, obtained from superovulated CF-1 mice, were exposed to ethanol and acetaldehyde through the culture medium for 60 min followed by a 105-h incubation period. Control and ethanol exposed embryos survived equally well in ethanol concentrations as high as 800 mg/100 ml medium and acetaldehyde levels up to 10 mg/100 ml medium.
Key words. Fetal alcohol syndrome; ethanol; acetaldehyde; pre-implantation mouse embryos.

Fetal alcohol syndrome (FAS) is a pattern of altered morphogenesis and function in infants born in chronic alcoholic mothers[1]. Numerous investigators have been able to demonstrate FAS-related effects in post-implantation rodent embryos exposed to ethanol both in vivo and in vitro[2,3]. The role of acetaldehyde, ethanol's primary metabolite, in producing the teratogenic effects seen in FAS is still under study. O'Shea and Kaufman[4,5] have found in their work with mice that acetaldehyde appears to parallel ethanol in its teratogenic effects on the growing post-implantation embryo. This work has been disputed by other workers. Blakely and Scott[6] failed to find acetaldehyde extremely teratogenic to post-implantation mouse embryos. They propose this conflict in results may be due to the different mouse strains used. Priscott[7] reported that rat fetuses in vitro were only affected at concentrations of 800 μM acetaldehyde, which he considers unrealistically high.

To date, almost all work using the rodent model has been performed on post-implantation (7-day-old or older) embryos. Little is known about the possible effects ethanol and acetaldehyde may have on pre-implantation embryos. Pennington et al.[8] found that a single dose of ethanol, 8 h after conception, suppressed growth in the pre-implantation embryos in vivo. Though this work is the first to recognize the effects of ethanol in the pre-im-