

during the reciprocal translocational may have carried a locus modifying recombination, thus the intact Y will prevent crossing-over. This may be further verified by the presence of a banding pattern found on the Y-chromosome of a wild-type strain with a normal XY complement in the male⁸.

Sullivan⁴ indicated that recombination, in test crosses, of heterozygous males may be due to factors (nondisjunction) other than crossing-over. Hiroyoshi⁷ agrees with Sullivan⁴, while Tsukamoto considers that crossing-over does not occur in the male housefly. The high recombination frequencies found in the test crosses involving the heterozygous *KIN* males are most readily explained as being due to crossing-over. Furthermore, these high values, plus the fact that autosome II was the autosome exhibiting recombination in the male, indicate that a reevaluation of recombination in male houseflies is required, involving a more

detailed study of the occurrence of recombination for autosomes I, IV and V. It now seems inadequate to base the system of recombination in male houseflies on the model of the *Drosophila* male.

- 1 Present address: Department of Zoology, Hebrew University of Jerusalem, Jerusalem, Israel.
- 2 R. Milani, in: Genetics of Insect Vectors of Disease, p.315. Ed. J.W. Wright and R. Pal. Elsevier; Amsterdam 1967.
- 3 I. C. McDonald, Can. J. Genet. Cytol. 12, 860 (1970).
- 4 R. L. Sullivan, J. Hered. 52, 282 (1961).
- 5 D.S. Lester, R.H. Crozier and E. Shipp, Experientia 35, 172 (1979).
- 6 D.E. Wagoner, Nature 223, 187 (1969).
- 7 T. Hiroyoshi, Genetics 50, 373 (1964).
- 8 D.S. Lester, R.H. Crozier and E. Shipp, Experientia 35, 174 (1979).

Chromosome aberrations in in vitro irradiated lymphocytes from human cord blood

D.C. Lloyd and E.J. Reeder¹

National Radiological Protection Board, Harwell, Didcot, Oxon OX11 0RQ (England), 7 July 1978

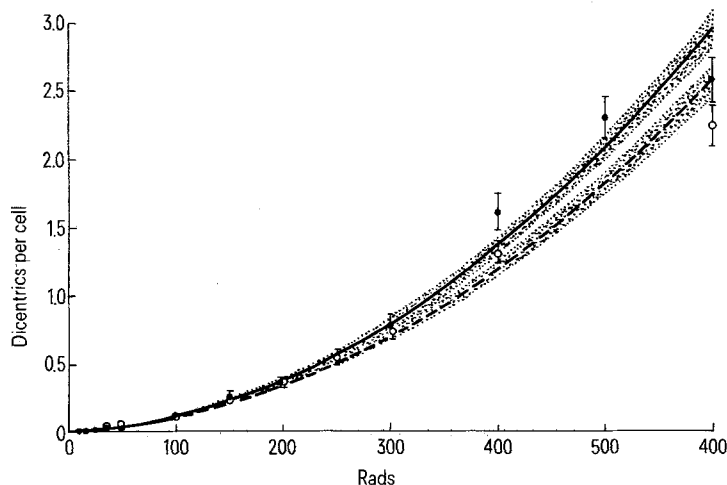
Summary. An in vitro dose effect curve of dicentric chromosome aberrations in human cord blood lymphocytes has been obtained for 250-kV X-rays. This is compared with a curve prepared in an identical manner using blood from adults. The comparison shows a marginally higher dicentric yield in blood of newborns at doses above about 250 rads.

There is a suggestion in the literature that when a peripheral blood sample from a child (<1 year) is irradiated in vitro the induced yield of dicentric chromosome aberrations is significantly higher than when the same dose is given to adult blood. The evidence is somewhat contradictory and comes from 3 studies²⁻⁴ of interdonor variability using samples from people ranging in age from newborns (cord blood) to 100 years. Each investigation used a single radiation dose and none examined the dose effect relationship for young children's blood in more detail.

In the present paper a dose response curve for dicentric chromosome aberrations is presented for cord blood and is compared with a curve previously produced in this laboratory using adult's blood.

Materials and methods. Blood samples from the umbilical cords of 12 healthy babies were received into 10 ml sterile heparin tubes (Stayne) in which they were exposed to X-radiation. The samples were taken following normal pregnancies in which no drugs or radiation were received

by the mothers and the births were full term and spontaneous. The blood was irradiated at 37 °C with various doses (see table 1) of 250-kV X-rays HVL 1.2 mm copper, at a dose rate of 100 rad per min. After irradiation the lymphocytes were separated and cultured for 48 h in a mixture containing 4 ml Eagle's basal medium (Wellcome), 1 ml bovine serum (Difco), 0.15 ml reconstituted phytohemagglutinin (Wellcome) and the buffy coat derived from 1 ml of blood. After 45 h 0.1 ml of colcemid (200 µg/ml) (Ciba) was added. Microscope slides prepared from cultures by a standard technique were stained in orcein (Harleco) and analyzed for dicentric, centric ring and acentric chromosome aberrations. Full details of the methods for culturing and fixing cells and of the criteria used in aberration analysis have been published elsewhere⁵. The aberration yields for adult blood used in the present comparison have been published elsewhere⁶. Apart from not being performed at the same time the exposure and culture conditions were identical. We have considerable



Dicentric yield plotted against radiation dose for adult blood (open symbols and dashed line) and cord blood (solid symbols and line) and fitted to the function $Y = aD + \beta D^2$. The shaded areas indicate 1 SE.

Table 1. Unstable chromosome aberration yields in human cord blood irradiated in vitro with 250-kV X-rays at 100 rad per min

Donor Sex	Dose (rad)	Cells scored	Dicentrics	Centric rings	Acentrics	Dicentrics per cell
1 M	10	2000	10	0	10	0.005
2 F	15	1000	10	0	7	0.010
3 M	25	1500	18	1	16	0.012
4 F	35	750	20	0	10	0.027
5 F	50	800	25	0	27	0.031
6 F	100	400	51	0	29	0.128
7 F	150	500	132	7	64	0.264
8 M	200	300	111	3	67	0.370
9 M	300	200	157	3	116	0.785
10 M	400	100	161	1	90	1.610
11 F	500	100	230	9	136	2.300
12 F	600	100	257	6	156	2.570

evidence (unpublished) spanning several years that the dose effect relationship for adult blood is reproducible when the same experimental conditions are observed.

Results and discussion. The aberration yields from the cord blood are given in table 1, and similar data for the adult blood have been tabulated by Lloyd et al.⁶. The yields were fitted to the equation $Y = aD + \beta D^2$ by a least squares fit using the Polyfit computer program of Edwards and Dennis⁷. This fit assigned weights to the data points assuming that the observed aberration yields carried Poisson errors. The resultant yield coefficients for dicentric and acentric aberrations are given in table 2. The coefficients for the adult blood vary slightly from those previously published. This is because in the present work only adult data up to 600 rad were considered so that the dose range was the same as that used for the cord blood. In comparing the coefficients in table 2 there is certainly no significant difference between the values of a for either dicentrics or acentrics but with the β -terms the differences are approximately 2 SE.

This variation is also reflected in the figure where the dose response curves for dicentric aberrations have been drawn. At doses below about 250 rad there is no significant difference between the 2 curves but at higher doses where the β -term in the yield equation predominates the curve for cord blood is elevated so that at 600 rad it indicates a dicentric yield $15\% \pm 6\%$ above that for adult blood. Where data points exist for both curves at the same dose it is interesting that at the 3 lower doses 10, 25 and 50 rad the cord blood dicentric yields are lower whilst at 4 higher doses, 100, 300, 400 and 600 rad, they are higher than the adult blood yields. At 600 rad there is a possibility that the linear quadratic model may break down due to saturation. However this is common to both sets of data where the points fall significantly below the fitted curves.

An elevated dicentric yield in irradiated blood from children has been demonstrated twice previously^{2,3}, although neither group found a similar effect for acentric aberrations. Bochkov and Pilosova⁸ have reported an increased ($14\% \pm 6\%$) aberration yield for 100-rad gamma rays in blood from newborns compared with adults although they did not distinguish between the various aberration types. Liniecki et al.² irradiated blood of 10 newborns to 300 rad of X-rays and obtained a mean level for dicentrics per cell of 0.96 ± 0.03 whilst the mean for 33 other subjects, teenagers and adults, was 0.81 ± 0.02 . By contrast Adams⁴ also using 300 rad of X-rays found no elevated yield in the blood of 5 newborns (0.81 ± 0.04 dicentrics per cell) when compared with 0.80 ± 0.01 in 47 adults. Sasaki and Tonomura³ using 160 rad of cobalt-60 gamma rays obtained a far larger disparity with the dicentric-plus-ring yield in neonate blood being about 70% higher and falling over the first year to a constant level for children > 1 year and adults. This effect was mirrored at higher dicentric yields in

Table 2. Values of the coefficients a and β obtained by fitting the aberration yields to dose in the quadratic function $Y = aD + \beta D^2$

	Coefficient a $\pm SD \times 10^{-4}$	Coefficient β $\pm SD \times 10^{-6}$
Dicentrics		
Cord	3.83 ± 0.86	7.58 ± 0.46
Adult	4.63 ± 0.54	6.36 ± 0.33
Acentrics		
Cord	2.35 ± 0.69	4.54 ± 0.31
Adult	3.76 ± 1.32	3.17 ± 0.65

a study reported in the same paper of patients with Down's syndrome.

Although Liniecki et al.² used a different dose and a slightly different quality of radiation it is difficult to reconcile the magnitude of their effect with that reported by Sasaki and Tonomura³ for normal subjects. Similarly, the present data do not support the findings of Sasaki and Tonomura for there is no significant difference between the adult and cord blood dose response data at 160 rad. However, at 300 rad the dose response curve for cord blood is $12\% \pm 5\%$ higher than for adults. This is in accord with Liniecki's et al. finding where the dicentric yield for cord blood was elevated by $18\% \pm 4\%$.

Conclusion. The present work therefore adds support to the possibility that at doses of a few hundred rad the in vitro sensitivity of neonate blood to the induction of dicentric chromosome aberrations is marginally higher than adult blood. However the data are not sufficient to reject the hypothesis that there is no difference in sensitivity.

- 1 Acknowledgment. We wish to thank Miss P. Orledge of the John Radcliffe Hospital, Oxford, for samples of cord blood, Mr M.J. Corp of the MRC Radiobiology Unit, Harwell, for irradiating the samples and Mr A.A. Edwards of NRPB, for help with statistics. The work was partly supported by Euratom Contract No. 171-76-1 BIO UK.
- 2 J. Liniecki, A. Bajerska and C. Andryszek, *Int. J. Radiat. Biol.* **19**, 349 (1971).
- 3 M.S. Sasaki and A. Tonomura, *Jap. J. Human Genet.* **14**, 81 (1969).
- 4 A.C. Adams, Ph. D. thesis, University of Aberdeen 1970.
- 5 R.J. Purrott and D.C. Lloyd, *The Study of Chromosome Aberration Yield in Human Lymphocytes as an Indicator of Radiation Dose 1*. Harwell: U.K. National Radiological Protection Board, NRPB-R2 (1972).
- 6 D.C. Lloyd, R.J. Purrott, G.W. Dolphin, D. Bolton, A.A. Edwards and M.J. Corp, *Int. J. Radiat. Biol.* **26**, 75 (1975).
- 7 A.A. Edwards and J.A. Dennis, *Polyfit - A Computer Program for Fitting a Specified Degree of Polynomial to Data Points*. Harwell: U.K. National Radiological Protection Board, NRPB-M11 (1973).
- 8 N.P. Bochkov and R.A. Pilosova, *Genetika* **4**, 144 (1968).