X-RAY INDUCED CHROMOSOME DAMAGE IN MAN.

Evidence that X-rays and other types of nuclear and allied radiation cause chromosome damage has been forthcoming from many years and has accumulated from the study of a variety of plant and animal tissues (Lea 1956). From these studies it had been generally assumed that the production of chromosome damage in man was an important mechanism in the establishment of the acute clinical effects of radiation exposure, and possibly also in the causation of such delayed effects as the induction of leukaemia (Court-Brown and Doll, 1960).

As far as direct observations on human tissues are concerned, Fliedner and his colleagues (1959), using simple squash preparations of bone marrow, noted evidence of chromosome 'stickiness', clumping of chromosomes and anaphase bridge formation in a number of persons accidentally exposed to a mixed neutron-gamma ray beam. Bender (1957), Peak and his colleagues (1957) Chu and Giles (1959) have studied the effects of radiation exposure in vitro on human tissue culture preparations. Only recently, however, has it become possible to undertake serial studies of chromosome changes in the directly irradiated human being. This present communication reports the results of such studies in two patients given x-ray treatment for ankylosing spondylitis.

TECHNICAL CONSIDERATIONS AND RESULTS.

The chromosome preparations were made from blood cultures using an adaptation of the technique of Hungerford and his colleagues (1959), the final spreading of the chromosomes being achieved by drying in air. With this technique a count of the chromosomes in 205 cells from six normal subjects showed 190 cells (92.68%) to have 46 chromosomes (Table 1). This proportion of modal cells did not differ significantly from that in a study of 489 cells from

sixteen patients suspected of having chromosome abnormalities but who were found to have apparently normal karyotypes (Table 2). 449 cells had a count of 46 chromosomes (91.8%) and variations from this modal number are considered due mainly to artefacts arising during preparation. Court-Brown, Jacobs and Doll (1960) have discussed the causes of variation from the modal number in chromosome counts/

.....counts made on bone marrow preparations. The improvement in the quality of the preparations, which has followed the introduction of the present technique, is evident from the finding that in 1602 cells from bone marrow preparations only 85.02% had a count of 46 chromosomes. A comparison of the count distributions in the pre-exposure blood samples from the two patients cited below with those in Tables 1 and 2, shows these distributions to be within the normal range of values for the present technique.

The two male patients studied were given X-ray treatment for ankylosing spondylitis. The first patient received treatment to the whole length of his spine and to his sacro-iliac joints between 18/7/60 and 29/7/60, a total skin dose of 1500 rads being given in 10 equal fractions. In the second patient, the effect of a single dose of X-rays, 250 rads to the skin over the spinal column alone given on 10/8/60, was studied over a period of ten days by serial blood cultures. The field arrangements and the X-ray physical characteristics are shown for both patients in Fig. 1A and 1B.

Cells were selected for counting provided that all the small chromosomes (Nos. 13 to 23 and the Y chromosome in the Denver Classification) were easily identified, and in every selected cell these were analysed. It will be seen from Tables 3 and 4 that polyploid cells were noted in most of the preparations. These polyploid cells are not included in the chromosome counts as they can be recognised without a precise count being made. For purposes of comparison, however, an approximate measure of the frequency of polyploids is indicated by the fact that only two were noted during the study of the 694 cells listed in Tables 1 and 2. The last technical point to be mentioned is that one of the common effects of radiation was the production of chromosome

fragments/

.....fragments, and in counting the chromosomes we have adopted the convention of scoring a fragment as a whole chromosome. Thus a cell with 46 chromosomes and a fragment would be recorded as as having 47 chromosomes.

The effect of X-ray exposure on the chromosome constitution of each patient is shown in Table 3 (the first patient) and in Table 4 (the second patient).

DISCUSSION.

The data from the first patient show clearly that a heavy accumulated dose of X-rays produces considerable chromosome damage. This is shown both in significant changes in the count distribution and in a considerable increase in the numbers of cells carrying chromosomes with structural abnormalities. These latter are of many types and no attempt will be made to describe them in this preliminary communication; it is worth noting, however, that chromosome fragments are common and that complex abnormalities such as ring chromosomes and dicentrics are seen (Figs. 2 and 3).

The data from the second patient are more informative. A single X-ray exposure was given on 10/8/60, and twentyfour hours later two significant changes had occurred. At this time the percentage of cells with 46 chromosomes had fallen from 93 to 72, and that of cells with structurally abnormal chromosomes had risen from 1 to 22. The fall in the modal cells was due to a remarkable increase in the number of cells with 47 chromosomes, which rose from 1% to 21%. During the five days from 12/8/60 until 17/8/60 six blood cultures were set up but only four were successful, and of the latter, those of 12/8/60 and 13/8/60 were of poor quality. This partial failure itself may be associated with some effect of irradiation. The unusually low count of modal cells was still present four days after exposure, and on the fourth day the percentage of cells with chromosome structural abnormalities reached a maximum.

.....By the fifth day, however, the percentage of modal cells had returned to within normal limits, and remained so, for the rest of the period of observation. Also the fifth day marked a decline in the number of cells with structural abnormalities, but even on the last two days of observation these were present in 9 to 10% of the cells.

In both patients unusual numbers of polyploid cells were seen. Perhaps the most striking increase was that occurring twentyfour hours after the X-ray exposure of the second patient. At this time 11 polyploids were noted during the finding and counting of 100 cells with diploid or near-diploid numbers.

Findings similar to those reported in this communication have also been noted by us in cases of chronic myeloid leukaemia following X-ray treatment. These will be discussed in a separate communication (Baikie et al, in preparation).

In conclusion, the data indicate, as might be expected, that X-rays readily produce chromosome damage which can be detected in cultures of human blood cells. Much more work will be necessary to achieve a fuller understanding of the pattern of the changes, particularly, the effect of varying such physical parameters of dose as the total dose and the dose-rate. The extent of the damage produced by a single dose of 250 rads, limited to the skin over the spinal column, gives grounds for hoping that the relationship between radiation dose and the extent of chromosome damage in man may be evaluated by direct observation over a wide range of dose.

We are indebted to Professor R. McWhirter and his staff for access to patients under their care, and to Miss M. Brunton and Miss S. Woodcock for technical assistance.

Isabel M. Brough. B.Sc. St. And.
Karin E. Buckton. B.Sc. St. And.
A.G. Baikie. M.B. Glasg. M.R.C.P.E.
W.M. Court-Brown, M.B. B.Sc. St. And.

M.R.C. Clinical Effects of Radiation
Research Unit,
Western General Hospital,
EDINBURGH. 4.

REFERENCES

BENDER, M.A. (1957) Science. 126. 974.

CHU, E.N.Y. GILES, N.H. (1959) Genetics. 44. 503.

COURT-BROWN, W.M. DOLL, R. (1960) in Modern Trends in Occupational Health. Butterworth & Co. Ld. LONDON.

COURT-BROWN, W.M. JACOBS, P.A. DOLL, R. (1960) LANCET. 1. 160.

FLIEDNER, T.M. CRONKITE, E.P. BOND, V.P. RUBINI, J.R. ANDREW, G. (1959) Acta Haemat. 22. 65.

HUNGERFORD, D.A. DONNELLY, A.J. NOWELL, P.C. BECK, S. (1959) Amer. J. hum Genet. 11.215.

LEA, D.E. (1956) Actions of Radiations on Living Cells. Cambridge University Press.

PUCK, T.T. MORKOVIN, D. MARCUS, P.I. CIECURA, S.T. (1957) J. exp. Med. 106. 485.

TABLE 1. CHROMOSOME COUNT DISTRIBUTIONS - SIX NORMAL SUBJECTS.

No.		CHROMOSOME COUNTS.							Total Cells counted.
		◄ 44	44	45	46	47	48	► 48	
B. 135	Male	-	-	2	32	-	-	-	34
B. 199	"	-	1	4	29	2	-	-	36
B. 210	"	-	-	1	30	-	-	-	31
B. 136	Female	-	-	4	39	-	-	-	43
B. 163	"	-	-	-	30	-	-	-	30
B. 198	"	-	-	1	30	-	-	-	31
	Totals	-	1	12	190	2	-	-	205
	Percent.	-	0.49	5.85	92.68	0.98	-		100.00

TABLE 2. CHROMOSOME COUNT DISTRIBUTIONS - SIXTEEN PATIENTS with NORMAL KARYOTYPES.

Chromosome counts

No.	Sex	Diagnosis.	≤44	44	45	46	47	48	>48	Total Cells counted.
B.151	M	Arthrogryposis	-	1	-	29	-	-	-	30
B.157	M	Anophthalmia	-	-	-	31	2	-	-	33
B.168	F	Spastic Diplegia	-	-	1	29	-	-	-	30
B.169	M	Pelger - Huet Anomaly	-	-	3	28	-	-	-	31
B.170	F	Pelger - Huet Anomaly	-	-	2	27	1	-	-	30
B.176	M	Hydrocephalus	-	1	3	26	1	-	-	31
B.179	M	Infertility	-	1	1	29	-	-	-	31
B.181	M	Infertility	-	-	3	28	-	-	-	31
B.182	M	Infertility	-	-	1	29	-	-	-	30
B.189	F	Primary Amenorrhoea	-	-	-	27	1	-	-	28
B.190	F	Testicular Feminisation	-	-	4	26	1	-	-	31
B.194	M	? Marfan's Syndrome	-	1	1	26	1	1	-	30
B.195	M	? Fanconi's Anaemia	-	-	1	30	-	-	-	31
B.196	M	? Mongolism	-	-	2	29	1	-	-	32
B.201	F	Primary Amenorrhoea	-	-	1	28	1	-	-	30
B.214	F	Primary Amenorrhoea	-	-	2	27	1	-	-	30
		Totals	-	4	25	449	10	1	-	489
		Per Cent.		0.89	5.11	91.82	2.04	0.20		100.06

TABLE 3. CHROMOSOME DATA from the FIRST PATIENT.

	CHROMOSOME COUNTS. 44	44	45	46	47	48	48	Total cells counted.	% Modal Cells.	% Cells with Chromosome Structural Abnormalities. Modal Cells	All cells.	Polyploids
18/7/60 (pre-exposure)	1	-	3	44	2	-	-	50	88	0	2	0
22/7/60	1	1	7	33	6	2	-	50	66	18	40	0
2/8/60	-	-	7	37	2	1	3	50	74	19	34	11
8/8/60	1	-	5	34	7	1	2	50	68	9	32	7
12/8/60	-	-	5	43	1	1	-	50	86	16	18	3

The changes in the proportion of modal and non-modal cells found on 22/7/60, 2/8/60 and 12/8/60 by comparison with the proportion in the control blood of 18/7/60 are highly significant. The value of X^2 and P (n-1) are respectively 24.92 and <0.001, 9.29 and <0.01, 18.96 and <0.001.

TABLE 4. CHROMOSOME DATA from the SECOND PATIENT.

	CHROMOSOME COUNTS.							Total Cells counted.	% Modal Cells	% Cells with Chromosome Structural Abnormalities.		Polyploids Tetraploids
	< 44	44	45	46	47	48	> 48			Modal Cells	All Cells	
10/8/60 pre-exposure)	-	-	5	93	1	-	1	100	93	0	1	1
11/8/60	1	1	4	72	21	1	-	100	72	7	22	11
12/8/60	1	-	3	27	4	-	-	35	77	18	23	1
13/8/60	-	-	1	19	2	3	2	27	70	27	41	0
15/8/60	-	-	2	90	6	1	1	100	90	11	16	3
17/8/60	-	-	3	93	2	1	1	100	93	6	11	0
19/8/60	-	2	3	89	4	-	2	100	89	4	10	6
21/8/60	-	-	1	90	9	-	-	100	90	2	9	8

The changes in the proportion of modal and non-modal cells found on 11/8/60 with the proportion in the control blood of 10/8/60 are highly significant. respectively 67.74 and <0.001, 13.52 and <0.001, 21.24 and <0.001.

12/8/60 and 13/8/60 by comparison
The values of 2 and P (n-1) are