described imbalances in R_F and R_E can be used. If one uses different types of chromatographic matrices and conditions, it should be possible to purify proteins, antibiotics, or other classes of compounds, and to execute the separations on the basis of different properties such as size, charge, hydrophobicity, or chemical affinity.

Another important feature of these methods is that good separation can be obtained even when multiple components focus on the same column. As a consequence of the conditions establishing an equilibrium zone, such multiple components will actually form distinct equilibrium zones stacked one on top of the other. Furthermore, a column that contains a continuous gradient (that is, two matrices mixed in continuously varying proportions) can be used to generate many distinct equilibrium positions.

At present, I project the potential of this technology as follows. Because of the experimental tests that are required to define the equilibrium conditions for any particular solute, the most likely use of CACE will be for the purification of materials that have already been characterized, and analytical applications will, at first, be limited. Although practical preparative purifications by CACE have not yet been examined, the measurements reported here indicate that the capacity and resolution of this method are as good as or better than those obtained in many of the established separation methods. Thus, with refinement, this technology could have major applications in a wide range of preparative fractionations.

PATRICK H. O'FARRELL
Department of Biochemistry and Biophysics, University of California, San Francisco 94143

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On the Nature of a Defect in Cells from Individuals with Ataxia-Telangiectasia

Abstract. *The cells and tissues of patients with ataxia-telangiectasia (A-T), an inherited disease characterized by a high degree of proneness to cancer, are abnormally sensitive to ionizing radiation. Noncycling cultures of normal human and A-T fibroblasts were exposed to x-rays so that the breakage and rejoining of prematurely condensed chromosomes in the G_1 phase could be compared. After a dose of 6.0 grays, both cell types had the same initial frequency of breaks and the same rate for rejoining of the breaks, but the fraction of breaks that did not rejoin was five to six times greater for the A-T cells. The results also show that progression of cells into the S phase is not a prerequisite for the increased frequency of chromosome fragments that appear in mitosis after A-T cells are irradiated in the G_1 or G_0 phase.*

Patients with the genetically inherited disease ataxia-telangiectasia (A-T) display various clinical disorders, including proneness to cancer, and their cells and tissues are abnormally sensitive to ionizing radiation (*1–4*). Although a number of suggestions have been made to account for the A-T defect (*2, 3, 5*), there is still no satisfactory explanation for it. Because the production of acentric chromosome fragments is perhaps the principal cause of cell reproductive death after exposure to ionizing radiation (*6, 7*), it is possible that differences in either the fragility of chromatin or the capacity for repairing its initial damage (or both) might account for the different sensitivities of normal and A-T cells to radiation. To test this possibility we compared the initial frequencies of breaks in the prematurely condensed (*8–10*) G_1 chromosomes of noncycling cells from confluent cultures of A-T and normal human fibroblasts (*11*); we also compared the rejoining of these breaks as a function of incubation time after an x-ray dose of 6.0 grays (Gy; 1 Gy is equivalent to 1 J of energy absorbed per kilogram) (Fig. 1).

The net number of prematurely condensed chromosome (PCC) fragments per cell, which is equivalent to the number of breaks per cell, was obtained by subtracting the average number of PCC's in unirradiated cells from the number of PCC's and their fragments in irradiated cells. The pooled data for the A-T cell line AT5BI and for the normal cell line AG1522 were each fitted to an equation of the form $Y = Ae^{-ct} + B$, where Y is the net number of fragments after a post-irradiation incubation time t, c is the rate constant for the rejoining of fragments, B is the net number of fragments remaining after a very long incubation (breaks that do not rejoin), and A is the number of initial breaks that take part in rejoining (*12*) (Table 1). The results from two different ataxia cell lines (AT5BI and GM2052) were in good agreement, as were those from two different normal human cell lines (AG1522 and AG6234).

These data show that both the initial number of breaks in the G_1 PCC's and the rate at which broken fragments rejoined were the same for both cell types. There was no significant difference in the curve intercepts for zero incubation time ($A + B$), or in the value of c. By the criterion measured in this assay system, chromatin from A-T cells is not more susceptible to breakage by x-rays than chromatin from normal cells. However, a larger proportion of the chromosome fragments in the A-T cells did not rejoin (27 percent for the AT5BI cells compared to about 5 percent for the normal AG1522 cells). Further, in these contact-inhibited cultures the increased residual damage in A-T cells was unrelated to their progression into the S phase. The half-times for the chromosome rejoining process were 1.56 ± 0.14 hours and 1.70 ± 0.13 hours for the A-T and normal cell lines, respectively (mean $\pm$ standard error of the mean). These values are consistent with the range of half-times (1 to 2 hours) for the rejoining of double-stranded breaks (dsb's) in DNA (*13–15*) and with the observation that the rate of rejoining is the same for A-T and normal cells (*14, 15*). The proportion of unrejoined chromosome breaks for A-T and normal cells is also similar to the proportion of unrejoined DNA breaks in hypersensitive and wild-type rodent cell lines (*16*).

Table 1. Parameters of the best-fit curve of the form $Y = Ae^{-ct} + B$ for A-T cell line AT5BI and normal cell line AG1522 (data from Fig. 1); S.D., standard deviation.

Cell line	Curve parameters ± S.D.			Curve intercepts $(A + B)^*$ ± S.D.	Points fitted (No.)
	A^*	B^*	c (hours^{-1})		
AT5BI	27.44 ± 1.77	9.64 ± 1.65	0.443 ± 0.041	37.08 ± 3.03	17
AG1522	32.11 ± 2.15	1.66 ± 1.65	0.408 ± 0.032	33.77 ± 3.29	25

*Net fragments per cell.

If acentric chromosome fragments are the principal lesion responsible for cell death induced by x-irradiation (*6, 7*), the proportion of cell killing from the type of residual damage we have measured after longer incubation times might correspond to all or part of a nonrepairing component sometimes associated with the initial slope of an x-ray survival curve (*17*). This is because asymmetric exchanges and their accompanying acentric fragments, which would contribute an increasingly greater proportion of lethal damage at higher doses of x-rays, were not visible by our method of measurement. Only acentric fragments from terminal or interstitial deletions, and rings, were detectable because they represented a discrete additional piece of stained chromatin.

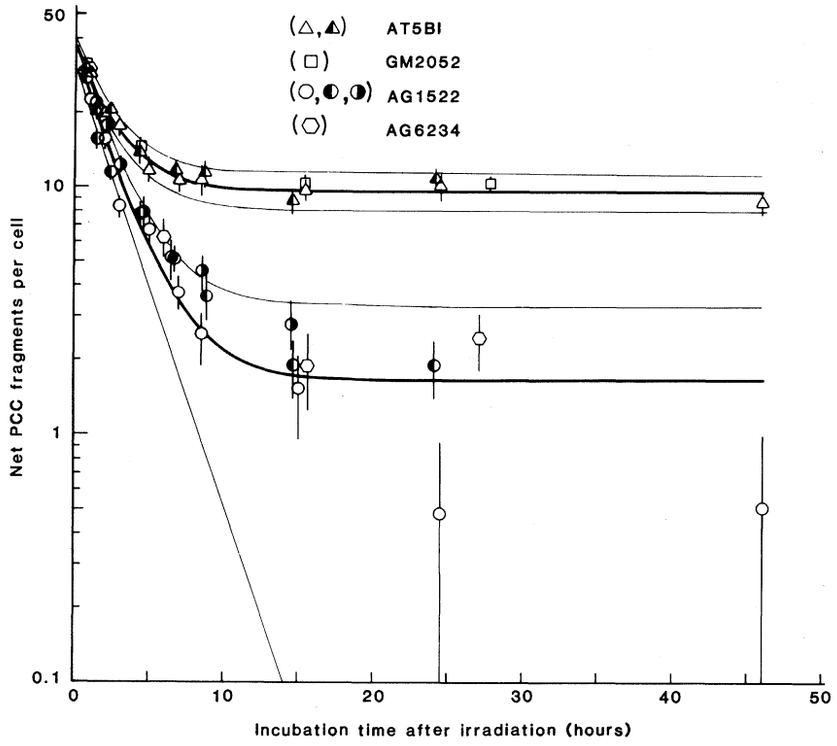

Fig. 1. The net number of PCC fragments per cell as a function of time after x-irradiation for cultured human fibroblasts from patients with ataxia-telangiectasia and from normal individuals. Density-inhibited cultures were given 6.0 Gy of x-rays generated at 280 kV (dose rate, 0.55 Gy/min; half-value layer, 0.3 mm of copper). Cultures were then allowed to incubate at 37°C for various lengths of time before being trypsinized and fused with mitotic HeLa cells to induce PCC (*8–10*). Autoradiographs of parallel cultures showed 1 to 4 percent labeled cells when tritiated thymidine (1.0 μCi/ml; specific activity, 80 Ci/m*M*) was added to the growth medium for 26 hours, indicating that an appreciable number of cells had not cycled through the S phase and reached the G_1 phase when samples were prepared for scoring. The fusion mixture contained approximately 10^6 mitotic HeLa cells together with 5×10^5 AG1522, AG6234, GM2052, or AT5BI cells and 150 hemagglutinating units per milliliter of Sendai virus (inactivated by ultraviolet radiation) (*9*). At least 20 cells were scored for each sample. The estimates of net fragments per cell were obtained by subtracting the average number of PCC's per cell in controls from that in irradiated cultures; vertical bars indicate the standard error of the difference between these two means. Dark lines represent curves for the best-fit parameters to the equation $Y = Ae^{-ct} + B$ for AT5BI and AG1522 cells only (*12*). The lighter lines show 68 percent confidence envelopes around each best fit. Two separate experiments were performed with AT5BI cells (from an 18-year-old male A-T patient), one with GM2052 cells (from a 15-year-old female A-T patient), three with AG1522 cells (from a 3-day-old male normal subject), and one with AG6234 cells (from a 15-year-old male normal subject).

We measured survival of cells over a range of x-ray doses for both the AT5BI and AG1522 cells. Cultures were irradiated in nondividing monolayers (plateau phase) and plated for the survival assay 24 hours later. Thus, all G_1 chromosome breaks capable of rejoining were given the opportunity to do so. Survival curves fitted to the data yielded estimates for the initial slope of $-1/4.2$ Gy^{-1} for the AG1522 cells and $-1/0.7$ Gy^{-1} for the AT5BI cells. Under the terms of the hypothesis, a dose of 6.0 Gy would be expected to yield 6.0/4.2 or 1.43 unrejoined breaks and 6.0/0.7 or 8.6 unrejoined breaks for the AG1522 and AT5BI cells, respectively. In view of the uncertainties, these values are in good agreement with our estimates of 1.66 and 9.64 unrejoined breaks for these two cell types.

The nature of the defect that results in hypersensitivity of ataxia cells to x-rays is controversial, even in its most superficial form. Paterson and co-workers (*3*) have attributed the hypersensitivity of A-T cells to a defective system of repair for different kinds of radiochemical lesions in DNA. Painter (*5*) has suggested that the defect may be associated with a difference in chromatin structure. The importance of chromatin structure in sensitivity to x-rays has been studied and discussed by several investigators (*5, 18*).

On the basis of studies of chromosomal aberrations induced by x-rays, Taylor (*2*) deduced that 1 to 3 percent of the dsb's in DNA produced initially do not rejoin in A-T cells compared to about 0.3 percent in normal cells. The number of chromosome breaks we observed in PCC's immediately after irradiation was only about 15 percent of the number of random dsb's in DNA produced initially (*13*). This result suggests that either DNA double-stranded breaks and chromosome breaks are functionally unrelated or that some feature of chromatin structure prevents the expression of about 85 percent of dsb's in DNA as breaks in PCC's. In the latter case, since the number of PCC fragments decreases as a function of incubation time and approaches the number of fragments seen after cells reach mitosis, it is unlikely that the structural state of chromatin harboring this large proportion of dsb's in DNA is a serious impediment to its rejoining. Ultimately, it may even facilitate it. Complete rejoining of dsb's in 85 or 90 percent of the chromatin, taken together with our estimates of 27 percent (A-T cells) and 5 percent (normal cells) being nonrejoinable in the remaining 10 to 15 percent, would be consistent with Tay-

lor's deductions (*2*). In this connection, it is also interesting that condensed and transcriptionally inactive heterochromatin constitutes about 90 percent of the chromatin in a typical mammalian cell (*19, 20*) and that virtually all break points induced by x-irradiation in mitotic chromosomes appear in their G–light-banded regions (*21*).

Normal cells irradiated with x-rays undergo a delay in the initiation of DNA synthesis, but A-T cells do not (*22–24*). It has been suggested that the delay occurs because initiation of DNA synthesis cannot take place while damaged DNA is undergoing repair and that the chromatin structure of A-T cells presents itself as a suboptimal substrate for such repair (*5, 23*). Our experiments with noncycling cells show that progression into the *S* phase is not a prerequisite for the increased frequency of chromosome fragments that appear in mitosis after ataxia cells are irradiated in the G_1 or G_0 phase (*2, 24*).

MICHAEL N. CORNFORTH
JOEL S. BEDFORD
Department of Radiology and Radiation Biology, Colorado State University, Fort Collins 80523

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12. Curves of the form $Y_T = Ae^{-ct} + Be^{-dt}$ were also fitted to the gross data (total PCC's plus fragments) for each experiment. In no case was the value of d significantly different from zero. For this reason, and because Y_T (the total number of PCC's plus fragments) would not be expected to decrease below that for unirradiated cells, even after very long incubation times, the exponent of the second term was set at zero and the net frequencies of fragments, Y (weighted according to the gross data), were fitted to the simplified expression.
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Diploid-Triploid Mosaicism: An Unusual Phenomenon in Side-Necked Turtles (*Platemys platycephala*)

Abstract. *Diploid and diploid-triploid mosaic individuals of* Platemys platycephala *were found in natural populations. In mosaic specimens, the blood, spleen, liver, and testis contained both diploid and triploid cells. The ratio of triploid to diploid cells was more variable among individuals than among somatic tissues within an individual. Only diploid cells underwent meiosis in males; haploid gametes were produced. There appears to be geographic variation for mosaicism in that only diploids were found in Bolivia, whereas diploids and diploid-triploid mosaics occurred in Surinam.*

Triploidy is rare in vertebrates but occurs as a natural condition in fishes (*1*), amphibians (*2*), and reptiles. Populations of triploid individuals are known in the lizard families Teiidae, Gekkonidae, and Agamidae (*3*), but have not been reported for any of the other major reptile groups (snakes, crocodilians, turtles, or the tuatara). Triploid vertebrate populations are invariably unisexual (all fe-

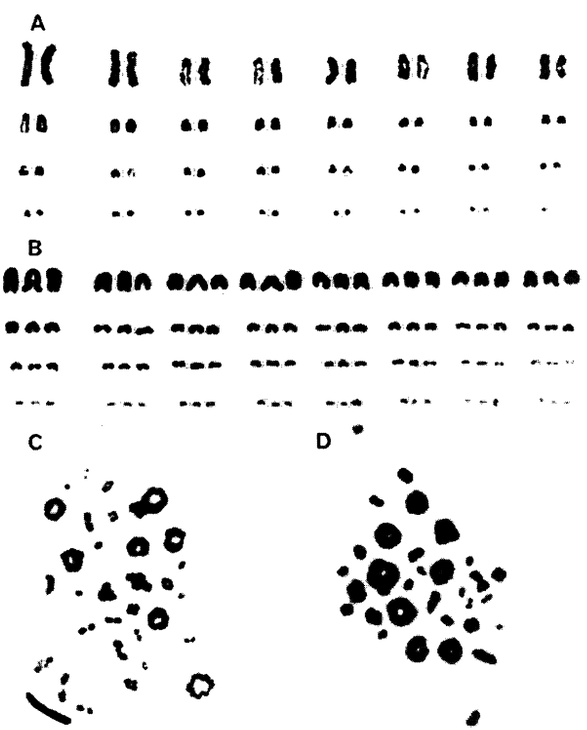

Fig. 1. Mitotic and meiotic chromosomes from *P. platycephala*. (A) Diploid ($2n = 64$) mitotic karyotype from the testis of a specimen from Bolivia. (B) Triploid ($3n = 96$) mitotic karyotype from the spleen of a diploid-triploid mosaic individual (AK1227). (C) Diakinesis in a diploid male from Bolivia with 32 bivalents. (D) Diakinesis in a diploid-triploid mosaic male (AK1227) with 32 bivalents.