however, if the cell and manometer are in a vacuum chamber (with window). A known drop in hydrostatic pressure in the chamber, brought on by a pump, will cause the bubble to expand, and the relative change in volume can be used to calculate the pressure in the bubble, regardless of its absolute volume. This calibration does not disturb the protoplasmic streaming of the cell.

The capillary method could be modified to give a method which would operate indefinitely. An oil drop could be put in an unfused capillary with one end in the cell and the other connected by a pressure valve to highly compressed gas. Changes in turgor would be matched by (recorded) changes in applied pressure needed to maintain a constant position for the drop.

Our method could be applied to large cells where the elasticity of the cytoplasm makes the methods requiring osmotic equilibrium all but impossible (for example, large cells of red algae). It also appears to be practical for measurements on cells not in osmotic equilibrium. It could thus provide data pertinent to the physics of the growth process (*7*).

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Chromosomal Breakage Induced by Extracts of Human Allogeneic Lymphocytes

Abstract. *A high frequency of chromosomal breakage and rearrangement has been found in fibroblasts treated with extracts of allogeneic human lymphocytes. The fact that these changes were not observed when extracts of autologous lymphocytes were tested indicates that mechanisms involving self-recognition are operative. Abnormalities, such as chromosome lagging and bridges, occurring in anaphase were also served.*

The increased frequency of thyroid antibodies in mothers of children with Down's syndrome could be interpreted as an indication that factors related to immunological aberrations in a parent predispose the offspring to chromosomal abnormalities such as Down's syndrome (*1*). For study of the effect of immunological or self-recognizing reactions on mitoses in vitro, fibroblasts were treated with extracts prepared from circulating human lymphocytes from either the same subjects who donated the fibroblasts (autologous) or from genetically unrelated subjects (allogeneic). In earlier studies, abnormalities in chromosomal distribution as evidenced by variability in chromosome number were noted in experiments with allogeneic extracts (*2*). We have since turned our attention to the question of whether lymphocyte extract causes more discrete chromosomal aberrations that can be scored in metaphase or anaphase.

Extracts of lymphocytes from seven subjects were tested in a total of 17 combinations of allogeneic extracts and fibroblasts (Table 1) (*3*). Three of these extracts were also tested on autologous fibroblasts (Table 2). The cultures were harvested at both 24 and 48 hours after the addition of extract. Anaphases were scored for chromosome lagging and bridges, and metaphases were scored for chromosomal breakage and rearrangements (*4*). Two kinds of breaks were scored, those in which both chromatids were broken (chromosomal breaks) and those in which a single chromatid was broken (chromatid breaks) (Fig. 1). In both instances the lesions were scored only if they were obvious (as opposed to gap lesions). The cells were further subdivided into those containing one and those having more than one broken chromosome. Included in the rearrangements are quadriradials and other complex chromosomal formations (Fig. 1). For each experimental point, a duplicate culture treated with buffer served as a control.

High frequencies of cells with broken and rearranged chromosomes were observed in all experiments with allogeneic extracts (Table 1). Extracts from the four mothers of children with Down's syndrome, each of whom had circulating thyroid antibodies, seemed to induce greater responses than those from the three subjects who lacked evidence of immunological aberrations. Approximately one-third of the abnormal cells contained more than one broken chromosome. Chromosomal rearrangements such as quadriradials were less common but occurred in up to 10 percent of the dividing cells (up to 21 percent of the abnormal cells) in some treated cultures. In the control cultures in these experiments, about 2 percent of dividing cells had one broken chromosome; only two cells with more than one broken chromosome were observed, and rearrangements were not seen.

In anaphase, 0.5 to 2.8 percent of cells in untreated, control cultures contained abnormalities. In treated cultures these frequencies increased three- to tenfold.

Extracts prepared from lymphocytes of the three subjects (Table 1, subjects 5, 12, and 13) without known immunological aberrations were tested autologously at the same time that they were tested allogeneically. All three produced changes in genetically foreign fibroblasts, but an increased frequency of chromosomal breakage in autologous fibroblasts was not observed (Table 2).

The maximum frequency of chromosomal aberrations was observed in the first mitosis following the addition of extract (24 or 48 hours). Thereafter, the frequency declined rapidly, reaching control levels 2 to 3 days later. The maximum increase in anaphase abnormalities paralleled the increase in chromosomal breakage observed in metaphase. When extract was incubated at 37°C for 3 days and then added to fibroblasts, it had only 50 percent of the activity of an equal amount of the same extract stored for 3 days at −20°C. Thus, the decline in the frequency of aberrant cells is at least partially due to inactivation of the extract at 37°C. The effect of lymphocyte extract on growth and viability was studied with duplicate cell counts made in a hemocytometer at the time of harvest immediately after treatment of cultures with trypsin. Most cultures exposed to lymphocyte extract contained 15 to 50 percent fewer cells than did untreated con-

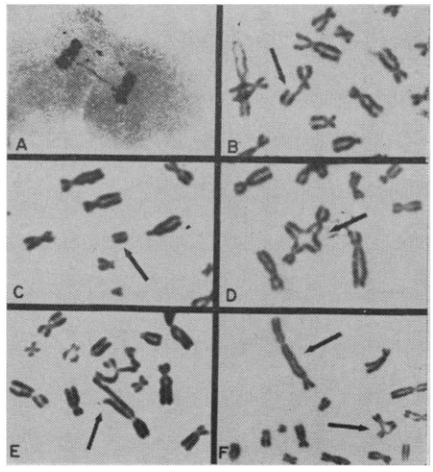

Fig. 1. Six cells showing anaphase abnormalities (A), chromosomal breaks (B, C, E), and rearrangements (D–F). (A) Bridge and lagging, (B) chromatid break, (C) chromosome break, (D) quadriradial, (E) complex rearrangement, and (F) dicentric chromosome and abnormal rearrangement.

trols. It seems likely that the lowered cell counts are related to the chromosomal aberrations. Hellström and coworkers (*5*) noted cytotoxicity in vitro when cellular extracts or intact lymphoma cells were added to tumor cells containing a foreign histocompatibility antigen. A cytopathic effect in vitro has been noted with intact sensitized lymphoid cells (*6*) and with normal, unimmunized lymphoid cells (*7*), especially if they are artificially aggregated to target cells (*8*). Conceivably cell death in these experiments could have been mediated by chromosomal changes.

In our experiments, a decrease in the number of intact cells in treated cultures was evident only in the harvest following the maximum frequency of chromosomal breakage. Shortly thereafter treated cultures contained more intact cells than control cultures did. This sequence of events may be the result of two opposing forces operating in the treated cultures—one, chromosomal aberrations favoring cell death or decreased growth and two, a nonspecific, growth-stimulating effect of cell extracts (*9*). Consequently, a decrease in cell number might be seen only after maximum breakage had occurred, but when the frequency of breaks declined, the stimulatory effect would predominate.

These experiments demonstrate that extracts prepared from human circulating lymphocytes can induce chromosomal aberrations in allogeneic fibroblasts in vitro. However, the nature of the active factor is not known, nor is it known whether it produces its effect directly or indirectly perhaps through the mediation of growth stimulation. In view of these uncertainties, the relationship of these observations to human disease remains conjectural (*10*).

Table 1. Chromosomal breakage and rearrangements induced by allogeneic lymphocyte extract in vitro. Cells were harvested at 24 and 48 hours. Subjects 1 through 4 have evidence of aberrant immunologic reactions as demonstrated by circulating thyroid antibodies, and each has a child with Down's syndrome; 5 is a 24-year-old male with treated lymphoma; and 12 and 13 are normal adult males. The skin cultures were obtained originally for biochemical studies from infant foreskins (subjects 10 and 11) or from adults (subjects 12–14). Cells (300,000 and 250,000) were seeded for 24 and 48 hours, respectively. A total of 24 cells with abnormalities were observed in the 1300 control cells analyzed. Twenty-two of these had a single broken chromosome, two had multiple breaks, and none had chromosomal rearrangements.

Extract from subject	Fibroblasts from subject	Abnormal cells (%) in first mitosis (24 or 48 hr)					Abnormal cells (%) in second mitosis (48 hr)	
		Control	Treated					
			Total	One break	>1 break	Rearrangements	Control	Treated
1	10	0	37	54	30	16	*	*
1	11	2	26	46	54	0	4	10
1	14	2	28	71	21	7	0	8
2	10	2	48	33	46	21	*	*
2	11	4	18	67	22	11	*	*
3	11	2	24	100	0	0	†	†
4	10	4	22	45	45	9	2	12
4	11	2	30	67	27	7	2	16
4	12	0	20	70	20	10	0	8
4	13	8	52	35	58	8	*	*
5	10	2	24	75	25	0	2	20
5	11	0	28	56	44	0	†	†
5	12	4	18	56	44	0	†	†
12	11	0	18	78	22	0	2	12
12	13	2	12	50	33	17	*	*
13	11	0	18	78	22	0	2	12
13	12	4	18	67	33	0	*	*

* First mitosis was observed at 48 hours; second mitosis was not studied. † First mitosis was observed at 24 hours; second mitosis was not scored.

Table 2. Chromosomal breakage and rearrangement induced by autologous lymphocyte extract in vitro compared to that obtained with allogeneic lymphocyte extract.

Extract from subject	Breaks and rearrangements (%)		
	Allogeneic*	Autologous	Not treated
5	18–24	3	4
12	12–18	4	4
13	18	2	2

* The details of these experiments are given in Table 1.

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3. Suspensions rich in human lymphocytes were prepared as previously described (*2*) and were diluted to 4×10^6 mononuclear cells per milliliter in an isotonic phosphate buffer (*p*H 7.1). We made crude extracts by rapidly freezing and thawing the cell suspensions ten times. No mycoplasmas were observed in extracts cultured for 2 weeks under both aerobic and anaerobic conditions. Extracts were added directly to freshly transferred fibroblasts in Waymouth's medium supplemented with fetal calf serum (15 percent). The fibroblasts were grown in either glass or plastic bottles, and the final concentration in the incubation mixture was that amount of extract obtained from 0.8×10^6 mononuclear cells per milliliter (2.5 ml total volume in plastic bottles, 5 ml in glass bottles). At the time of use the cultures varied in age from two to thirteen transfers.
4. The cell culture and cytological techniques used for the examination of metaphases have been described (*2*). The same procedures and concentrations of extract were used in the study of anaphases, except that the fibroblasts were grown on glass cover slips in plastic petri dishes, colchicine was omitted, and the cells were fixed and stained directly on the cover slips. For each experimental and control point, 50 to 100 metaphases that appeared diploid under low magnification were scored for chromosomal breaks and rearrangements under oil immersion. Similarly 250 to 500 anaphases were scored for each experimental and control point.
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