believed to be transmitted as an autosomal recessive trait. Parents of patients can generally be considered to be obligate heterozygous carriers of the disease. If lack of galactocerebroside β-galactosidase activity is the genetically determined defect, parents are expected to have low enzyme activity in all somatic cells and probably also in serum, because the deficiency of this enzyme in homozygous patients is systemic and not limited to the brain (*1*). Four of the six parents we examined gave, as expected, low enzyme activity. However, the two exceptions require careful evaluation.

The mother of family 1 is exceptional, because she showed normal activity of galactocerebroside β-galactosidase not only in leukocytes but possibly also in serum. She was pregnant when the blood sample was drawn. We plan to follow up the enzyme activity of this individual in order to rule out the possible effect of pregnancy or any other extraneous factors which might have affected the enzyme activity. We cannot exclude another rare possibility, germinal mosaicism, in which only the gonad carries the mutation (*8*).

The father of family 3 had low enzyme activity in serum, but his leukocytes showed normal galactocerebroside β-galactosidase activity. This may be the result of technical problems associated with leukocyte preparations. Leukocytes prepared from normal individuals in different laboratories showed significantly different enzyme activities, despite the fact that presumably the same isolation procedure was used. Unlike serum, there are a number of factors which can affect the final calculated enzyme activities of leukocytes, such as a partial blood clotting due to incomplete anticoagulation, contamination by plasma protein due to incomplete washing, incomplete elimination of red cells, errors in cell count or protein determination, or the variable proportions of granulocytes and lymphocytes.

Our study on heterozygous carriers of Tay-Sachs disease also demonstrated better reproducibility in hexosaminidase assays in serum than in leukocytes (*4*). Unlike galactosidases, hexosaminidase was stable in serum, and heterozygous carriers of Tay-Sachs disease could be detected by assaying hexosaminidase A in serum, without overlap of values with normal controls. When hexosaminidase A was assayed in leukocytes, however, there was an overlap between heterozygous carriers and normal controls, although the difference between the two groups was statistically significant.

We conclude tentatively, therefore, that, despite exceptions, the activity of galactocerebroside β-galactosidase is generally lower than normal in parents of patients with Krabbe's disease, as predicted from the assumption that the deficient activity of this enzyme is the genetically determined defect underlying globoid cell leukodystrophy. One of the two siblings of the patient in family 3 is asymptomatic but had partially deficient activity of galactocerebroside β-galactosidase both in serum and leukocytes, and we believe that this individual is heterozygous. For the detection of heterozygous carriers of the disease, serum, if available fresh, appears to give more consistent data.

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Hybrid Cell Line from a Cloned Immunoglobulin-Producing Mouse Myeloma and a Nonproducing Mouse Lymphoma

Abstract. *A hybrid cell line was established by cell fusion between a cloned Balb/c myeloma that is resistant to 8-azaguanine and produces immunoglobulin (γG and free kappa chain) and C57BL/6N lymphoma that is resistant to bromodeoxyuridine and does not produce immunoglobulins. The hybrid cells contained the membrane antigens of both parents; they synthesized free kappa chain; no synthesis of γG (γ_{2a}) heavy chain was detected.*

Recent experiments support the notion that the nucleus of the differentiated cell contains large numbers of unexpressed genes (*1, 2*). Discovery of repressors that control gene expression in bacteria has stimulated the search for evidence of similar nature, which may explain the selective gene expression observed in the differentiated cells of the higher organisms. One approach to this problem has been hybridization of two cells which differ in their expression of specific genes. Experiments along this line have provided evidence both in favor of and against the "repressor mechanism" (*3, 4*). We have examined this question with respect to immunoglobulin synthesis by hybridizing a cloned immunoglobulin G (γG; also called γ_{2a}) producing mouse myeloma and a nonproducing mouse lymphoma cell line. The hybrid cells were capable of continuous growth in culture. They produced kappa light chain but not the γG heavy chain of the immunoglobulin synthesized by the parental myeloma clone.

An RPC-5 myeloma tumor of Balb/c origin (*5*) was adapted to tissue culture and grown in Eagle's minimal essential medium supplemented with glutamine, sodium pyruvate, nonessential amino acids, penicillin, and 10 percent fetal calf serum (MEM+) (*6*). Ten clonal lines were isolated (*7*). Clone number 4 (CL4), one of the two parents in this hybridization study, produced γG and free kappa chain with no qualitative change in immunoglobulin synthesis after 2½ years of continuous culturing. The other parent, E.L.4, was a suspension culture of a lymphoma that was induced in a C57BL/6N mouse by 9,10-dimethyl-1,2-benzanthracene (*8*). The E.L.4 was maintained in MEM+ with 10 percent fetal calf serum, and did not produce any immunoglobulins. 8-Azaguanine-resistant CL4 (CL4-

Table 1. Detection of histocompatibility antigen of E.L.4 and CL4 on E.L.4 × CL4 hybrid cells by ^{51}Cr cytotoxicity assay. Three Balb/c mice (female 10 weeks old) were immunized by intraperitoneal injection of 3×10^7 E.L.4 cells. Conversely three C57BL/6N mice were similarly immunized with LSTRA lymphoid cells. The E.L.4 and LSTRA tumor cells were derived from their ascitic tumors carried in C57BL/6N and Balb/c mice, respectively.

Assay	Lysis of target cells (mean percent ± 1 S.D.)		
	E.L.4	CL4-8AZA	Hybrid E.L.4 × CL4
Immunized Balb/c anti-E.L.4 spleen cells	58 ± 5	10 ± 1	58 ± 4.4
Normal Balb/c spleen cells	6 ± 0.6	10 ± 1.2	10 ± 3.7
Immunized C57BL/6N anti-LSTRA spleen cells	6 ± 0.4	22 ± 2	38 ± 3
Normal C57BL/6N spleen cells	6 ± 0.6	11 ± 0.1	9 ± 0.6

8AZA) and bromodeoxyuridine-resistant E.L.4 (E.L.4-BUdr) were developed as described by Littlefield (*9*). The CL4 subline thus developed was resistant to 15.2 μg of 8-azaguanine per milliliter and the E.L.4 subline was resistant to 100 μg of BUdr per milliliter. The modal chromosome number of CL4 decreased from 57 to 54 after development of resistance to 8-azaguanine. However, the type of immunoglobulin synthesized did not change in this drug-resistant subline. The modal chromosome number of E.L.4 was 40 and decreased to 39 in the BUdr-resistant subline. The CL4-8AZA had two marker chromosomes: one metacentric, another submetacentric. The E.L.4-BUdr had one submetacentric marker chromosome.

Cell fusion was performed (*2*) with 1×10^7 cells of each parental cell line and 2000 hemagglutination units of Sendi virus inactivated by β-propriolactone (*10*). Cells were resuspended in HAT medium (MEM+ medium with 20 percent fetal calf serum, $1 \times 10^{-4}M$ hypoxanthine, $5 \times 10^{-7}M$ aminopterin, $2 \times 10^{-5}M$ thymidine) and incubated at 37°C (90 percent air and 10 percent CO_2). After 2 weeks in HAT medium, nonfused parental cells died and lysed. Many fused giant cells were observed. A month later, the large monolayer cells began to divide and increase in number. The original E.L.4 parent was a suspension culture, while CL4 was 85 percent suspension and 15 percent monolayer culture. The hybrid cells, however, grew as a complete monolayer. After 2.5 months in culture, the hybrid cells became more uniform in size. Doubling time of CL4-8AZA was 23 hours, and that of E.L.4-BUdr was 21 hours. However doubling time of the hybrid was 39 hours.

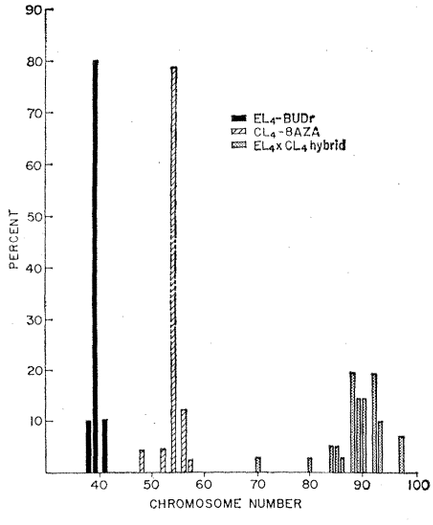

Fig. 1. Chromosome numbers for each cell type were obtained by counting 50 photomicrographs of stained chromosome preparations. E.L.4-BUdr had one submetacentric marker, while CL4-8AZA had one submetacentric and one metacentric marker chromosome. The hybrid cells had all three marker chromosomes.

Chromosome analysis 3 months after hybridization showed that the hybrid cells in general had the sum of the chromosome numbers of the respective parent lines (Fig. 1). The metacentric and submetacentric chromosomes of CL4-8AZA and the submetacentric chromosome markers of E.L.4-BUdr were present in the hybrid cells. In vitro cytotoxicity tests were performed (*11*), by ^{51}Cr release from labeled target cells. Spleens of Balb/c mice immunized against E.L.4 and spleens of C57BL/6N mice immunized against LSTRA, a Balb/c lymphoma (*12*), were used as the source of attacking cells against the hybrid and both parental cell lines. Normal Balb/c and C57BL/6N spleens were used as controls. The hybrid cells contained both of the parental cell lines' membrane antigens (Table 1).

Ouchterlony analysis of intracellular and secreted protein showed that the hybrid cells synthesized kappa chains; but no γG heavy chain was detected. No other immunoglobulins were detected in either the hybrid cells or the parental cell lines when other monospecific antiserums or when antiserum to mouse serum was used. The E.L.4-BUdr was a nonproducer, and CL4-8AZA produced γG and free kappa chain. Immunoelectrophoresis supported these findings (Fig. 2). Antiserums used were monospecific when tested against various purified mouse immunoglobulins by Ouchterlony analysis and immunoelectrophoresis (*13*, *14*).

Our experiments have demonstrated the feasibility of fusion of myeloma cells for studies of control mechanisms of immunoglobulin synthesis. Absent or markedly decreased production of γG heavy chain in hybrid cells derived from producing and nonproducing cells is consistent with the observation (*3*) that melanin production and synthesis of dopa oxidase ceased in hybrid cells of melanin-producing cells and several nonproducing cell lines. However, the synthesis of light chain in our hybrid cells is not consistent with these observations and is in agreement with the finding (*4*) that hyaluronic acid production continued in hybrids of hyaluronic acid producers and nonproducers.

Several explanations are possible for

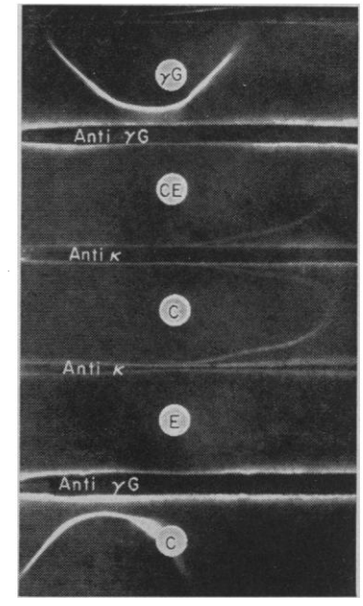

Fig. 2. Ouchterlony analysis at cell concentrations from 5×10^5 to 4×10^7 cell/ml were performed with the lysates of the three cell types. These analyses were in complete agreement with the immunoelectrophoresis results. The *γG* was from MC821 myeloma tumor purified by diethylaminoethyl cellulose chromatography (*13*); *C* is CL4-8AZA, one application of cell lysate from 1×10^6 cell/ml; *E* is E.L.4-BUdr, one application of cell lysate from 4×10^7 cell/ml; and *CE* is CL4 × E.L.4 hybrid, one application of cell lysate from 4×10^7 cell/ml. *Anti γG*, antiserum to γG; *Anti κ*, antiserum to kappa chain.

expression of light chain gene in these hybrids while the heavy chain gene is not being expressed.

1) The population of hybrid cells is made up of cells that contain the CL4 chromosome for light chain but not the heavy chain, and, perhaps, also of cells that do not contain either chromosome. The modal chromosome number in the hybrid cells was 92. This was one less than the sum of the two parent lines. However, the range was 70 to 97. Although chromosome dropout may be responsible for the lack of heavy chain synthesis in the hybrid cells, it seems rather an unlikely explanation. Of the hybrid cells, 83 percent had 88 to 97 chromosomes, while 15 percent had 80 to 86 chromosomes. Only 2 percent had 70 chromosomes. This observation implies that dropout of the heavy chain chromosome, if it occurred, has been a highly selective process. If chromosome dropout were responsible for lack of heavy chain synthesis in the hybrid cells, then cloning of these cells should show that the cells with highest chromosome number are light chain producers, whereas those with low chromosome numbers may be totally nonproducing. Furthermore, the light chain producing clones may also become nonproducing after further chromosome dropout.

2) The lack of heavy chain synthesis in the hybrid cells may be due to interference with its synthesis during either transcription or translation possibly by "repressor" or other control molecules that may have been contributed by the nonproducing E.L.4 parent line. Such an explanation would permit us to predict that clones of cells with highest chromosome numbers may be totally nonproducing, whereas those with low chromosome numbers produce light chains. In addition, further chromosome dropout from the hybrids may cause the cells to revert and become heavy chain producers.

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14. Monospecific antiserums to heavy chains were provided by Dr. Richard Asofsky.
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Repression of Interferon Action: Induced Dedifferentiation of Embryonic Cells

Abstract. *Cells cultured from young (6-day) chicken embryos differ from those of older (13-day) embryos in having a greater susceptibility to infection by certain viruses and a considerably lesser sensitivity to the action of interferon. These circumstances parallel those observed in the intact embryo. The addition of a small percentage of cells from young embryos alters the response of cells cultured from older embryos by increasing viral susceptibility sevenfold and decreasing sensitivity to interferon 25-fold. We postulate that a repressor which inhibits the expression of interferon in older embryonic cells is elaborated by cells from young embryos.*

Susceptibility to viruses decreases in general from early life to adulthood (*1*). This decrease has been thought to be due in part to maturation of specific immune mechanisms and in part to interferon (*2*). Interferon is a protein produced by appropriately stimulated vertebrate cells. It appears to be one of a group of biologically active peptides which includes hormones and bacterial colicins (*3*). The antiviral action of interferon is thought to be expressed by a newly formed polypeptide, the synthesis of which is induced by exposure to interferon (*4*). Mammalian fetuses during the latter half of pregnancy have the capacity to produce interferon (*5*). During chicken embryonic life, the progressive development of antiviral resistance, interferon production, and interferon sensitivity is manifest not only in the intact embryo but also in cells cultured from it (*6–9*). Since the chicken embryo remains relatively isolated from maternal influence and immunologically immature throughout its development, in contrast to mammalian fetuses (*10*), the roles of cellular resistance and interferon in natural age-related susceptibility to viruses can be readily studied. Using this embryonic model, we have shown that a small proportion of cells from young embryos (during the first trimester) will markedly influence a culture of predominately older cells (from embryos at the end of the second trimester) by (i) increasing viral susceptibility and (ii) decreasing interferon action. These findings suggest that young embryonic cells elaborate a substance which represses the expression of interferon such that "old" cells react to interferon like "young" cells.

Dispersed cells were cultured (*8*) from chicken embryos on day 6 and day 13 of embryonic life, a period encompassing much of the development of the reticuloendothelial system (*11*). Mixtures of cells from embryos of the two ages were then made in order to study the cellular interactions possibly involved in the changing viral susceptibility observed during embryogenesis. Cells from embryos of the two different ages were dispersed by trypsin, then counted in a hemocytometer, and, finally, mixed in the various ratios desired in growth medium (*12*). The population densities were such that either 6- or 13-day cells grown separately would form confluent cell layers in 24 hours, a time chosen to avoid further possible aging in vitro. Such cell layers were washed, and vesicular stomatitis virus (VSV) was adsorbed to replicate cultures either of unmixed 6- or 13-day cells or of mixtures of the two cell populations prepared in varying ratios. As shown in Fig. 1, fewer plaques formed in cultures of 13-day cells (mean of 11.7) than in 6-day cell populations (mean of 56.7). In cultures of mixed populations, an increasing proportion of 6-day cells resulted in