Differentiated Rat Glial Cell Strain in Tissue Culture

Abstract. *Rat glial tumors, induced by injections of* N-*nitrosomethylurea, were plated and propagated in culture. Among a few cell strains obtained, one clone contains S-100 protein, which is unique to brain in vertebrates. Stationary-phase cultures contain approximately ten times more S-100 protein per cell than exponentially growing cells. When injected into newborn rats, cells producing S-100 grew as a glial tumor, which contained S-100 protein.*

A highly acidic protein unique to the vertebrate brain has been detected in and purified from brain extracts *(1)*. This protein was named S-100 on the basis of its solubility in 100 percent saturated ammonium sulfate at neutral *p*H. About 30 moles per 100 moles of its amino acid residues are acidic amino acids. It has a molecular weight of about 30,000 *(1)*. Serologic studies have shown that S-100 protein appears to be strongly conserved among various species *(2)*. Antiserum to S-100 appears to have a detrimental effect on the transmission of nerve stimuli and causes ultrastructural changes in nervous tissue *(3)*.

A number of rat brain tumors induced by *N*-nitrosomethylurea *(4)* and consisting of more or less differentiated astrocyte-like cells *(5)* were found to contain S-100 protein. Attempts to transplant these primary tumors into new rats either intracranially or subcutaneously were rarely successful. However, if the tumors were first grown in culture before transplantation, injection of newborn rats with the cultured cells resulted in a high efficiency of tumor formation.

The method of alternate culture and animal passage was used to establish clonal strains of cells that produce S-100 *(6)*. For tissue culture, primary and transplanted tumors were plated on plastic petri dishes and incubated in a humidified atmosphere of 5 percent CO_2 and 95 percent air. The growth medium was F_{10} *(7)*, supplemented with horse serum (15 percent) and fetal calf serum (2.5 percent). For estimating S-100 in tumor cultures, we froze and thawed the washed cells several times and removed the cellular debris by centrifugation. Using microcomplement fixation *(8)*, we assayed soluble protein for S-100. The protein concentration of the extracts was determined by the method of Lowry *et al. (9)*, with bovine serum alubumin being used as a standard.

Each clonal strain was studied for its S-100 content and its ability to grow as a tumor when injected into rats. Five morphologically distinct clonal cell strains were established from these tumors. Of these five strains, only one (C-6) (Fig. 1) contains appreciable amounts of S-100. A second strain ($C\text{-}2_1$) contains traces of S-100, whereas the other three (C-3, $C\text{-}2_2$, and $C\text{-}2_8$) had no detectable S-100 protein. Approximately 10^6 cells from each strain were injected into rats. Only strains C-6, $C\text{-}2_1$, and $C\text{-}2_2$ produced tumors. Approximately 0.2 percent of the soluble protein of tumors derived from the C-6 cells was S-100, whereas tumors derived from $C\text{-}2_1$ and $C\text{-}2_2$ contained only traces of S-100 protein (0.004 and 0.001 percent, respectively).

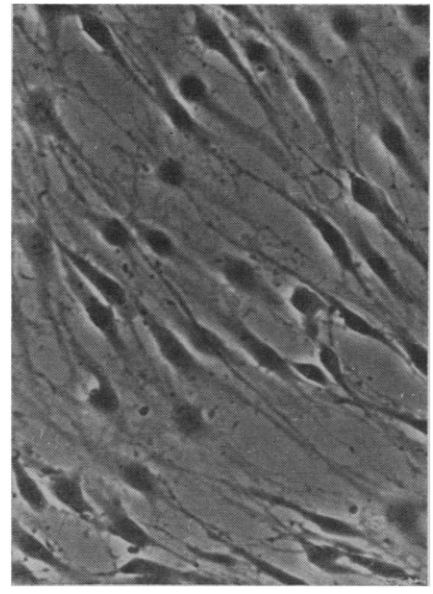

Fig. 1. Phase-contrast photomicrographs (× 96). Clonal glial cells derived from rat glial tumor, our strain C-6, which synthesizes the brain protein S-100.

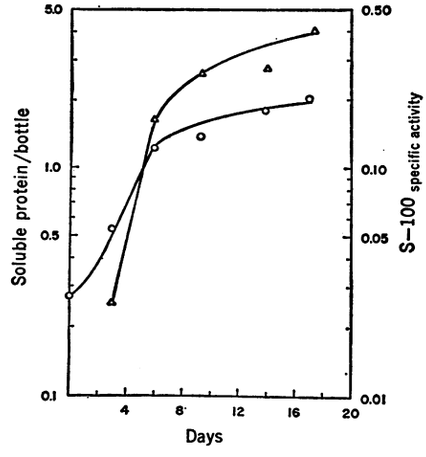

Fig. 2. Cell growth and S-100 synthesis of our clonal strain C-6. At 0 time, pooled cells were divided evenly into 20 bottles. At various times bottles were removed, and the cells were assayed for S-100 (△) and soluble protein (○). S-100 specific activity (S.A.) is expressed as micrograms of S-100 per microgram of soluble protein times 10^3. Soluble protein is expressed as milligrams per bottle.

Growth curves of the clonal strain producing S-100 (C-6) were obtained (Fig. 2). This cell line has a generation time varying from 35 to 41 hours. The ratio of S-100 to soluble protein increases approximately tenfold as the cells grow from a low cell density to confluency. When the cells reach confluency, the total amount of S-100 per cell remains constant. When cells are subcultured, the amount of S-100 per cell goes down drastically. This is not due to the effect of trypsin on the cell; this sudden decrease in S-100 per cell may be due to extremely rapid protein turnover. It is uncertain whether the increase of S-100 during the logarithmic phase of growth is due to increased synthesis of S-100 or decreased protein turnover. S-100 has been found in numerous brain tumors, both from man and other animals (with the exception of meningionas, which are intracranial tumors of mesenchymal origin).

Our experiments show that, at least in rats, serially propagated brain cells are capable of performing at least one organ-specific function over a period of a year.

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Nonrandomness of Translocations in Man: Preferential Entry of Chromosomes into 13-15/21 Translocations

Abstract. *Lymphocytes from 20 individuals with Down's syndrome due to 13-15/21 centric-fusion translocations were studied by autoradiography after continuous late labeling with tritiated thymidine. In no case was chromosome 13 involved; chromosome 14 was involved in 18 cases, and chromosome 15 in two cases. These results are similar to those from 13 previously studied cases and indicate that the entry of chromosomes 13-15 into translocations is nonrandom. This nonrandomness is not a simple function of chromosome size or shape, since chromosomes 13-15 are acrocentrics of similar size.*

Translocations in the general population and in criminal populations (*1*) nonrandomly involve acrocentric chromosomes (Nos. 13, 14, 15, 21, and 22). This tendency is also apparent in trisomy 21 (Down's) syndrome (*2*) and in trisomy 13 (D_1) syndrome (*3*). Translocations in patients with these syndromes tend to involve chromosome 21 or chromosome 13 in centric-fusion with other acrocentric chromosomes (*3*). The tendency for any chromosome to be observed in translocations can reflect either the preferential entry of that chromosome into the translocation event or selection for individuals receiving the translocation with that chromosome (or selection against individuals receiving translocations involving other chromosomes).

The present investigation was designed to provide information as to whether the entry of chromosomes 13-15 into a translocation is nonrandom. The translocation chosen for study was the 13-15/21 centric-fusion translocation in Down's syndrome. All patients studied had the same abnormal phenotype (Down's syndrome), the same chromosomal abnormality (13-15/21 centric-fusion translocation), and presumably the same chromosomal imbalance (triplication of the long arm of chromosome 21). As far as is known, loss of the short arm of chromosomes 13, 14, or 15 does not have phenotypic effects upon the individual (*4*). Differences between the frequencies of 13/21, 14/21, and 15/21 translocations in Down's syndrome should therefore reflect different tendencies on the part of chromosomes 13, 14, and 15 to enter into centric-fusion translocations with chromosome 21.

Twenty patients with Down's syndrome with 13-15/21 translocation were studied to determine the identity of the chromosome from the 13-15 group. These patients were ascertained in Washington, Oregon, Idaho, southern California, and Michigan. They included all such patients available to us. To our knowledge, the patients were not related to each other.

Peripheral blood lymphocytes were cultured for 48 to 72 hours and continuously labeled with tritiated thymidine (specific activity 2.0 c/mmole at a concentration of 1 μc/ml medium) beginning 6 hours before harvest. Well-spread metaphases were photographed before and after autoradiography with Kodak AR-10 stripping film, as described by Schmid (*5*).

Although pairs 13, 14, and 15 are morphologically very similar, they have been shown to be distinguishable by autoradiographic analysis of their DNA replication patterns (*5, 6*). Chromosomally normal cells labeled late in their DNA synthetic period have been found to show a pair of chromosomes (Nos. 13) with heavy label over the middle and distal portions of the long arms, a pair of chromosomes (Nos. 14) with heavy label over the centromere and short arms, and a pair of chromosomes (Nos. 15) with very light or no label. Individuals with Down's syndrome due to a 13-15/21 translocation have been shown to have five free chromosomes in the 13-15 group, which on autoradiography consist, as would be expected, of two pairs of chromosomes and one unpaired chromosome (*5, 7*). The results of the autoradiography are given in Table 1. In 12 cases all cells analyzed were consistent with the missing chromosome's being No. 14 and in one case with its being chromosome 15. In six cases 90 percent or more of cells were consistent with the missing chromosome's being No. 14, although in each case one or two cells showed discordant labeling patterns. The same was noted in one case with chromosome 15 missing. Similar minor discrepancies in labeling patterns have been observed by other workers (*6, 7*).

The results in our 20 patients thus indicate that chromosome 13 was not involved in any case, chromosome 14 was involved in 18 cases, and chromosome 15 in two cases (Table 1). The

Table 1. Autoradiographic analysis of 13-15/21 translocations in Down's syndrome.

Patient's laboratory No.	Parents' karyotype*		No. of Total cells scored	Unpaired chromosome						Chromosome in translocation
	Father	Mother		13	13 or 14	14	14 or 15	15	13 or 15	
171/66	T	N	11	0	0	6	5	0	0	14
255/66		N	27	0	2	8	15	1	1	14
45/67	N	N	19	0	2	8	8	1	0	14
94/67	N	N	5	0	0	4	1	0	0	14
131/67	N	T	13	0	3	5	5	0	0	14
167/67			41	0	3	15	22	0	1	14
183/67	N	N	25	2	2	17	4	0	0	14
184/67	N	N	15	0	0	9	6	0	0	14
193/67			16	0	1	8	7	0	0	14
194/67	N	N	14	0	2	2	10	0	0	14
230/67	N	N	21	0	0	1	10	9	1	15
235/67	N	N	20	0	5	8	7	0	0	14
247/67			19	0	1	10	8	0	0	14
258/67			9	0	1	8	0	0	0	14
2/68			12	0	0	10	2	0	0	14
3/68			18	0	2	7	8	1	0	14
6/68			45	0	0	0	37	8	0	15
8/68			20	1	4	5	9	1	0	14
24/68	N	N	24	0	2	18	4	0	0	14
60/68	N	N	25	0	3	15	7	0	0	14

* Key: N, normal karyotype; T, translocation carrier; blank, unstudied.