in some instances could be seen arising from the inner aspect of the fibrous capsule surrounding the plastic plate.

A separate cell line established in monolayer culture from subcutaneous tissue obtained by scraping with a scalpel became tumorigenic when inoculated in saline suspension after 84 to 98 days in vitro: no tumors developed in ten mice inoculated with 1×10^7 cells at 84 days, but one mouse out of ten developed tumors after being inoculated with 1×10^7 cells at 98 days. Thirty mice implanted with plates alone never developed tumors after more than $1\frac{1}{2}$ years of observation (the frequency of tumors induced by a flat foreign body depends on the area of its smooth surface; the size of plates we used appeared to be below that required for tumor induction).

Table 1 shows that the latent period before the appearance of tumors from the plastic-attached cells generally became shorter as time in tissue culture increased. From all of the data in Table 1, the correlation coefficient between these two variables was -0.60. After 1 to 30 days in vitro, cells produced tumors in 18 of 192 implants (9.3 percent), whereas after 3 to 4 months in vitro, cells produced tumors in 14 of 50 implants (28 percent).

In view of the close analogy between our results and those of Brand (*4*), in particular the increase in tumor frequency and decrease in tumor latent period with time spent in tissue culture, we tend to accept the hypothesis that the smooth plastic surface of the tissue culture vessel per se was acting as a carcinogen during the period spent by the cells in vitro, and that the surface of the plastic plate continued the same type of carcinogenic effect later in vivo. This hypothesis is more readily acceptable when one considers the behavior of connective tissue cells in vitro in relation to their pathophysiological role in vivo. When connective tissue cells (mostly fibroblasts and endothelial cells) are explanted into tissue culture, they react as they would if confronted in the host with an open wound in which is embedded a smooth plastic surface covered with medium containing serum: by the two programs of foreign body rejection (*4*) and of wound healing (*7*). The foreign smooth surface of the tissue culture flask stimulates them to proliferate and form a confluent "capsule" that tends to "wall off" the foreign body. From this viewpoint, the apparent "anchorage dependence" of fibroblasts cultured in vitro could be termed "foreign body stimulation," and their serum growth requirement termed "serum stimulation," since serum represents an acute emergency fluid formed after adjacent hemorrhage and clotting that contains a mitogenic factor released from blood platelets (*8*). In fact, serum produced from platelet-free plasma will not support the exponential growth of mouse fibroblasts (*9*). Thus, "spontaneous" neoplastic transformation in vitro appears to be the natural counterpart of foreign body (smooth surface) tumorigenesis in vivo, since in each case tumor cells arise from a population of connective tissue cells that are attempting to wall off a foreign surface by forming a multilayered sheet of cells. Additional evidence supporting this idea is the parallelism between the susceptibility of various species to foreign body tumorigenesis and the tendency of their cells to undergo "spontaneous" neoplastic transformation in vitro. Foreign body tumorigenesis occurs readily in mice, rats, and hamsters, but practically never in guinea pigs and humans (*4*). Correspondingly, spontaneous neoplastic transformation in vitro occurs almost universally in cultured connective tissue or embryo cells of mice and rats, and frequently in hamsters (*10*), but never in those of guinea pigs (*11*) or humans (*1*).

The detailed mechanism by which smooth surfaces are sarcomagenic for murine connective tissue has been much debated (*4*, *5*). In the case of plastic film tumorigenesis, the initial carcinogenic event occurs away from contact with the plastic; tumors arise from one to three parent pre-neoplastic cells originally located in the connective tissue capsule adjacent to the film. However, the cells of these clones must later become attached to the plastic surface before their conversion to tumor cells will occur (*4*).

It thus appears that for both smooth surface tumorigenesis and "spontaneous" neoplastic transformation in vitro, factors related to the geometry and mechanics of cell attachment to a flat surface are somehow associated with an increased frequency of genome replication errors and the consequent buildup of phenotypic variants that are capable of escaping growth control.

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Nicotiana Chromosome Coding for a Specific Polypeptide of the Small Subunit of Fraction 1 Protein

Abstract. *Fraction 1 protein has been isolated from leaves of a male sterile* Nicotiana tabacum *plant containing an extra* N. debneyi *chromosome. The extra chromosome induces appearance of a third polypeptide composing the small subunit of fraction 1 protein, which otherwise contains two polypeptides as is shown by analysis of numerous different cultivars of* N. tabacum.

A specific chromosome of *Nicotiana debneyi* has been added genetically to the chromosome complement of *N. tabacum*. This alien chromosome is transmitted to a portion of the progeny and alters the visible phenotype of plants containing it. The presence of this chromosome also results in the addition of a small subunit polypeptide of fraction 1 protein; this polypeptide has otherwise been found only in fraction 1 proteins of *N. debneyi*.

Fraction 1 protein (ribulose bisphosphate carboxylase/oxygenase) is com-

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posed of eight large subunits and eight small subunits to form a macromolecule of approximately 550,000 daltons (*1*). The polypeptides of the large and small subunits can be resolved by electrofocusing the carboxymethylated fraction 1 protein in 8*M* urea. In higher plants, genetic information coding for the primary structure of the large subunit is contained in extranuclear DNA (*2*). In contrast, coding information for the amino acid sequence of the small subunit is contained in nuclear DNA (*3*).

For the small subunit, electrofocusing has shown that it is composed of from one to four individual polypeptides of different isoelectric points, the number depending on the species of plant from which the protein was isolated (*4*). The presence of multiple small subunit polypeptides in a species of *Nicotiana* is due to gene duplication by amphiploidy and sequestering genetic information on heterologous chromosomes. This has been demonstrated in two rounds of amphiploidy which started from a cross of *N. sylvestris* × *N. tomentosiformis* giving rise to *N. tabacum* (*5*), with *N. digluta* subsequently being generated from the cross of *N. glutinosa* × *N. tabacum* (*6*). In the process, the number of small subunit polypeptides increased from one to four while the haploid chromosome number multiplied from 12 to 36. The difference in isoelectric points is correlated with four differences in amino acid composition between the two *N. tabacum* small subunit polypeptides (*7*). Segregation of the small subunit polypeptide coding information of a different order now has been found in plant material that segregates for the presence of an alien chromosome.

Sand (*8*) has created male sterile cultivars of *N. tabacum* with *N. debneyi* cytoplasm. Male sterility is manifested by abnormalities in flowers. Sand and Christoff (*9*) selected several different male sterile types based on degree of abnormality in morphology, the 1A type (*10*) being the most abnormal flower and the 4H type almost indistinguishable from normal flowers. Burns *et al.* (*11*) found the usual complement of 24 *N. tabacum* haploid chromosomes in the 1A type plants, whereas an extra chromosome containing a nucleolar organizer was present in the 4H type plants.

The 4H type plants constitute only about 4 percent of the investigated population of the male sterile *N. tabacum* cultivar with *N. debneyi* cytoplasm. Sixty male sterile plants were grown to flower and three 4H type individuals were identified among 57 of the 1A type. Crystalline fraction 1 protein (*12*) was isolated from the three 4H individuals, from several individuals of the 1A type, from *N. debneyi*, and from the male fertile cultivar of *N. tabacum* used to create and maintain the male sterile lines. The proteins were subjected to carboxymethylation in 8*M* urea and isoelectric focusing (*13*). An example of the results is shown by the photograph and densitometer tracings of the small subunit regions of the gel in Fig. 1. The *p*H gradient runs from *p*H 7 at the top to *p*H 5 at the bottom of the picture.

Since the male steriles had cytoplasm from *N. debneyi*, as was expected from previous studies (*14*), the isoelectric points of the large subunit polypeptides of the fraction 1 protein from male sterile lines are the same as those from *N. debneyi*, which are quite different from those of *N. tabacum*.

The *N. tabacum* fraction 1 protein has two small subunit polypeptides (*d*, *e*) which are completely different in isoelectric points compared to the three small subunit polypeptides (*a*, *b*, *c*) of *N. debneyi* protein. The same *d* and *e* small subunit polypeptides, somewhat reduced in staining intensity, are found in the 4H type plant but in company with a third polypeptide. The latter has an isoelectric point corresponding to polypeptide *a* in the small subunit of *N. debneyi* fraction 1 protein. It is thus highly probable that the coding information for the third polypeptide was contained on the extra *N. debneyi* chromosome present in addition to the *N. tabacum* genome in the unusual 4H type of male sterile cultivar.

Since all the male steriles and *N. debneyi* contained 30 μg of protein per sample, a semiquantitative estimate of gene dose can be made with the aid of the densitometer tracings of the staining intensity of the individual polypeptides at the bottom of Fig. 1. Considering the sum of the staining intensities of polypeptide *a*, *d* and *e* in sample 4, the amount of polypeptide *a*, judged by staining, appears to be about one-fifth of the total amount of the three polypeptides. This is consistent with the idea that polypeptide *a* in male sterile 4H type is coded by the equivalent of a single gene dose compared to a dose equivalent to four genes coding for the two *N. tabacum* type polypeptides.

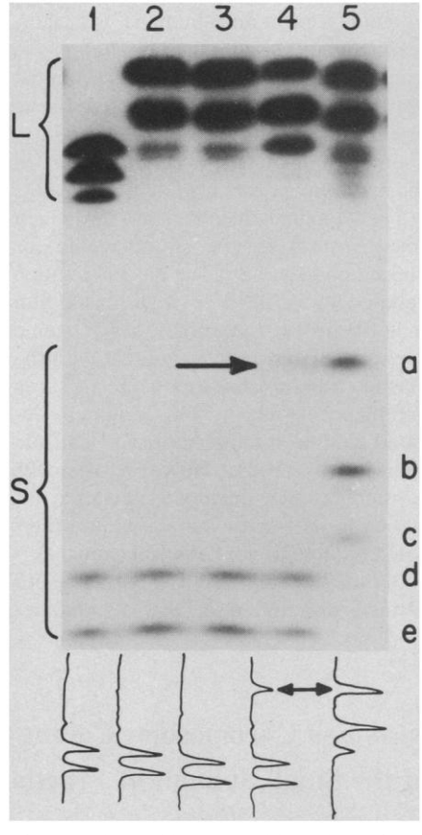

Fig. 1. Polypeptide composition of fraction 1 proteins from three (1, 2 and 3, 4) *Nicotiana tabacum* cultivars and *N. debneyi* (5). S-Carboxymethylated fraction 1 proteins were subjected to electrofocusing in a 5 percent polyacrylamide slab gel containing 1 percent ampholine, *p*H 5 to 7, and 8*M* urea; polypeptide bands were stained with bromophenol blue (*L*, large subunit; *S*, small subunit). A densitometer tracing of small subunit area is shown below. (Lane 1) *Nicotiana tabacum*, 25 μg of protein; (lanes 2 and 3) 1A type male sterile *N. tabacum*, 30 μg of protein; (lane 4) 4H type male sterile *N. tabacum*, 30 μg of protein; and (lane 5) *N. debneyi*, 30 μg of protein.

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