majority of soluble HeLa cell proteins labeled by [^{3}H]cysteine. We have previously characterized the second radioactive peak, with an elution volume to void volume ratio of 2, as MT (*10*). The third peak is composed of low-molecular-weight cysteine compounds and free cysteine eluting in the included volume of the gel.

Incubation of HeLa cells for 24 hours with $10^{-6}M$ dexamethasone caused a marked increase in incorporation of [^{3}H]cysteine into the MT peak, when compared to controls with no glucocorticoid (Fig. 1A). Dexamethasone ($10^{-6}M$) caused a sixfold increase in MT synthesis over the control (Fig. 1D). Incubation of HeLa cells with $10^{-5}M$ progesterone had no effect on the incorporation of [^{3}H]cysteine into MT (Fig. 1B). Inclusion of $10^{-5}M$ progesterone in the incubation mixture together with $10^{-6}M$ dexamethasone reduced the incorporation of [^{3}H]cysteine into MT (Fig. 1B) when compared to the large increase seen with $10^{-6}M$ dexamethasone alone (Fig. 1A). Inhibition of the response to $10^{-6}M$ dexamethasone by $10^{-5}M$ progesterone was close to 50 percent (Fig. 1D). This inhibition by progesterone suggests that dexamethasone exerts its effect by binding to the glucocorticoid receptor, since progesterone is known to compete with binding of glucocorticoids to their receptor (*13*). Cox and Ruckenstein (*7*) reported complete inhibition by $10^{-5}M$ progesterone of the stimulation of Zn^{2+} uptake achieved by $10^{-6}M$ HC. The partial inhibition observed in our experiment may be due to the stronger potency of dexamethasone as an inducer, since it is not metabolized. Both progesterone and HC are metabolized. Neither progesterone nor dexamethasone treatment had any marked effect on the incorporation of [^{3}H]cysteine into high-molecular-weight soluble proteins of HeLa cells (Fig. 1C).

The increased synthesis of MT in HeLa cells in response to incubation with dexamethasone might be explained as follows. Dexamethasone may increase the uptake of Zn^{2+} in HeLa cells. This might then increase the availability of Zn^{2+} to the metal-mediated MT induction mechanism, when the cells are grown in concentrations of metal less than optimal for maximal MT induction. The uptake of Zn^{2+} in primary cultures of parenchymal liver cells, the main organ for MT synthesis, can be stimulated by dexamethasone (*14*). Alternatively, the stimulation by dexamethasone of [^{3}H]-cysteine incorporation into MT may be a direct effect of dexamethasone on MT metabolism. Dexamethasone might directly either stimulate MT synthesis or inhibit its degradation.

Ten years ago Cox (*6*) proposed, as a possible mechanism for Zn^{2+} uptake, the existence of a Zn^{2+} carrier or Zn^{2+}-binding molecule containing sulfhydryl groups. He proposed that prednisolone (another glucocorticoid agonist) "may enhance Zn^{2+} uptake in certain mammalian cell cultures by increasing the level or activity of carrier molecules." Our studies suggest that MT is this Zn^{2+}-binding molecule.

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"Spontaneous" Neoplastic Transformation in vitro: A Form of Foreign Body (Smooth Surface) Tumorigenesis

Abstract. *Explants of subcutaneous connective tissue from adult BALB/c mice into plastic petri dishes were serially subcultured and tested for tumorigenicity in two ways: by the subcutaneous implantation of cells attached to plastic plates (1 by 5 by 10 millimeters), and by the subcutaneous injection of cells suspended in saline. Cells grown in vitro for 18 or more days before being implanted attached to a plastic plate (2.4×10^4 to 3.4×10^5 cells per plate) formed tumors after 24 to 79 weeks. The latent period before tumor appearance correlated inversely with the time spent by the cells in tissue culture. Cells inoculated in saline suspension (10 to 100 times the above number per plate) did not form tumors until after 84 days in vitro; plates alone did not induce tumor formation within more than 1 1/2 years of implantation. The tumors arising from the plate-attached cells were transplantable without plates and histologically appeared to be undifferentiated sarcomas. It is well established that smooth-surfaced foreign bodies, regardless of their chemical composition, will produce sarcomas when transplanted subcutaneously in rodents. We interpret our data, particularly the decrease in tumor latent period with time spent in tissue culture, as indicating that a smooth surface was acting as a carcinogen first in vitro (the surface of the tissue culture dish) and then in vivo (the surface of the plastic plate).*

The etiology of "spontaneous" neoplastic transformation in tissue culture exhibited by mammalian cells from a number of species, particularly rodents, has not been determined (*1*). Neoplastic transformation occurs in cells grown in serum-free medium (*2*), and in the absence of oncornavirus gene products (*3*). Stimulated particularly by the work of Brand on plastic film tumorigenesis in mice (*4*) we tested the hypothesis that "spontaneous" neoplastic transformation of mouse cells in vitro is a form of foreign body (smooth surface) tumorigenesis. Plastic film is representative of a large number of chemically different substances that are sarcomagenic only if they possess an extended flat surface. For example, subcutaneous implants of glass, metals, plastics, and silicone rubber all produce sarcomas in sheet form but not in particulate form (*5*). We postulated that just as association in vivo between the smooth surface of a plastic film implant and subcutaneous connective tissue could give rise to tumors,

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so association in vitro between the smooth surface of the tissue culture vessel and explanted subcutaneous connective tissue cells could also give rise to tumors by a similar mechanism.

Brand (*4*) used a two-step experimental procedure in vivo. In the first step, he implanted plastic films (0.2 by 15 by 22 mm) subcutaneously in mice possessing T6 marker chromosomes. In the second step, at various times he excised the film plus the surrounding fibrous capsule, removed the film, and tested the fibrous capsular tissue for tumorigenicity by transplanting it subcutaneously into congenic T6-negative mice either with or without attachment to fresh plastic film. He found that when the plastic film had remained in the T6-positive mice for longer than 4 to 8 weeks, the transplanted capsular tissue attached to fresh plastic film consistently produced sarcomas; the transplants of capsular tissue without plastic film practically never produced sarcomas. A noteworthy finding was an inverse correlation between the time spent by the capsular tissue in the primary host and the latent period before appearance of tumor in the secondary host after transplantation of the capsular tissue attached to the fresh plastic film. In addition, the frequency of tumors in the secondary host correlated directly with the time spent by the capsular tissue in the primary host.

We also used a two-step experimental procedure analogous to that of Brand. For the first step, we explanted and grew mouse subcutaneous cells in vitro, thereby exposing them to the smooth plastic surface of tissue culture flasks. For the second step, at various times we tested the cultured cells for tumorigenicity either by injecting them subcutaneously in saline suspension or by implanting them subcutaneously when they were attached to plastic plates (1 by 5 by 10 mm). The results corresponded closely to those obtained by Brand. When the subcutaneous connective tissue cells had been cultured in vitro for longer than 3 to 4 weeks, they produced sarcomas in vivo when they were implanted attached to plastic plates; cells injected subcutaneously in saline suspension did not produce sarcomas until they had been in tissue culture for 12 weeks. Evidence in favor of the hypothesis that "spontaneous" neoplastic transformation in vitro is a form of foreign body (smooth surface) tumorigenesis was the occurrence of an inverse correlation between the time spent by the cells in tissue culture and the latent period before appearance of sarcomas after the cells had been implanted in vivo attached to plastic plates. In addition, the frequency of tumors correlated directly with time in tissue culture.

The procedures we used were as follows. Six male BALB/c mice were killed by cervical fracture and depilated by immersion in laundry bleach for 15 minutes. Hanks solution (3 ml) containing 0.5 percent collagenase (Sigma type 2) and 0.5 percent trypsin (Gibco) was injected subcutaneously into each of the dead mice to produce a bulla. Forty-five minutes later, approximately 1.5×10^7 connective tissue cells were obtained from each mouse by needle aspiration of the bulla. The cells were seeded into a T75 plastic flask and grown under standard tissue culture conditions. A confluent sheet of cells formed within 2 to 12 days. At the time of all subsequent subcultures, the cells in all flasks were trypsinized, pooled, and seeded into new flasks at a density of 4×10^6 cells per T150 flask. Cells were attached to polycarbonate plates (1 by 5 by 10 mm) (*6*) at near confluency by overnight incubation in vitro. The mean number of cells per plate was determined by trypsinizing and counting the cells on ten extra plates. Plates with attached cells were implanted subcutaneously into male mice, one plate per mouse, cells facing the skin.

In Table 1 we compare the tumorigenicity of cells in saline suspension with that of cells attached to plastic plates after increasing intervals of time in culture. Seven separate lines were tested. From the earliest time tested (18 days) onward the cells were tumorigenic at relatively low cell concentrations when implanted attached to plastic plates (2.4×10^4 to 3.4×10^5 cells per plate), whereas they were not tumorigenic in saline suspension even at 10 to 100 times these concentrations (Table 1). The tumors could all be transplanted without having to be further attached to plates. Histologically, they were anaplastic sarcomas that

Table 1. Tumorigenicity of monolayer cultured connective tissue cells inoculated while in suspension or attached to plastic plates. The data for tumor incidence show the number of animals with tumors compared to the number of animals inoculated. When plates were implanted subcutaneously without any attached cells none of the animals ($N = 30$) developed tumors within 480 days of observation. The abbreviation ACT denotes adult connective tissue.

Passage number	Number of		Cells in saline suspension injected subcutaneously		Cells attached to plate implanted subcutaneously		Latent period (weeks)
	Days in culture	Population doublings	Number of cells	Tumor incidence	Number of cells	Tumor incidence	
			Line ACT 1				
2	19	5.6			3.6×10^4	0/15	
4	27	7.4			4.2×10^4	0/12	
6	34	8.6	1.0×10^7	0/8	7.8×10^4	2/12	63, 67
			Line ACT 2F				
4	23	7.2	1.0×10^7	0/7	2.4×10^4	1/13	31
6	31	8.3			2.1×10^4	1/10	46
9	50	11.2	9.5×10^6	0/7			
			Line ACT 2S				
4	23	7.4			2.0×10^5	0/12	
6	30	8.3			1.0×10^5	0/10	
7	36	8.5	1.0×10^7	0/6	4.1×10^4	2/10	47, 79
			Line ACT 3				
2	18	5.7	9.5×10^6	0/6	7.0×10^4	3/10	70, 79, 79
			Line ACT 7				
4	26	4.2	1.0×10^7	0/5	7.0×10^4	3/19	45, 55, 76
4	33	6.0	5.0×10^6	0/6	5.0×10^4	0/15	
			Line ACT 8				
7	75	7.8	5.0×10^6	0/5	3.0×10^4	3/11	29, 29, 50
14	130	18.8			7.0×10^4	7/9	24, 24, 24, 31, 35, 40, 40
			Line ACT 9				
3	35	7.5	1.0×10^7	0/5	5.0×10^4	3/20	45, 57, 70
4	44	12.3	1.1×10^7	0/5	4.5×10^5	0/10	
5	48	13.5			2.5×10^5	3/12	42, 46, 52
6	54	16.8	1.0×10^7	0/10	3.5×10^5	0/12	
7	66	21.6			3.4×10^5	1/10	39
13	126	48.4			8.0×10^4	2/10	52, 59
14	134	53.3	1.0×10^6	0/10	1.0×10^5	1/10	49

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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