produces prolonged cardiovascular effects in shock (Fig. 2). Thus, the centrally mediated cardiovascular effects of TRH, like those of naloxone, should also result in improved tissue perfusion and survival in shock states (*18*).

Depending on the dosage, naloxone may either diminish or enhance clinical pain in humans (*19*). Although some evidence suggests that naloxone may also effectively reverse shock in humans (*20*), it has not been established whether clinical pain in such states would be altered by this opiate antagonist at the doses required for the therapeutic effect. In animal experiments TRH has been shown to be devoid of effects on nociceptive latencies (*8*), and in the experiments described here TRH appears to be as effective as naloxone in improving the cardiovascular pathophysiology in experimental shock. Collectively, these findings suggest that TRH or TRH metabolites and analogs may be useful therapeutically for shock or acute hypotension, acting in a manner similar to naloxone but without intensifying pain perception.

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Epidermal Growth Factor Enhances Viral Transformation of Granulosa Cells

Abstract. *Kirsten sarcoma virus produced a low incidence of transient morphological transformation in primary cultures of rat ovarian granulosa cells. In the presence of epidermal growth factor, the incidence of transient transformation increased severalfold and two continuous cell lines were established. Epidermal growth factor, a naturally occurring polypeptide hormone, appears to act here as a tumor promoter in the retrovirus-induced transformation of a mesodermally derived epithelium.*

Kirsten murine sarcoma virus (Ki-MSV) produces stable morphological transformation of several connective tissue cell types in culture, and such transformed lines form sarcomas when injected into sublethally irradiated rats (*1*, *2*). An epithelial tissue of mesodermal origin, the adrenal cortex, is susceptible to transformation by Ki-MSV, and differentiated carcinomas result as well as sarcomas (*3*). We attempted to extend these observations by infecting rat ovarian granulosa cells, another steroid-secreting epithelial cell type of mesodermal origin, with Ki-MSV.

Transformation foci appeared in all cultures within 1 week of infection (*4*). Without epidermal growth factor (EGF), very few foci were produced, usually fewer than ten per dish. The same virus preparations, used here at 1.0 ml per culture, contained between 2.3×10^4 and 2.8×10^6 focus-forming units per milliliter when assayed on comparable NRK (normal rat kidney) cultures (*3*). Unlike Ki-MSV–induced foci in cultures of adrenocortical cells or fibroblasts (*3*), foci in granulosa cell cultures invariably reverted to normal morphology within 3 weeks (Fig. 1). In no case did a stably transformed cell line result.

Addition of EGF to cultures at the

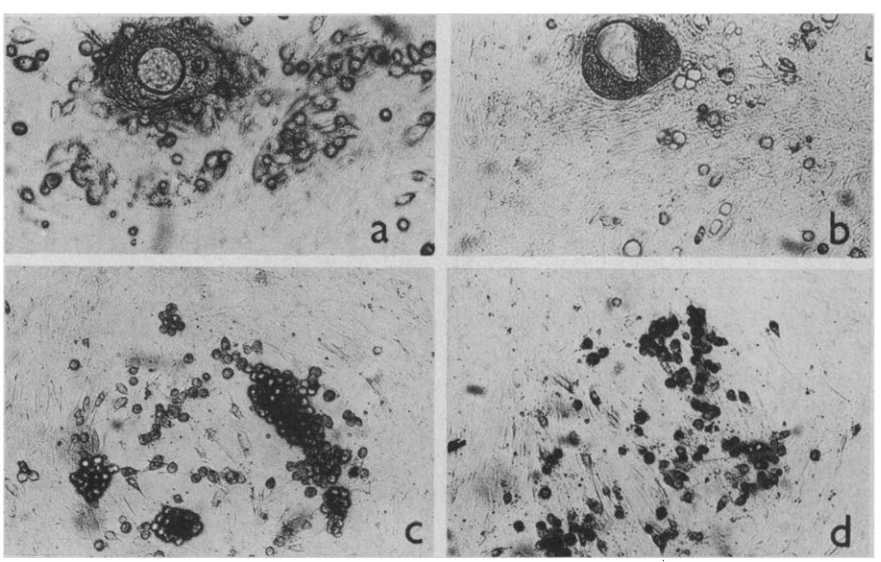

Fig. 1. Various stages of morphological transformation of Ki-MSV–infected granulosa cells. (a) Morphologically transformed cumulus oophorus cells surrounding an oocyte 6 days after infection (×50). (b) The same focus 58 days later. The transformed morphology has been lost. The degenerating oocyte is still prominent (×50). (c) A focus of transformation 8 days after infection. The cells are rounded and form clumps and are retracting from the substratum (×40). (d) The same area 6 days later. The focus is reverting (×40).

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time of viral infection and regularly thereafter produced a dramatic increase in the incidence of transformed foci (Table 1). This enhancement of transformation was not due to a generalized proliferation-stimulating effect of EGF. Epidermal growth factor has been shown to increase the growth rate and replicative life-span of granulosa cells of other species in culture, but has only a marginal mitogenic effect on rat granulosa cells (*5*). This was confirmed under our culture conditions: except for sporadic instances of slight, transient growth acceleration, EGF had no effect on the growth rate and replicative potential of the cells in medium supplemented with 1 or 10 percent fetal bovine serum. Furthermore, EGF did not selectively accelerate the cell cycle of potentially transformable cells. Had such been the case, foci would have appeared earlier in cultures with EGF than in those without it. This was never observed.

Without EGF, all foci reverted within 3 weeks and the cultures became stationary and morphologically similar to uninfected controls. Recognizable foci persisted for longer in cultures with EGF, although most of these also reverted to normal morphology after 4 weeks. One group of transformed cells grew slowly over 80 days in the presence of EGF, eventually giving rise to a population of rapidly growing morphologically transformed cells. Another line of rapidly growing morphologically transformed cells was obtained after EGF was first added to a quiescent culture 105 days after infection with Ki-MSV. Cells subcultured and plated at low density 6 days after infection invariably reverted to normal morphology in the absence of EGF; foci formed in secondary culture only if EGF was present in the medium.

In this culture system, then, transformation seems to be a two-step process. Infection with Ki-MSV is necessary but not usually sufficient for transformation. The effect of the virus thus resembles that of an "initiator" as described for chemical carcinogenesis. Similarly, EGF appears to act here as a chemical promoter. Such an action would be in keeping with in vivo studies suggesting that EGF promotes chemically induced tumors (*6*).

There is considerable evidence that RNA tumor virus–transformed rodent cells produce sarcoma growth factor that binds to the cells and maintains the transformed state (*7*). Also, diffusible factors that confer the transformed phenotype on normal cells were recently isolated from human tumors (*8*). The factors from both sources bind specifically to EGF receptors (*8, 9*), hence it has been suggested that they are EGF-related polypeptides, possibly originating from common ancestral proteins (*8*). It seems highly significant that EGF in its normally occurring form also has a promoting effect and that it enhances the transformation of a differentiated cell type in primary culture.

Table 1. Effect of EGF on the incidence of transformed foci in Ki-MSV–infected granulosa cell cultures. Foci were counted 6 to 12 days after infection, when their number was maximal. In experiment 4 infection was carried out with supernatant medium from Ki-MSV–transformed NRK cultures, Millipore-filtered directly onto the cells (2 ml per culture), rather than with viral concentrate. A different virus preparation and different animals were used for each experiment; FFU, focus-forming units; S.E.M., standard error of the mean.

Experiment	Cultures (No.)	Ki-MSV (FFU/ml)	EGF (ng/ml)	Foci per culture (mean ± S.E.M.)
1	3	0	0	0
	3	0	10	0
	3	4.0×10^5	0	8.7 ± 1.21
	3	4.0×10^5	10	86.3 ± 5.25
2	8	2.8×10^6	0	7.0 ± 1.43
	8	2.8×10^6	10	52.3 ± 7.30
3	6	2.3×10^4	0	0
	6	2.3×10^4	10	2.5 ± 0.51
4	7	Filtrate	0	51.3 ± 8.70
	7	Filtrate	10	180.6* ± 21.52

*This value is an underestimate because foci in parts of the cultures were too numerous to be counted accurately.

Epidermal growth factor did not stimulate proliferation of uninfected granulosa cells. It is possible, therefore, that its promoter-like properties are unrelated to its action as a mitogen. This would be in keeping with the ability of EGF to alter the viral infectivity of cells (*10*), enhance anchorage independence of cells already transformed by a DNA virus (*11*), and increase the transformation frequency of polyoma virus, possibly through changes in the cytoskeleton (*12*). Alternatively, viral infection may render the granulosa cells responsive to EGF mitogenesis. Such an influence would be similar to the increased responsiveness to tumor promoter of fibroblasts infected with temperature-sensitive Rous sarcoma virus and maintained at the nonpermissive temperature (*13*). Epidermal growth factor increased both the number and stability of foci induced by Ki-MSV. Whether these foci were transient or progressed to continuous lines may have depended on influences superimposed on those of the virus and EGF—for example, on culture variables that regulate granulosa cell differentiation (luteinization) and thereby influence the proliferative potential of the cells. However, it is also possible that the rare continuous cell lines produced under the combined action of virus and EGF arose in a very limited subpopulation of genetically susceptible cells in which the molecular mechanisms of induction, as well as the final product of transformation, are fundamentally different from those associated with the formation of transient foci. Our study supports the hypothesis that viral transformation is a multistep process which may have much in common with the initiation/promotion model of chemical carcinogenesis (*13*).

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