control of cell surface area and nutrient uptake, a theory which now seems unlikely[34,35], and "wound healing" in monolayer culture. This wound healing, once thought to be density dependent, now seems to involve the destruction of an external diffusion barrier near the cell surface[36]. In certain culture systems, growth inhibition definitely does not involve density dependence[27].

Holley defines density-dependent growth regulation as a tendency for cells "to grow to a 'saturation' density and then stop growing." This definition is unsatisfactory. Density dependence is a change per cell in the value of a cellular property as population density changes. As a rule, a density dependence of growth operates at all population densities and may involve either or both the promotion and inhibition of growth rate[10,26,27]. In culture, the inhibition of cell growth usually occurs even when nutrients and diffusable factors are in excess and not rate limiting[3-5,10,27-33]. Although in well fed cultures growth inhibition is occasionally observed[4], it occurs at much higher densities than are normally employed and probably reflects the restriction of diffusional transport either by an external diffusion barrier[36] or by multilayering. Growth inhibition and its density dependence in culture are not usually the result of resource depletion except for the special situation in which the investigator fails to provide adequate nutrient to his cultures. In general, both 'saturation' density and quiescence are starvation artefacts[3-6,10,27-33].

Holley's acceptance of the physiological significance of certain diffusable growth affectors is uncritical. Of the materials he cites, only the nerve and epidermal growth factors (NGF, EGF) are clearly growth regulators *in vivo*. Plant lectins certainly are not, cyclic AMP probably is not[37-40], while the role of fibroblast growth factor, insulin, hydrocortisone, prostaglandins, antigens, and proteolytic enzymes *in vivo* is unknown. Since blood vessels are selectively permeable, the mere presence of a growth affector in serum does not mean that the substance regulates cell growth. Its presence in the interstitial fluid surrounding a suspected target cell must also be demonstrated. Similarly, a growth affector from one species of vertebrate cannot be considered physiological when applied to cells from another, as is the case with several of the studies Holley cites. Even Holley's use of the term 'polypeptide factors' is in error. Such factors (S_2, erythropoietin, EGF, NGF, S_1) are often multimers and often of very high molecular weight (26, 46, 74, 140, 600 kdalton respectively); or very small and not polypeptides at all (putrescine, uridine, adenine).

Finally, recent evidence raises the possibility that the extracellular matrix may contribute to growth regulation in some systems[41-43], and that contact interactions may also[27], though not in the manner postulated by Todaro and others.

In summary, growth regulation is complex, confusing, and not at all understood. Certain diffusable substances (NGF, EGF, erythropoietin, colony-stimulating activity) are clearly physiological growth regulators. And certainly density-dependent growth regulation occurs in many culture systems, though it is far more complex and often differs fundamentally from Holley's description of it. While density-dependent growth regulation is sometimes mediated by diffusable substances, it is probably at times mediated by matrix and contact interactions as well. There is at present no evidence for density-dependent increases by cells in their resource requirements, nor is there evidence that resource depletion regulates growth *in vivo*. Even in culture, resource depletion seldom contributes to growth regulation of well fed cells. Finally, no general difference between normal and malignant cell growth regulation in culture has ever been rigorously proved.

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Matters arising

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HOLLEY REPLIES—Growth regulation is complex, but I believe Skehan makes the subject unnecessarily confusing.

It is unlikely that all tumors are identical, so it is unrealistic to expect the absolute correlation of tumorigenicity with a single property as Skehan demands[1]. Nevertheless, there are differences between "normal" and tumorigenic cells and the two types of cells often differ greatly in their growth behaviour in cell culture. It is important to understand the differences that are observed and this was the subject of the review[2].

Skehan is influenced by what he considers to be a discovery of "growth inhibition" at low cell density[3]. In my view, Skehan has not discovered a growth inhibition but rather uses the term incorrectly, and has confused the literature. Cell cultures that begin with quiescent cells normally show a lag period, then a period in which there is one relatively synchronous cell division, and then an extended period of asynchronous growth. Skehan plots[3] such a growth curve in the form of growth rate per day and concludes that there is "growth inhibition" at the end of the period of synchronous cell division, since the rate of appearance of new cells falls. In my view, the transition from a brief period of synchronous cell division to asynchrony in a growing population is not properly called "growth inhibition."

There are several inaccuracies in Skehan's present comments[1] but they will be detected by anyone who reads the review[2].

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