COOK ET AL. REPLY—There is no doubt that some tissues possess the enzymes necessary to metabolise extracellular purine nucleotides to their equivalent nucleosides, so that there is the likelihood of adenosine being formed from any nucleotide which is based on the adenosine moiety. Although not specifically mentioned in our letter[1], it is likely that CoA gives rise to adenosine at some point in its breakdown and this might have been inferred both from the enhancement of the potency of CoA by dipyridamole and by the demonstration of the efficacy of adenosine-3′5′-diphosphate, a compound also likely to be formed during the breakdown of CoA. The problem, therefore, is not whether adenosine could be present but whether it is necessary. The comment seems to suggest that the ability of any purine nucleotide to reduce acetylcholine output might depend on the prior production of adenosine and that this would be the sole active agent. Although this could conceivably be the case it is unlikely that the receptor requires the nucleotides to be degraded to the nucleoside for agonist activity. Experiments using the ATP analogues α,β-, and β,γ-methylene ATP have shown[2] approximately equal potency to ATP, no difference in onset time, and sensitivity to theophylline. In addition, the offset time for these analogues was very much prolonged. It is unlikely that these compounds present a rapidly metabolisable substrate to 5′-nucleotidase and therefore it seems that the receptor, although referred to as the 'adenosine' receptor, may not require formation of the free nucleoside *per se*. It may recognise and also interact with the 6-aminopurine-9-β-D-riboside moiety wherever a compound contains it, provided that there is no steric hindrance preventing its presentation to the receptor.

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Diffusion-limitation of cell growth

THE paper by Whittenberger and Glaser[1] is entitled 'Cell saturation density is not determined by a diffusion-limited process'. Its refutation might similarly be entitled: 'The Stokes–Einstein equation should not be extrapolated to solutions of chain-polymers'. They assume that a 25-fold increase in bulk viscosity of a gel must proportionately entail a 25-fold decrease in molecular diffusivity. This is incorrect both in principle (Stokes–Einstein was derived for homogeneous media) and in practice[2,6]. For a small molecule like insulin the decrease in D would be nearer 25% than 25-fold.

Quantitative studies on diffusion diffusion require at least four parameters: D, Q, C and a penetration depth[3]. The authors[1] would have made a welcome start by measuring these.

Qualitatively speaking, diffusion-limitation is probably not the only limitation to cell growth in crowded conditions. But surely, even on a qualitative level, Occam's razor should be used? Thus it is necessary to eliminate the effect of stretch-limitation[5], a phenomenon which has been independently demonstrated on single cells, and the possible interaction between stretch-limitation and diffusion-limitation for crowded cells in a gelled medium[4] before proceeding to invoke yet a third hypothesis, namely contact inhibition[1].

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WHITTENBERGER AND GLASER REPLY—We thank Maroudas for pointing out to us a potentially very important reservation regarding our observations[1]. As the applicability of the data cited by Maroudas[2] to our system is not clear[3], we have measured in a synthetic boundary cell[4] the diffusion coefficient of several proteins in the molecular weight range of potential growth factors in the presence and absence of the polymers used in our work on cell growth. The measurements were carried out in calcium and magnesium free Hank's solution buffered with HEPES[5], and the results are shown in Table 1.

Although the effect mentioned by Maroudas is quite real, its magnitude is not as large as he anticipated. The effect of the polymers we used on the diffusion of small proteins is large enough for us to have observed an effect on the final cell density or on the rate of growth of the cells if the growth of our cells was diffusion limited. Therefore, the conclusion of the paper is still valid. The reference by Maroudas to gelled medium is not clear as none of our media are gelled. Regarding the general comments of Maroudas with regard to contact inhibition of growth we would like to point out the following: Swiss 3T3 cells remain at constant density in spite of daily medium changes. In collaboration with D. Raben and M. Lieberman, we have shown that a plasma membrane-enriched fraction from 3T3 cells blocks cell growth early in G1, can bind to 3T3 cells on a dish, and mimics the effect of high density on the rate of uptake of smaller molecular weight nutrients. Membranes do not deplete bulk medium of growth factors[5]. The inhibitor activity from membranes has recently been solubilised from membranes (B.W., D. Raben, M. Lieberman & L.G., *PNAS*, in press); it is maximally active at levels of 10 μg protein ml^{-1} and is totally reversible. At this level it is unlikely to interfere with the surface available for cells to grow. We believe that in this instance Occam's razor suggests that one of the elements that controls cell growth in Swiss 3T3 cells is cell contact.

The original wounding experiments by Dulbecco[6] clearly distinguish between the serum requirements at the wound and topo-inhibition, in agreement with the notion that cell to cell contact is involved in growth control. More recent experiments by Westermark[7] on a human glial cell line and epidermal growth factor also agree with this notion.

All proteins were obtained from Sigma.

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Table 1 Diffusion constants of proteins in the presence of various polymers

	Buffer	+10% Dextran	+10% Ficoll	+2% Methyl Cellulose
Ribonuclease A	11.4	3.85	5.0	6.60
Soybean trypsin inhibitor	10.2	2.83	4.85	5.15
Pancreatic trypsin inhibitor	11.4	3.8	—	—

Diffusion constant cm^2 s^{-1} × 10^7.

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