

This assumption seems to be based on the growth pattern of a single infant, aged 4 to 7 months, who grew in 11 saltuses during 118 days of observation, or 8% of the time [figure 1 of (7)]. The actual skewness coefficient calculated from our experimental data for that infant is 1.265 (Fig. 2).

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the effect is similar to the methods cited here. While blind measurement is a protocol to be recommended for future studies, clinical studies have documented significant errors introduced from the locking mechanism on the Harpenden-Holtain instrument itself, a mechanism designed for portability, not blind read-out accuracy.

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Variability Among Human Umbilical Vein Endothelial Cultures

Endothelial cell (EC) cultures have been extensively used in models of different vascular phenomena such as inflammation and angiogenesis. One of the most widely used sources for cultured ECs is human umbilical veins. Until recently, most investigators have isolated human umbilical vein endothelial cells (HUVECs) in their own laboratories and propagated the cultures for brief periods (that is, several weeks) under standardized (STD) conditions using Medium 199 supplemented with 10 to 20% serum (usually fetal calf or human) plus heparin-binding growth factor 1 [also called acidic fibroblast growth factor or endothelial cell growth factor (ECGF)] stabilized by heparin as originally described by Levine and colleagues (1). Recently, several commercial sources of HUVECs have become available, often provided with variant (VAR) conditions of media and growth factors that may differ significantly from STD conditions. Few, if any, data are available that compare cultures of HUVECs propagated in these different conditions with cells from STD cultures.

In 1992 A. E. Koch *et al.* reported, in part, that HUVECs obtained from a commercial source responded to human interleukin-8 (IL-8) as a mitogen (2). We had been unable to reproduce this finding using HUVECs isolated and cultured in our lab-

oratory (3). We decided to investigate whether differences in the culture systems used could contribute to this discrepancy. We isolated HUVECs in the standard manner and split the cells into two groups: a STD group cultured in Medium 199, 20% fetal calf serum (FCS), 50 µg/ml ECGF, 100 µg/ml heparin, and a VAR group cultured in the medium used by Koch *et al.* (2). In multiple experiments, involving three different paired isolates, we found that cells propagated under STD conditions did not proliferate to IL-8 whereas those cultured

under VAR conditions did (Table 1). Cells switched from STD to VAR conditions did not acquire responsiveness, but cells switched from VAR to STD conditions showed diminished responsiveness to IL-8 (Table 1). These data are most consistent with the interpretation that the cultures grown under VAR conditions contain an IL-8 responsive cell type that is lost in STD cultures. Unexpectedly, even cultures under VAR conditions that respond to IL-8 do not appear to display high affinity binding of [¹²⁵I]-IL-8, using an assay (3) easily able to quantify binding to neutrophils (data not shown).

There are several possible explanations for lack of detectable high affinity receptors: (i) IL-8 could be mitogenic for HUVECs

Table 1. Mitogenic actions of IL-8 on HUVEC cultures.

Culture conditions*	Cell number†		Significance‡
	No IL-8	+IL-8	
STD→STD	22.3 ± 9.9	18.7 ± 4.1	n.s.
VAR→VAR	15.3 ± 7.6	34.8 ± 5.5	P < 0.005
STD→VAR	31.03 ± 7.3	28.3 ± 5.7	n.s.
VAR→STD	22.8 ± 10.5	31.8 ± 9.8	P = 0.08

*HUVEC isolates were serially subcultured in parallel in standard medium (STD = Medium 199, 20% FCS, 50 µg/ml ECGF, 100 µg/ml heparin, 0.4 mg/ml L-glutamine, 100 U/ml penicillin-G, and 100 µg/ml streptomycin sulfate), or variant medium [VAR=Clonetics (San Diego, California)-optimized Endothelial Growth Medium, 2% FCS, 10 ng/ml human epidermal growth factor, 1 µg/ml hydrocortisone, bovine brain extract, 40 ng/ml heparin, 50 µg/ml gentamicin, and 50 ng/ml amphotericin B] for two passages. Each culture was then split (1:3) into two groups; one remained in the same medium (STD→STD or VAR→VAR) and one was crossed over to the other medium (STD→VAR or VAR→STD). †10 × 10³ cells were plated into 2.0-cm wells containing fresh STD or VAR medium ± 1 × 10⁻⁹ M IL-8. Cells were harvested and counted at 48 hours. Each condition was tested in six replicates, and cell number is the mean ± SD of 10⁻³ cells per well. ‡P values from paired Student's *t* test. Only cells maintained in VAR show a significant mitogenic response; the response of cells transferred from VAR to STD medium is diminished. n.s., not significant.

through a low affinity receptor; (ii) IL-8 could be active on HUVECs that express a very low number of high affinity receptors; or (iii) only a small subpopulation of cells in the HUVEC culture express high affinity IL-8 binding sites, but this subpopulation can trigger proliferation of other HUVECs by releasing a secondary mitogen in response to IL-8. To investigate this last possibility, we stimulated a HUVEC culture grown under VAR conditions with an optimal concentration of TNF plus interferon- γ to induce E-selectin, and we positively selected E-selectin-expressing cells by fluorescence-activated cell sorting. In two such experiments, the positively selected cells lost their responsiveness to the mitogenic action of IL-8 (not shown). These observations favor the interpretation that a small population of cells that cannot be induced to express E-selectin mediates the IL-8 mitogenic response to HUVEC cultures under VAR conditions. Such cells may be HUVECs or may represent a nonendothelial population (for example, T cells or mast cells) that survives better under VAR than under STD conditions.

Our specific experiments suggest that IL-8 does not act directly on (most) cultured HUVECs. However, the importance of our observations lies in the more general point: not all HUVEC cultures are alike. As cultured HUVECs have a limited life-span, routine exchanges of cultures among laboratories to resolve discrepant findings is not feasible. Investigators are advised to note the isolation procedure and culture conditions used in different studies.

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Response: We read with interest this comment about our report (1). Pober and co-workers point out that while using standard

isolation and culture techniques, they were unable to show an increase in HUVEC proliferation in response to IL-8. However, when cells were grown in commercially available tissue culture medium, they were able, as we did, to show a significant HUVEC proliferative effect in response to IL-8. Moreover, HUVECs cultured with the use of commercial media and then switched to standard media still retained some of the capacity to proliferate. It appears that the source of HUVECs and conditions in which they are grown do indeed influence the outcome of experiments. However, with the advent of commercial sources of HUVECs and culture medium in which to propagate these cells, the commercial culture conditions are rapidly becoming the standard. While individual labs may modify their isolation and culture procedures, the advantage of using commercial sources of cells and their media is precisely the standardization of these conditions and the availability of reagents.

Watson *et al.* propose that cultures grown with the use of commercially available HUVEC culture medium contain an IL-8 responsive cell type that is lost in culture under standard conditions. They stimulated HUVEC cultures grown under commercial culture conditions with optimal concentrations of IFN- γ and tumor necrosis factor (TNF) to induce E-selectin. These cells lost their responsiveness to the mitogenic action of IL-8. Cytokines such as TNF- α induce endothelial IL-8 production (2). It is thus possible that HUVECs are maximally activated as a result of endogenous IL-8 production and thus cannot display further responsiveness to exogenous IL-8.

Our experiments did not address the question of whether a subpopulation of cells was responsible for the proliferation induced by IL-8. It is, however, a distinct possibility. It seems unlikely that contaminating cells, such as T cells, would be responsible for this proliferation, as we have performed the experiments using HUVECs at many passage levels and it is unlikely that T cells would survive several passages. Also, the HUVECs used bear endothelial markers, such as factor VIII-related antigen, on their surface. Hence, if a subpopulation of cells is responsible for the proliferative effect, we agree with Watson *et al.* that it is likely to be a population of HUVECs.

Watson *et al.* also propose that IL-8 may act on endothelial cells by releasing a secondary mitogen in response to IL-8. IL-8

appears to have a direct effect, rather than an indirect effect, on endothelial cells because in chemotaxis assays employing this cytokine, IL-8 was directly chemotactic for HUVECs. In these assays, if IL-8 induced an indirect effect, HUVECs, which are on the opposite side of the chemotaxis membranes, would not migrate in response to IL-8, as release of a secondary factor by the HUVECs would reverse the chemotactic gradient. Schonbeck and co-workers (3) have recently demonstrated low affinity IL-8 receptors on HUVECs, which may contribute to the observed effect. Nonetheless, we cannot, however, entirely exclude the possibility that IL-8 also induces a secondary mitogen in HUVECs, perhaps by activating a latent endothelial cell-derived mitogen or one in the media.

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