match the λp50 of the downwelling illumination in the rivers and lakes of South America where they are found. A visual pigment matched in this way would maximize the apparent contrast when viewing objects silhouetted against the downwelling light. There are proportionally more cells containing the shortwave PCP's in the upward-looking ventral retina. The longwave PCP's are more common in the down and forward-looking dorsal retinas whose field of view contains a higher proportion of longwave light. The λ_{max} of the guppy longwave pigment is only 546 nm, rather close to the "shortwave" PCP of the cichlids and notopterids, is concentrated in the same area of the retina, and could function in a similar manner. The functional significance of the ellipsosomes contained in certain of the cones of poeciliids has yet to be determined (*19*).

If such intraretinal specializations occur in most fishes, they might provide a partial explanation for certain discrepancies encountered in studies of piscine color vision. Radically different photopic spectral sensitivities are often obtained for the same species when behavioral methods that differ in the location of the stimulus in the visual field are compared.

The differential distribution of colored oil droplets in the retinas of pigeons (*20*), while operating on a different principle, results in similar alterations of the relative spectral sensitivities of dorsal and ventral regions.

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9. Courtesy of Dr. Albert Szent-Györgyi.
10. The prism of a Leiss quartz prism monochromator was replaced with a 600 groove/mm reflection grating blazed for 400 nm. The much greater dispersion of the grating allows the use of wide entrance and exit slits to illuminate an area of approximately 1 cm^2 with a flux of greater than 200 $\mu W/cm^2$, while retaining a narrow passband.
11. Our low-calcium Ringer solution contained 113.5 m*M* NaCl, 11.9 m*M* $NaHCO_3$, 3.3 m*M* NaH_2PO_4, 3.4 m*M* KCl, 21 m*M* $MgCl_2$, 11.1 m*M* glucose, adjusted to *p*H 7.4.
12. The composition of NBT incubation medium (*7*) was 39.0 m*M* NaCl, 0.7 m*M* KCl, 0.7 m*M* $CaCl_2$, 0.4 m*M* $MgSO_4$, 0.4 m*M* $NaHCO_3$, 5.5 m*M* NaH_2PO_4, 21.0 m*M* Na_2HPO_4, 6.0 m*M* NBT, and 13.0 m*M* disodium succinate.
13. The λ_{max} was 594 and 548 nm (J. S. Levine, in preparation).
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Human Skin Fibroblasts Derived from Papillary and Reticular Dermis: Differences in Growth Potential in vitro

Abstract. *Papillary fibroblasts, when compared to reticular fibroblasts from the same skin specimen, exhibit greater proliferative capacities in vitro. These results demonstrate a difference in function between morphologically similar cells obtained from the same tissue. Such findings represent an important consideration in the study of cell aging in vitro.*

Fibroblastlike cell lines derived from human dermis are being extensively used in the diagnosis of genetic disorders as well as studies in vitro of the aging process (*1*). Investigators have presumed that those cell lines derived from the dermis of normal individuals will behave similarly when propagated in vitro. However, recent data have raised the possibility that normal human dermis contains several fibroblastlike cells which may differ in behavior while retaining similar morphology. For example, different responses to hydrocortisone were observed among fibroblastlike cell lines from a common site in normal individuals (*2*); qualitative differences in testosterone metabolism exist between sister lines derived from a single foreskin explant (*3*); and finally, both precursor forms of collagens I and III are synthesized by human skin fibroblasts cultured from the same biopsy (*4*).

In obtaining specimens for the study of

Table 1. Population doublings of human skin fibroblasts at passage four.

Normal fibroblast cell line	Age	Race	Sex	Site	PD*		Δ†
					Papillary	Reticular	
9	48	W	M	Thigh	7.8	5.3	−32.1
14	50	W	M	Thigh	8.3	7.2	−13.3
16	48	W	M	Abdomen	7.6	6.8	−10.5
17	63	W	M	Abdomen	7.4	4.0	−45.9
19	49	B	F	Buttock	8.1	6.2	−23.5
23	16	W	F	Abdomen	10.0	9.1	− 9.0
32	68	B	F	Abdomen	8.7	7.8	−10.3
33	19	W	F	Thigh	12.2	10.5	−13.9
40	79	B	F	Abdomen	9.8	8.8	−10.2
44	67	B	M	Abdomen	11.3	10.5	− 7.1
48	66	B	M	Abdomen	9.8	8.4	−14.0
50	32	W	M	Thigh	10.7	8.9	−17.0
52	45	W	F	Thigh	11.1	8.9	−20.0
Mean							−17.4

*The PD's were calculated by the equation $\log_2 \times (N/N_0)$ where N is the final cumulative cell number and N_0 is the initial cell number. †Δ = (PD reticular − PD papillary/PD papillary) × 100 percent.

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human skin fibroblasts in tissue culture, cell biologists have frequently neglected to consider the structural organization of human dermis in vivo. Human dermis is geographically divided into two main areas. The uppermost region lying directly beneath the epidermis is called the "papillary" layer, and the region extending from the papillary layer to the subcutaneous fat is referred to as the "reticular" layer (*5*). In vivo, these two regions of the dermis are structurally and functionally different. The structural appearance of the collagen, elastic, and reticulin fibers, as judged by histochemical techniques, is quite different (*5, 6*). Functionally, the papillary dermis is metabolically more active and has many more fibroblasts than the reticular layer. The fibroblasts in the papillary layer are more basophilic when stained and show greater enzymatic activity than those in the reticular layer (*5*). For example, leucine aminopeptidase exhibits strong enzymatic activity in the cells of the papillary dermis but virtually no activity in the reticular layer (*7*).

In most studies concerning aging and proliferative behavior of human skin fibroblasts in vitro, skin of full thickness has been used (*8*). Since human dermis in vivo is structurally and functionally organized into two separate regions, it is important to determine whether fibroblastlike cells from these two regions exhibit differences in growth behavior in vitro. To investigate this problem, we propagated parallel cultures of papillary and reticular fibroblastlike cells which had originated from the same biopsy.

A skin specimen was taken with a 6.0-mm biopsy punch and the uppermost area of the dermis, which lies just below the epidermis, and the lowermost region, located just above the subcutaneous fat, were dissected out and placed in separate 60-mm petri dishes. The purity of each specimen was monitored by staining with hematoxylin and eosin. After 30 days in the primary culture, the cells were trypsinized and subcultured. The cells were then trypsinized once every 7 days.

A comparison of the rate of growth during a single passage between three different cell lines of papillary and reticular fibroblasts is shown in Fig. 1. Although the three lines display growth curves typical of density-inhibited populations, the saturation level achieved by the papillary cultures is always greater than the corresponding reticular cultures. Table 1 shows the population doublings (PD's) achieved at the fourth weekly passage with trypsin. In every case the PD for the reticulum-derived cells from each cell line was less than the corresponding papilla-derived ones. These results were evident regardless of the site of biopsy or donor age and sex. Three of the 13 cell lines were subcultured for their entire life-span in vitro and the PD's were calculated at termination of the culture (Table 2). It is evident that the proliferative differences measured at passage 4 are maintained throughout the life of these cells in vitro.

Table 2. Population doublings of human skin fibroblasts at termination, where termination is defined as less than one PD per week for two consecutive weeks.

Normal fibroblast cell line	Age	Race	Sex	Site	PD		Δ*
					Papillary	Reticular	
40	79	B	F	Abdomen	28.5	19.2	−32.6
44	67	B	M	Abdomen	32.2	24.9	−22.7
48	66	B	M	Abdomen	40.1	32.7	−18.5
Mean							−24.6

*Δ = (PD reticular − PD papillary/PD papillary) × 100 percent.

One of the characteristic changes associated with the aging process in human skin is dermal atrophy, especially in the papillary region. Thus, cultures of fibroblastlike cells derived from older individuals originate from tissue composed mainly of reticular dermis, resulting in less proliferative cells in vitro. This observation raises the possibility that the shortened life-span in vitro of human skin fibroblasts obtained from older adult donors (*8*) is due to the absence of papillary fibroblasts which are present in the dermis of young individuals. We cannot form any conclusions concerning the replicative capacities of papillary and reticular fibroblasts in relation to donor age. Nevertheless, our data show clearly that the papillary and reticular regions of adult human dermis can give rise to fibroblastlike cells in vitro which have different proliferative capacities while retaining similar morphological features. In addition, our results may be important in the study of certain connective tissue diseases which involve either papillary or reticular dermis. A good example is papular mucinosis, which affects only the papillary dermis.

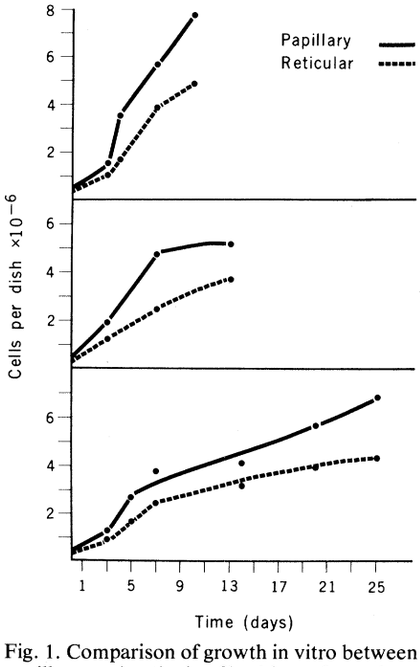

Fig. 1. Comparison of growth in vitro between papillary and reticular fibroblasts from three different cell lines. At passage three, 400,000 cells were seeded into 100-mm Falcon petri dishes containing 6.0 ml of Eagle's minimal essential medium containing 10 percent fetal calf serum, 100 units each of penicillin and streptomycin per milliliter, and 50 μg per milliliter of Mycostatin. Cells were allowed to grow at 37°C in a high-humidity incubator containing a mixture of 5 percent CO_2 in air. On the days indicated, two dishes from each line (papillary and reticular) were removed and combined and were counted in a hemacytometer. Therefore, each point on the curves is an average count of two dishes. The culture fluid was changed every third day.

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