increases in translation rates of those messages with the slowest binding constants. How changes in the relative breakdown rates could occur is not known, but, if the present results are valid, then it may be that protein breakdown in muscle can be satisfactorily explained in terms of a single system with varying affinities for different proteins according to the activity of the system rather than in terms of a two-component system with separate, selective and non-selective properties as suggested by several authors (Schimke, 1970; Knowles & Ballard, 1976; Dice & Walker, 1978).

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The Relationship between Synthesis of Ribonucleic Acid and the Decline in Proliferative Ability during Aging in vitro

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It is now well established that human mesenchymal cells have a finite lifespan in culture. After a period of rapid cell division the culture growth rate decreases, mitoses eventually cease and the culture degenerates. Hayflick (1965) termed these periods phase II and phase III respectively. It has been suggested that the loss of proliferative ability during aging in vitro may be caused by an alteration in the ability to transcribe genetic information from chromatin (Ryan & Cristafalo, 1975). To investigate this possibility, changes in RNA metabolism have been studied during various stages of the lifespan of human mesenchymal cells in vitro.

Human mesenchymal cells were cultured as described by Hill et al. (1978). The human mesenchymal cell line He125 was derived from primary culture of human embryonic lung tissue. Cells were routinely passaged by splitting cultures 1:8 when 'quiescent' (Hill, 1977). The cultures survived a total of 20–31 passages. WI-38 cells were obtained from American Type Culture Collection at passage 17 and were split 1:2 when 'quiescent'. The cultures survived a total of approx. 43 passages.

A decrease in the growth rate of the cultures is a primary expression of aging in vitro. This decline expressed as a decrease in the labelling or Cristafalo index (Cristafalo & Sharf, 1973), which represents the proportion of cells in the population that synthesize DNA during a 24 h period of labelling with [3H]thymidine (Fig. 1). The Cristafalo index of He125 cells declines gradually during the lifespan until about passage 21/25, where a sharper decline in Cristafalo index occurs. These two stages correspond to Hayflick's phase II and phase III. In contrast with He125 cells the Cristafalo index of WI-38 cells...
Passage

Fig. 1. Percentage of nuclei labelled as a function of passage number for two different human embryo diploid mesenchymal cell lines

He125 cells (a) were split 1:8 throughout their lifespan. WI-38 cells (b) were split 1:2 throughout their lifespan. Labelling index was determined by the method of Cristafalo & Sharf (1973).

hardly changes until passage 39/43, after which a sharp decline in Cristafalo index occurs. Thus, in contrast with these WI-38 cells, in the He125-cell system the kinetic properties of the cultures change progressively throughout the culture lifespan.

A decrease in chromatin-template activity has been found when comparing phase-III cultures of WI-38 cells with early phase-II cultures (Ryan & Cristafalo, 1975; Bowman, 1976). However, few reports have examined cells at different points in phase II to establish whether any alterations in chromatin-template activity precede the onset of phase III. In these studies we have investigated transcription in cultures of He125 cells throughout the culture lifespan in an attempt to determine: (1) is there a decrease in RNA synthesis during aging in vitro?; (2) if so, are the changes progressive throughout the culture lifespan, i.e. do they correlate with the kinetic properties of the cultures?

There is evidence of a decrease in the ability of cells to incorporate [3H]uridine into RNA when comparing phase-III WI-38 cells with early phase-II cells (Macieira-Coelho et al., 1966). Incorporation of [3H]uridine into RNA of 'quiescent' He125 cell populations at various stages of the culture lifespan was measured as described by Hill et al. (1978). The incorporation of [3H]uridine into acid-insoluble material consistently increased after passage 19 up to two passages before the end of the culture lifespan relative to that of early phase-II cells. However, there was a rise in uptake of precursor into the acid-soluble precursor pool. Thus alterations in incorporation into RNA may have been due to differences in precursor pool specific radioactivity and may not reflect the true rate of RNA synthesis. There was, however, no evidence for an impairment in transcription when comparing 'quiescent' cells of different passages before the onset of phase III.

To extend these studies, nuclei were prepared by a slight modification (Hill et al., 1978) of the procedure of Marzluff et al. (1973) and their ability to synthesize RNA was estimated. There was no decrease in the total RNA-synthesizing capacity of nuclei isolated from late phase-II cultures (passage 21/25) compared with those of early phase-II cultures (passage 10/25) when comparing either exponentially growing or 'quiescent' populations of cells (Table 1). Thus the decreased Cristafalo index of passage-21 cells was not accompanied by a decrease in RNA synthesis. The RNA-synthesizing capacity

Vol. 6
Table 1. Incorporation of UMP into RNA in nuclei isolated from HeI25 cultures

Passage numbers are expressed as a fraction of the total lifespan of the cultures. Results are obtained from separate experiments comparing cultures at various stages during the lifespan with passage-10 cultures. Incorporation has been normalized to that obtained from passage-10 cultures. The overall scatter did not exceed 10%.

<table>
<thead>
<tr>
<th>Passage Index</th>
<th>Cristafalo Index (%)</th>
<th>UMP Incorporation (c.p.m./µg of DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/25</td>
<td>90</td>
<td>Exponentially growing cultures: 2000</td>
</tr>
<tr>
<td>21/25</td>
<td>68</td>
<td>2542</td>
</tr>
<tr>
<td>23/25</td>
<td>62</td>
<td>1443</td>
</tr>
<tr>
<td>24/25</td>
<td>42</td>
<td>1670</td>
</tr>
</tbody>
</table>

of nuclei isolated from passage-23/25 cultures was decreased compared with that of passage-10/25 cells from exponentially growing but not 'quiescent' cultures. By passage 24/25, the RNA-synthesizing capacity of nuclei isolated from both exponentially growing and 'quiescent' cultures was significantly lower. Therefore in 'quiescent' cultures the decrease in Cristafalo index of passage-21/25 and passage-23/25 cells was not accompanied by a fall in template activity. There was no evidence of a significant change in the types of RNA produced in 'quiescent' cultures, since the proportion of total activity due to RNA polymerase II (assayed by sensitivity to 1 µg of α-amanitin/ml) was unchanged throughout the lifespan.

The results comparing RNA synthesis in isolated nuclei of phase-II cells are consistent with earlier reports on the aging of WI-38 cells in vitro (Ryan & Cristafalo, 1975; Bowman, 1976). However, for HeI25 cells, the decline in template activity in both exponentially growing and 'quiescent' cultures is expressed after a decline in Cristafalo index. These data suggest that transcriptional changes in these late-passage cultures are terminal events occurring after the expression of a decreased-growth potential.

The following conclusions can be drawn. (1) In this system there is a progressive decline in growth ability during the culture lifespan. (2) There is no evidence of a decrease in the ability of 'quiescent' cells to incorporate [3H]uridine into RNA up to the onset of phase III. (3) The transcriptional ability of nuclei isolated from exponentially growing cultures declines in late phase-II cultures; this may be an effect of a decreased growth rate. (4) The transcriptional ability of nuclei isolated from 'quiescent' cultures does not decline until phase III. (5) These data are not consistent with the suggestion that a decline in growth potential is caused by altered gene activity.

Marzluff, W. F., Murphy, E. C. & Huang, R. C. C. (1973) *Biochemistry* 12, 3440–3446

1978