Decreased Sensitivity of Old and Progeric Human Fibroblasts to a Preparation of Factors with Insulinlike Activity

C. B. HARLEY, S. GOLDSTEIN, B. I. POSNER, and H. GUYDA, *Departments of Medicine and Biochemistry, McMaster University, Hamilton, Ontario, Canada L8N 3Z5; McGill University, Departments of Medicine and Pediatrics, Montreal, Quebec, Canada H3A 1A1*

ABSTRACT To determine whether old cells have a reduced response to a preparation of factors from human plasma with insulinlike activity (ILA), we analyzed the response to ILA of early and late passage human fibroblasts from young, old, and progeric donors in the acute stimulation of [^{3}H]2-deoxy-D-glucose (2dG) uptake and the delayed stimulation of [^{3}H]thymidine (TdR) incorporation into DNA. The ILA concentration required to produce equivalent, relative stimulation of TdR incorporation was increased two- to threefold in late passage cells and cells from old and progeric donors ($P < 0.01$). 50 and 95% of maximal stimulation (ILA_{50}, ILA_{95}) was achieved by 0.26±0.07 and 1.38±0.13 ng insulin equivalents/ml (mean±SD) respectively, in cells from young adults at early passage. Corresponding values were 0.54±0.05 and 2.90±0.25 in cells from old donors; >0.9±0.1 and >3.1±0.1 in cells from a 9-yr-old progeric donor; and 0.4±0.05 and 1.1±0.04 in cells from normal children (9–13 yr). For two cell strains from young adults, ILA_{50} and ILA_{95} were 0.30±0.02 and 1.0±0.3 ng eq/ml at 30% of their in vitro lifespan completed (%LC) and these values increased at rates of 0.005 ng eq/ml per %LC and 0.04 ng eq/ml per %LC, respectively.

The mean stimulation of 2dG uptake ratio (ILA/control) decreased from early to late passage from 2.1±0.6 to 1.3±0.1 in young adult donors ($P < 0.05$), but there were no significant differences between young and old donors at either early or late passage. The mean stimulation ratio in progeric cells (1.2±0.2) did not change with in vitro passage, but was significantly lower than that of age-matched normal cells (2.1±0.8, $P < 0.001$). In progeria cells, the reduced stimulation of 2dG uptake upon addition of ILA was due to an increased basal rate of uptake (0.19±0.01 pmol [^{3}H]2dG/min per mg protein vs. 0.13±0.01 in age-matched normal cells), and not to a decline in the maximal rate of uptake (0.26±0.01 vs. 0.27±0.02, respectively). Similar results were found for in vitro aging in cells from an old donor.

INTRODUCTION

Biological aging is accompanied by a deterioration of glucose tolerance (1) and it has now been shown that this is due to reduced tissue sensitivity to insulin in the face of normal or elevated levels of circulating insulin (2). In recent years, attention has been directed to a group of insulinlike growth factors that stimulate metabolism and proliferation of various cell types (3, 4) but it is still not known whether aging diminishes tissue sensitivity to these factors in vivo. Part of the problem relates to the low levels of these hormones in blood but most importantly to their crossreactivity on radioimmune and radioreceptor assays with insulin and with each other (3, 4). Knowledge of the circulating levels of these hormones and tissues sensitivity to their actions would clearly be important in exploring the basis of the age-dependent diminution in the ability to carry out tissue growth and repair (5, 6).

We have previously investigated the response of cells in vitro to a partially purified preparation of

Dr. Harley was the recipient of a Science Scholarship from the National Research Council of Canada. His present address is Department of Biochemistry and Biophysics HSE 1504, University of California, San Francisco, California 94143. Dr. Guyda is a Research Scholar from the Province of Quebec. Dr. Goldstein's present address is Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205.

Received for publication 28 January 1981 and in revised form 27 April 1981.

peptides with insulinlike activity (ILA)[1] prepared from human plasma (7, 8). ILA produced a large, acute stimulation of glucose uptake and a large but delayed and transient stimulation of DNA synthesis. We have now attempted to determine the effect of aging on the acute and delayed response of cells to ILA. We have compared normal diploid fibroblasts from young and old donors at both early stages of passage and at late passage ("aging in vitro") (9, 10) as well as from a patient with the Hutchinson-Gilford (progeria) syndrome that manifests several features of premature aging plus severe stunting of somatic growth.

METHODS

Subjects. Normal individuals aged 9–76 yr consisted of subjects in excellent health and no known family history of diabetes mellitus or growth disorders. The progeric subject was a 9-yr-old male with the classic syndrome as described (11, 12).

Cell culture. All cultures were established in our laboratory following a 4-mm skin punch biopsy of the anterior forearm and propagated as described (13, 14), in a 37°C 5% CO_2/95% air humidified atmosphere. The culture medium consisted of Eagle's minimal essential medium supplemented with 15% fetal calf serum, nonessential amino acids, glucose to a final concentration of 15 mM, and 1 mM pyruvate. Table I lists the cell strains used and their in vitro lifespan (mean population doublings from the first passage until termination) (9). Since ~10 population doublings are involved in establishing the primary culture (15), these doublings are included in determining the percent of the in vitro lifespan completed.

Preparation of the cells for assay. Cells at various stages of the replicative lifespan were inoculated into 35-mm plastic dishes (Corning Glass Works, Science Products, Corning, N. Y.) at a split ratio of 1:4 or 1:8. Subconfluent cells were rinsed once with phosphate-buffered saline, and the growth medium was replaced with serum-free medium (growth medium minus calf serum plus 0.1% albumin) or test medium containing various concentrations of ILA. Control medium contained an appropriate volume of normal saline.

Thymidine (TdR) incorporation. Cells were prepared as above. 48 h after cells were rinsed and refed with serum-free medium, TdR incorporation was reduced to a minimum (8). ILA or control medium (normal saline) was added to the serum-free medium and a small volume of serum-free medium containing [^{3}H]TdR was then added over a 1–8-h interval centered between 16–24 h following ILA addition. Cells were then prepared for liquid scintillation counting as described (8).

Uptake of 2-deoxy-D-glucose (2dG). Cells were prepared as above but allowed to reach confluence. After incubation with the test medium for 3–4 h, cells were rinsed and assayed for uptake of [^{3}H]2dG as described (8).

[1] *Abbreviations used in this paper*: 2dG, 2-deoxy-D-glucose; ILA, insulinlike activity; ILA_{50}, ILA_{95}, concentrations of ILA required to achieve 50 or 95% maximal stimulation, respectively; %LC, percent lifespan completed; TdR, thymidine.

TABLE I
Fibroblast Cultures Used in This Study

	Cell	Donor age	In vitro lifespan (MPD)*
		yr	
Children			
Normal	A2	9	70
	RE	13	65
Progeria	P5	9	42
Young adults	EM	28	44
	TM	24	52
	J004	22	68
	CBH	23	50
Old adults	J088	76	48
	J069	67	49
	J087	76	32
	J046	67	34

* MPD = mean population doublings accumulated from time of first cellular outgrowth from explants to cessation of growth. Cultures were subdivided each time they reached confluence at a 1:4 or 1:8 split ratio counting two or three MPD, respectively, each time in the cumulative total.

Preparation of ILA. A preparation of partially purified ILA was obtained from Cohn fraction IV of human plasma by acid-ethanol extraction and Sephadex chromatography under acid conditions as described in detail (7). At this stage of purification, the ILA migrated with a molecular mass of 6–12,000 daltons on Sephadex G-75. It contained <0.01% insulin and consisted of three insulinlike peptides (acidic, near neutral, and basic) as judged by isoelectric focusing (7). Because the material was quantified during purification by an insulin radioreceptor assay, its potency has been expressed in nanogram equivalents (ng eq) of insulin in which 1 ng eq equals 25 μU of porcine insulin in the radioreceptor assay (7). The minimal specific activity of pure ILA, based on the activity of our purest preparations, is 1,075 mU/mg. The material used in the studies reported here was 2–4% pure, but it contained essentially no insulin.

Statistical tests. A two-tailed Student's *t* test for unpaired data was used to ascertain the significance of observed differences between cell strains. When testing the effect of ILA on a single strain, the alternative to the null hypothesis was that ILA stimulated growth or uptake, and hence a one-tailed Student's *t* test was used.

RESULTS

Stimulation of DNA synthesis. The time-course for incorporation of [^{3}H]TdR into late-passage cells and cells from older and progeric donors was synchronous with that of early-passage cells from young donors[2] (8). Fig. 1 shows the dose-response curve for ILA stimulation of DNA synthesis in cells from a young donor. It may be seen that quantitative differences exist in both the maximum extent of stimulation and the concentration of ILA required to achieve this

[2] Harley, C. B., and S. Goldstein. Unpublished data.

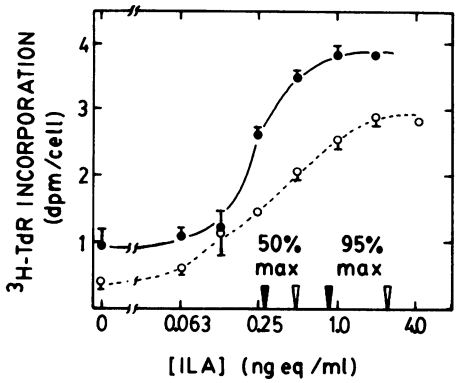

FIGURE 1 Response to ILA of early-(● —— ●) and late-passage (○ —— ○) fibroblasts (A2) to increasing doses of ILA. DNA synthesis was measured as [^{3}H]TdR incorporated into DNA during the synthetic peak from 18–24 h after addition of ILA. ILA_{50} and ILA_{95} are indicated by the arrows (▼ early passage; ▽ late passage). Mean (±SD) for replicate cultures in a single experiment are shown.

maximum. The ratio of maximally stimulated DNA synthesis in ILA-treated cells to that of control (saline-treated) cells was greater in early-passage cultures compared to late-passage cultures from all donors, but there was considerable variability in this parameter (Table II). Serum starvation inhibits DNA synthesis to varying degrees day to day, and the stimulation ratio is very sensitive to the "basal" rate since it is small and appears in the denominator of the ratio. Although cells from old and progeric donors had lower absolute incorporation of [^{3}H]TdR than young, normal cells[2], the interpretation of these data requires a comparison of the efficiency of serum starvation and the intracellular specific activity of [^{3}H]TdR triphosphate in the different cell strains.

A reliable index for comparison of the various cells is the relative sensitivity to ILA expressed as the concentrations required to achieve 50 or 95% of the maximal stimulation (ILA_{50} and ILA_{95}, respectively) (Fig. 1). These parameters correlated with both cellular age in vitro (Fig. 2) and donor age (Fig. 3). For two young normal strains (Fig. 2), ILA_{50} was 0.3±0.02 ng eq/ml insulin at 30% of their lifespan completed (30%LC) and increased at a rate of 0.005 ng eq/ml per %LC ($P < 0.001$). This gives a doubling time for ILA_{50} of 60% of the total in vitro lifespan. ILA_{95} was 1.0±0.3 ng eq/ml at 30% LC and increased at a rate of 0.04 ng eq/ml per % LC ($P < 0.005$), a doubling time of 25% of the lifespan. The relatively greater rate of increase in ILA_{95} reflects a flattening of

TABLE II
ILA Stimulation of [^{3}H]TdR Incorporation into Fibroblast DNA Expressed as the Ratio to Control

Donors (N)*	Passage level	Mean ratio±SD (n)*
Young (6)	Early	6.6±3.8 (9)
	Late	3.0±1.8 (7) ($P < 0.05$)
Old (4)	Early	11±10 (3)
	Late	6±2 (4)
Progeria (1)	Early	8.0±2.5 (3)
	Late	2.5±1.6 (2) ($P < 0.10$)

Data represent the mean ratio for the ILA-stimulated incorporation [^{3}H]TdR into acid-insoluble material vs. untreated controls during a 1–8-h interval centered about the peak of DNA synthesis following exposure to ILA. Early- and late-passage levels refer to <40% and >75% of the in vitro lifespan completed, respectively. Differences between donor groups at either early or late passage were not significant. Differences between individual strains within a donor group at corresponding passage levels were not significant.

* N is the number of strains tested and *n* is the number of independent experiments.

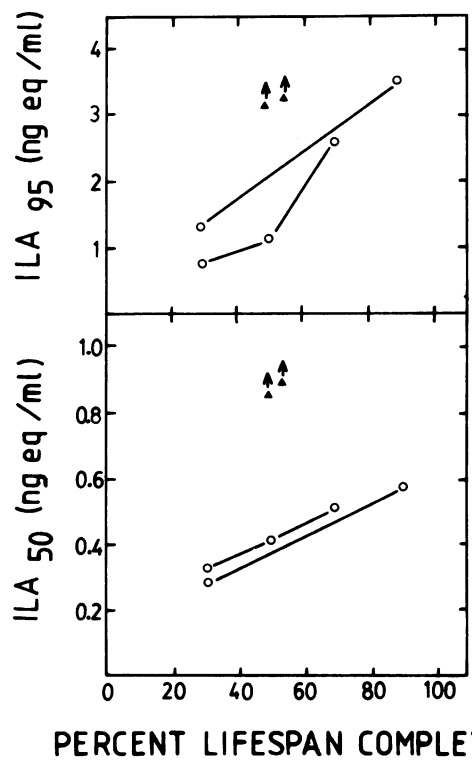

FIGURE 2 Effect of cell passage on ILA_{50} and ILA_{95} for DNA synthesis in cultured cells. Dose-response curves were generated as in Fig. 1 and the results for ILA_{50} (bottom panel) and ILA_{95} (top panel) were plotted against the percent lifespan completed. Arrows indicate a minimum estimate as maximum stimulation could not be attained at the top dosage of ILA used (4.1 ng eq/ml). Two strains of normal fibroblasts (○ —— ○), were used (A2 and J004). The rate of increase in ILA_{50} and ILA_{95} was determined by linear regression on the pooled data for these strains (see text). The single progeric strain (▲) was examined in two experiments at mid-lifespan.

the dose-response curve with in vitro aging, as seen in Fig. 1. Progeria cells at mid-lifespan were significantly less sensitive to ILA than the age-matched normals, requiring >0.9 ng eq/ml ($P < 0.001$) and 3.1 ng eq/ml ($P < 0.01$) to obtain 50 and 95% of the maximal stimulation, respectively. Fig. 3 shows the relative sensitivity to ILA of cells from young, old, and progeric donors at early passage. There was a significant increase in ILA_{50} and ILA_{95} in both progeria and old adults. In some assays of cells from progeric and old donors, the maximal response to ILA could not be obtained from the dose-response curve. In such cases, minimum estimates of ILA_{50} and ILA_{95} were used in the statistical comparisons. The true values of these parameters would show even greater differences than those reported here. Differences between children (9–13 yr) and young adults (22–28 yr) were not significant. Between young and old adult donors, the mean increase in ILA_{50} was 0.007 ng eq/ml per yr and for ILA_{95}, 0.04 ng eq/ml per yr. The doubling time for both ILA_{50} and ILA_{95} was ~40 yr. These rates are remarkably similar to the increases seen per percent lifespan completed during the in vitro passage of cells from young donors.

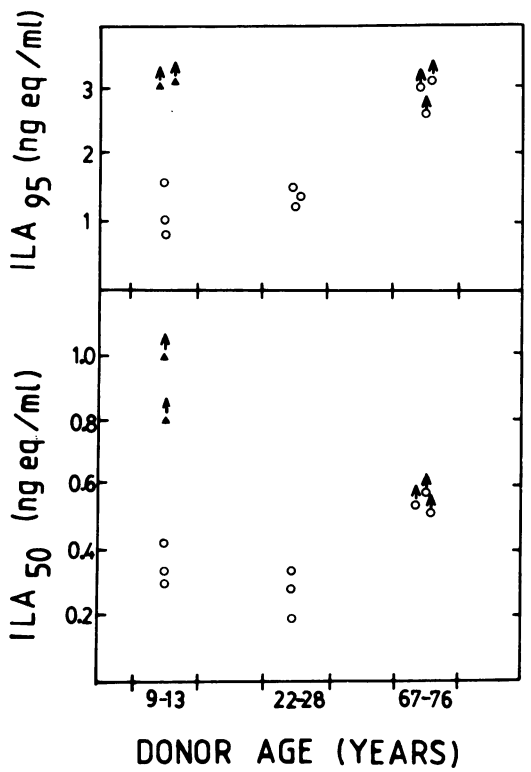

FIGURE 3 Effect of donor age on ILA_{50} and ILA_{95} for DNA synthesis. Cells from donors of various ages indicated were assayed at early passage (<60% LC). Arrows indicate minimum estimates because maximum stimulation was not attained at the uppermost ILA dose of 4.1 ng eq/ml. Data from all 11 donors (Table I, except EM and J088, which were not assayed at early passage) are shown. Two or three different cell strains were used in each normal age group (○) while the progeric strain (▲) was examined on two separate occasions.

TABLE III
Effect of ILA on Stimulation of 2dG Uptake by Fibroblasts

Donor (N)	Age (yr)	In vitro age (%LC)	Uptake ratio (ILA/control) mean±S.D.	(n)
Normal child (1)	9	<40	2.0±0.4	(9)
		40–55	2.3±1.0	(11)
		>75	1.7±0.4	(3)*
			mean 2.1±0.8	(23)
Progeria (1)	9	<40	1.3±0.2	(2)
		>75	1.2±0.1	(3)
			mean 1.2±0.2	(5)*
Young adults (3)	22–28	<50	2.1±0.6	(4)*
		>85	1.3±0.1	(2)‡
Old adults (4)	67–76	<45	2.7±1.3	(5)
		>55	1.6±0.2	(2)‡

Uptake of 2dG was measured 3–4 h after addition of ILA (4 ng eq/ml) or control medium as described in Methods. The mean ratio of the treated to untreated uptake rates±SD of n experiments is reported for cells from N donors at the indicated stages of their in vitro lifespan.

* Effects of ILA on progeria cells were significantly less than effects on age-matched or adult normals at early passage ($P < 0.01$).

‡ There was a significant decline in the uptake rate as a function of %LC for young adults ($r = -0.997, P < 0.0005$) and old adults ($r = -0.671, P < 0.05$) but not for the normal child. Differences between normal donors were not significant.

Stimulation of 2-deoxy-glucose (2dG) uptake. Because DNA synthesis is a delayed and transient response to ILA exhibited by a relative minority of the cell population (8), we determined the effects of aging on the stimulation of 2dG uptake.

Although ILA stimulation of the rate of 2dG uptake occurs as an acute cellular response, i.e. within minutes, maximal stimulation of uptake requires 2–4 h of incubation with ILA (8). In the experiments reported here, we have compared the rates of 2dG uptake during a 15 min interval, 3–4 h after addition of ILA. ILA had a significant effect on the rate of 2dG uptake for each strain ($P = 0.0001$–0.04). However, late-passage cells were stimulated less than early-passage cells for all donor types, with significant differences observed in cells from young and old adults (Table III). The progeria response was significantly less than either the age-matched controls or all normal cells combined ($P < 0.01$).

Because higher rates of glucose consumption have been observed in late-passage cells and in cells from

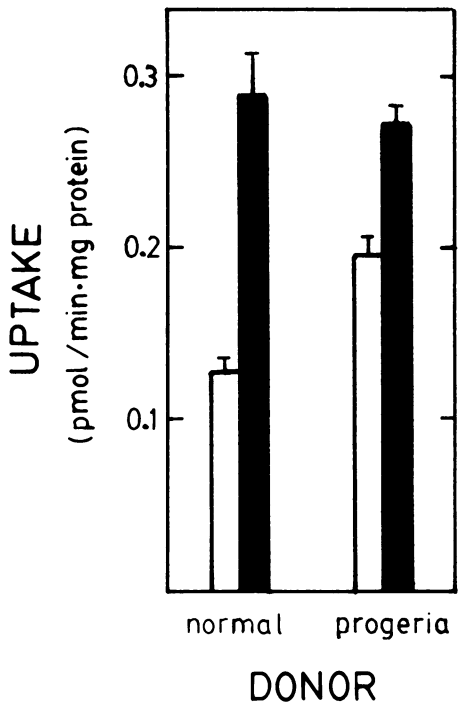

FIGURE 4 Basal and stimulated rates of 2dG uptake in normal and progeric cells. Confluent, early-passage cells from the progeria donor (40% LC) and early-passage cells from the age-matched normal child (A2, 26% LC) were assayed for the rate of 2dG uptake during a 15-min interval 3 h after addition of ILA or control medium (Methods). The bars represent mean rates (±SD) of three or four determinations in a single experiment.

progeria and old donors[3] (16, 17) we compared the basal (saline-treated) and ILA-stimulated rates of 2dG uptake in progeria and the age-matched normal strain (Fig. 4). There was no significant difference between normal or progeria cells in the maximal ILA-stimulated rate of 2dG uptake. The overall reduced response of progeric cells was due to a significantly elevated basal rate ($P < 0.001$). Qualitatively similar results were obtained with cells from an old donor (J069). The reduced response to ILA as a function of in vitro age (29 vs. 72% LC) resulted from a small (12%) decrease in the stimulated rate of uptake ($P < 0.05$) and an elevated basal rate (21% increase, $P < 0.07$). Comparison of absolute rates of 2dG uptake as opposed to stimulated/basal ratios (Table III) requires precise knowledge of specific activity and cellular densities and was not done in the other strains listed in Table III.

DISCUSSION

We have analyzed the sensitivity of early- and late-passage human fibroblasts from young, old, and progeric donors to a preparation of factors with ILA using two parameters, the acute response of glucose uptake and the delayed response of DNA synthesis. Our observations indicate (*a*) that the sensitivity of cells from young and old donors to ILA stimulation of DNA synthesis decreased with in vitro age. That is, the concentration of ILA required to produce equivalent, relative stimulation of [^{3}H]TdR incorporation increases with passage, doubling in an interval representing ~50% of the in vitro lifespan. (*b*) The sensitivity of cells from old donors and a subject with progeria is less than that of cells from young, normal donors. Old and progeria cells at early passage require 2–3 times as much ILA as young cells to produce the same relative stimulation of DNA synthesis. (*c*) At early passage, there is no significant difference in stimulation of 2dG uptake between normal donors of any age group (9, 22–28, and 67–76 yr), but the response of progeric cells is greatly reduced. (*d*) The stimulation of 2dG uptake decreases as a function of in vitro age in cells from young and old adult donors. (*e*) The apparent reduced response of progeric and late-passage cells to ILA stimulation of 2dG uptake is due primarily to an elevated basal rate of uptake.

The reduced sensitivity of late-passage cells and cells from old and progeric donors to stimulation of DNA synthesis by ILA cannot be ascribed to a reduced acute metabolic response to glucose because cells from old donors responded as well, or better than cells from young donors in the assay for 2dG uptake. Thus, the decreased proliferative capacity of old, progeric and late-passage cells is likely to be a primary defect rather than a consequence of decreased glucose entry. A recent report (18) shows that while insulin and multiplication stimulation activity, a related insulin-like growth factor, exert their effects on glucose metabolism in rat adipocytes via the insulin receptor, their effect on DNA replication in human fibroblasts acts through a different (unidentified) receptor. Our data, then, suggest that there may be a diminution in the number, affinity and/or sensitivity of this particular receptor with donor age and with tissue culture age. The status of insulin receptors has been measured on cultured fibroblasts as a function of donor age (19, 20), and no significant change was found in receptor number, but an age-dependent increase in affinity was observed in one report (19). No studies have yet been published on the status of insulin receptors on cultured fibroblasts as a function of passage level. However, Ladda (21) has reported that receptors for epidermal growth factor increase per cell but not per surface area in the progressively larger late-passage cells. He also reported a shift to the right in the epidermal growth factor dose-response curve for DNA synthesis at late-passage (21).

Our data show a diminished sensitivity of cultured

[3] Goldstein, S., S. R. Ballantyne, A. L. Robson, and E. J. Moerman. In preparation.

human fibroblasts in their response to ILA stimulation of DNA replication as a function of culture age, donor age, and in progeria. This may explain the decreased ability of elderly people to repair tissue, and the increased repair time at sites of chronic cell turnover in persons of any age. In this latter situation, we speculate that cells in vivo have consumed more of their replicative lifespan and hence behave like late-passage cells in vitro. The data may also explain, at least in part, the poor growth of mesenchymal tissues in progeria and the severe stunting of growth. The reduced sensitivity of progeric and late-passage cells to ILA may not be specific for this group of peptides since the growth rate and net protein synthesis in response to serum stimulation are also reduced in these cells.[2] It is of interest that somatomedin-C levels in the progeric subject were within normal limits of age-matched children.[4]

The increased basal uptake of 2dG in late-passage and progeric cells could result from a higher rate of passive diffusion in these cells. We have previously shown that ILA does not affect this component of uptake, but rather stimulates specifically facilitated transport (8). Because the maximal rate of uptake in late-passage and progeric cells was slightly reduced in comparison to young normal cells, the facilitated transport component of uptake would show an even greater reduction if passive diffusion were in fact elevated in these cells. It would be of interest to determine the reason for the increased basal rate of 2dG uptake in late-passage and progeric cells. There are at least five possibilities. First, these cells may be unable to "down-regulate" metabolism in response to conditions that slow growth. Second, if termination of the replicative lifespan of human fibroblasts is a form of differentiation (22–25) then such post-mitotic fibroblasts may simply require a higher metabolism of glucose. Third, the increased basal activity of late-passage and progeric cells may be the normal response of cells to prolonged interference of cell replication: they may be poised for growth, but blocked by a defect in completing the cell cycle. Fourth, there may be a defect in oxidative energy metabolism, thus increasing the demand for glucose to supply energy via the alternate pathway of glycolysis. Indeed, late-passage and progeric fibroblasts consume more glucose than early-passage or age-matched normal cells (16, 17). Finally, the integrity of the cell membrane may deteriorate in late-passage and progeric cells, allowing greater passive diffusion of small molecules. As noted above, however, this is not a complete explanation since total uptake in the presence of ILA is not increased. Although it remains difficult to distinguish between these alternatives, our data clearly demonstrate the dichotomy that exists in fibroblast responsiveness to ILA on an acute metabolic effect vs. a delayed effect on DNA synthesis. Further metabolic studies, for example at different stages of growth in young and old cells, may clarify the mechanisms whereby ILA, insulin, and related peptides exert their effects on these two parameters and how biological aging impairs one and not the other.

[4] Bala, M., and S. Goldstein. Unpublished data.

ACKNOWLEDGMENT

This work was supported by the Medical Research Council of Canada grants MT-3515, MT-4182 and MT-4403.

REFERENCES

1. Andres, R. 1971. Aging and diabetes. *Med. Clin. N. Am.* **55:** 835–845.
2. DeFronzo, R. A. 1979. Glucose intolerance and aging. Evidence for tissue insensitivity to insulin. *Diabetes.* **28:** 1095–1101.
3. Van Wyk, J. J., M. E. Svobova, and L. E. Underwood 1980. Evidence from radioligand assays that somatomedin-C and insulin-like growth factor I are similar to each other and different from other somatomedins. *J. Clin. Endocrinol. Metab.* **50:** 206–208.
4. Rechler, M. M., H. J. Eisen, O. Z. Higa, S. P. Nissley, A. C. Moses, E. E. Schilling, I. Fennoy, C. B. Bruni, L. S. Phillips, and K. L. Baird. 1979. Characterization of a somatomedin (insulin-like growth factor) synthesized by fetal rat liver organ cultures. *J. Biol. Chem.* **254:** 7942–7950.
5. Goldstein, S. 1971. The biology of aging. *N. Engl. J. Med.* **295:** 1120–1129.
6. Goldstein, S. 1979. Senescence. *In* Endocrinology. L. J. Degroot, G. F. Cahill, W. D. Odell, L. Martini, J. T. Potts, D. H. Nelson, E. Steinberger, and A. I. Wingrad, editors. Grune & Stratton, Inc., New York. **3:** 2001–2028.
7. Posner, B. I., H. J. Guyda, M. T. Corvol, R. Rappaport, C. Harley, and S. Goldstein. 1978. Partial purification, characterization, and assay of a slightly acidic insulin-like peptide (ILAs) from human plasma. *J. Clin. Endocrinol. Metab.* **47:** 1240–1250.
8. Harley, C. B., S. Goldstein, B. I. Posner, and H. J. Guyda 1980. Insulin-like peptides stimulate metabolism but not proliferation of human fibroblasts. *Am. J. Physiol.* **239:** E125–E131.
9. Hayflick, L. 1965. The limited in vitro lifetime of human diploid cell strains. *Exp. Cell Res.* **37:** 614–636.
10. Goldstein, S. 1978. Human genetic disorders which feature accelerated aging. *In* The Genetics of Aging. E. L. Schneider, editor. Plenum Publishing Corp., New York. 171–224.
11. Singal, D. P., and S. Goldstein 1973. Absence of detectable HL-A antigens on cultured fibroblasts in progeria. *J. Clin. Invest.* **52:** 2259–2263.
12. Goldstein, S. and E. Moerman 1975. Heat-labile enzymes in skin fibroblasts from subjects with progeria. *N. Engl. J. Med.* **292:** 1306–1309.
13. Goldstein, S., and J. W. Littlefield 1969. Effects of insulin on the conversion of glucose-C-14 to C-14-0_2 by normal and diabetic fibroblasts in culture. *Diabetes.* **18:** 545–549.
14. Goldstein, S., E. J. Moerman, J. S. Soeldner, R. E. Gleason, and D. M. Barnett 1979. Diabetes mellitus and genetic prediabetes: decreased replicative capacity of cultured skin fibroblasts. *J. Clin. Invest.* **63:** 358–370.

15. Goldstein, S., J. W. Littlefield, and J. S. Soeldner 1969. Diabetes mellitus and aging: diminished plating efficiency of cultured human fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* **64:** 155–160.
16. Goldstein, S., and G. Trieman 1975. Glucose consumption by early- and late-passage diploid human fibroblasts during growth and stationary phase. *Experientia (Basel).* **31:** 177–180.
17. Goldstein, S., S. R. Ballantyne, A. L. Robson, and E. J. Moerman 1980. Energy metabolism during aging of human fibroblasts in vitro. Proceedings of the Annual Meeting of the Canadian Association on Gerontology. Saskatoon, Canada.
18. King, G. L., C. R. Kahn, M. M. Rechler, and S. P. Nissley 1980. Direct demonstration of separate receptors for growth and metabolic activities of insulin and multiplication-stimulating activity (an insulinlike growth factor) using antibodies to the insulin receptor. *J. Clin. Invest.* **66:** 130–140.
19. Rosenbloom, A. L., S. Goldstein, and C. C. Yip 1976. Insulin binding to cultured human fibroblasts increases with normal and precocious aging. *Science (Wash. D. C.).* **193:** 412–415.
20. Hollenberg, M. D., and E. L. Schneider. 1979. Receptors for insulin and epidermal growth factor-urogastrone in adult human fibroblasts do not change with donor age. *Mech. Aging Dev.* **11:** 37–43.
21. Ladda, R. L. 1979. Cellular aging in vitro: altered responsiveness of human diploid fibroblasts to epidermal growth factor. *In* Recent Advances in Gerontology. Proceedings of the XI International Congress of Gerontology, Tokyo, Japan. H. Orimo, K. Shimada, M. Iriki, and D. Maeda, editors. *Excerpta Med. Int. Congr. Ser.* 85–86.
22. Cristofalo, V. J. 1972. Animal cell cultures as a model system for the study of aging. *Adv. Gerontol. Res.* **4:** 45–79.
23. Martin, G. M., C. A. Sprague, T. H. Norwood, and W. R. Pendergrass 1974. Clonal selection, attenuation, and differentiation in an in vitro model of hyperplasia. *Am. J. Pathol.* **74:** 137–150.
24. Bell, E., L. F. Marek, D. S. Levinstone, C. Merrill, S. Sher, I. T. Young, and M. Eden 1978. Loss of division potential in vitro: aging or differentiation? *Science (Wash. D. C.).* **202:** 1158–1163.
25. Goldstein, S. 1980. Do we differentiate ourselves to death? *Cell.* **20:** 571–573.