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Aging at the Cellular Level

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Aging, as a biological phenomenon, has most often been treated in connection with death. Commonly, it is conceived as an explanation and a cause of natural death. However, since natural death is very rare, a connection between aging and death is not always obvious. For that reason, aging is often defined as a process leading to decrease in the survival capacity of the individual organism[1,2]. Age changes are assumed to be a consequence of differentiation and resulting specialization of cells[3,4]. They have generally been tied to the postreproductive period[2]. Aging and death have often been considered a price paid by organisms for differentiation and higher organization[3,5].

Since there is no differentiation or natural death in unicellular organisms, the very existence of the aging process in microbial cells has been denied[2]. However, with proper technique, the beginning of aging can be discerned at an early stage of cell development at which no differentiation takes place. Observations on microbial cells actually indicate that aging is not a consequence of differentiation and does not necessarily involve death as its outcome. The basic aspects of aging, as a universal biological process, can be studied under controlled conditions and without any resort to teleological motivations. One of the most salient characteristics of aging at the cellular level is a change in metabolic pattern in the course of cell development.

Photosynthesis in the Course of Cell Development. Observations on metabolic activity in microbial cells have indicated that the activity does not remain constant in the course of the life cycle of the cells but undergoes variations whose direction, timing, extent, rate, and dependence on external factors are closely bound with the developmental status of the cells[6–12]. The course of photosynthetic activity during the life cycle of algal cells is described in Figure 1.

Measurements were made on cells of the high-temperature strain of the green unicellular alga Chlorella 7-11-05[13] synchronized by light:dark technique[7,11]. After 3 or 4 cycles of light and dark, more than 99% of the cells in existence at the end of the dark period were small daughter cells. In this condition, the algal suspension was transferred into light. Manometric determinations of photosynthetic gas exchange were then made at predetermined intervals on aliquots of synchronized cells. The developmental stages of cells were indicated in hours as time passed from the beginning of the growth period (beginning of illumination) to the moment of harvesting cells from the growth vessels[7,8].

Photosynthetic activity was usually low at the beginning of the life cycle of cells. It generally increased with progress in cell development, reached a peak, and then declined toward the end of the life cycle. Re-

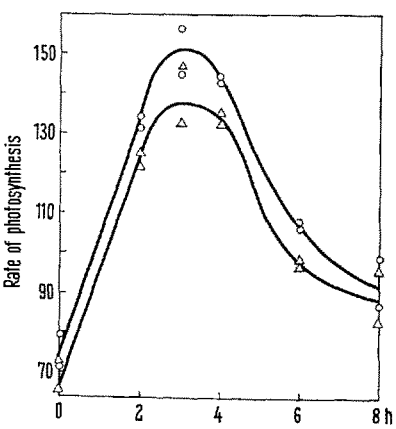

Fig. 1. Rates of apparent photosynthesis in mm^3 O_2 or CO_2/mm^3 packed cells and h in the course of cell development of *Chlorella* 7-11-05. Measurements in 0.02 *M* KH_2PO_4 buffer, pH 4.5, by indirect two-vessel method[7]. Circles, oxygen evolution; triangles, carbon dioxide consumption.

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covery of photosynthetic capacity took place during the period when cells were dividing in the dark and extended over a portion of the subsequent light period[7,9].

Similar curves were obtained by a number of investigators[14–16]. In other studies, the initial rising portion was missing from the time-course curve and the activity seemed to decline from the very beginning of cell development through the whole life cycle[17–19]. A decline of the time-course curve and occurrence of the lowest rate at the end of the life cycle are the most common and, therefore, most characteristic features of change in photosynthetic activity in the course of cell development.

The photosynthetic capacity of older cells was found to be inferior in both nitrogen-containing and nitrogen-free media[7,9], and in such diverse suspending fluids as phosphate buffer at pH 4.5[7], bicarbonate buffer at neutral pH[20], and carbonate-bicarbonate buffer at pH above 9[21]. Determinations were made by manometric[7,9,14,18], polarographic[15,16], and ^{14}C techniques[16,19] used under various conditions as to temperature[22] and illuminance[23]. The decline in photosynthetic activity as the cells advanced in age was demonstrated both for low-temperature strains[15,16,19] and a high-temperature strain[6,7,9–12] of *Chlorella pyrenoidosa*, as well as for *Ch. ellipsoidea*[18,24], *Scenedesmus*[14], and colonial green alga *Hydrodictyon*[17]. In various studies the decline in photosynthetic activity has been ascertained by calculations on packed cell volume basis, dry weight of cells, or on the basis of nitrogen and chlorophyll content.

Data on *Hydrodictyon* are particularly interesting because this organism does not need to be artificially synchronized for studies on metabolism throughout its life cycle. The development of the individual cells of the colony, highly synchronized at the beginning of the life cycle, is believed to proceed with a high degree of synchronization also at later developmental stages up to the moment of reproduction. NEEB[17] maintained this alga on intermittent, 12h light:12 h dark, regimen over the period of 34 days. Photosynthetic activity changed during each light:dark cycle, reaching its peaks during the light periods. However, followed over the life cycle as a whole, photosynthetic capacity of cells clearly showed a decline toward the time of reproduction.

Photosynthetic activity in older cells was found to become saturated at lower light intensities than that in younger cells and to be more subject to deleterious effects of strong light[9]. Studies at light intensities below saturation also proved the inferior photosynthetic capacity and, therefore, the lower quantum efficiency of older cells[25].

In prolonged measurements, cells of different developmental stages showed peculiarities characteristic of their age (Figure 2)[10–12,20–23]. Vigorously metabolizing young cells were capable of increasing their photosynthetic rate with time even in the absence of nitrogen from the suspending fluid. In sluggish old cells, photosynthetic activity invariably declined with time. In cells of intermediate age suspended in nitrogen-free phosphate buffer, the activity first decreased to a low level and then recovered; depending on the age of the cells, it regained the level observed at the beginning of the experiment or rose above that level.

Variations in activity with time were clearly influenced by external factors such as temperature and light intensity[22,23]. Under a given set of experimental conditions, however, the pattern of variation was dependent on the developmental stage of the cells. The decline in activity with time generally intensified and the capacity to recover to a higher level of activity invariably declined as the cells became older.

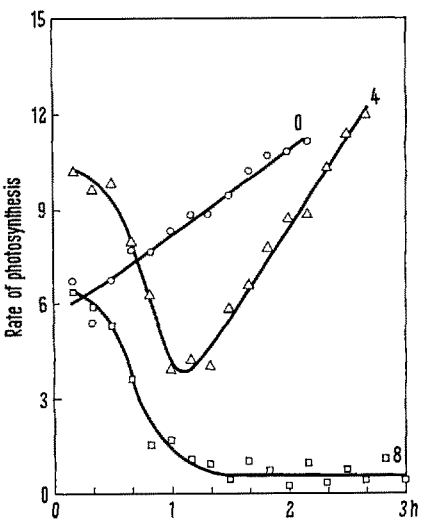

Fig. 2. Rates of photosynthetic gas exchange in the course of a photosynthesis experiment for cells of successive developmental stages of *Chlorella* 7-11-05. Increments in manometric readings (mm change) for 10-min intervals were corrected for the volume of the gas phase and the packed cell volume. Measurements in 0.02 *M* KH_2PO_4 buffer, pH 4.5, by one-vessel method. Developmental stages of synchronized cells are indicated in h on the curves[11].

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Respiration in the Course of Cell Development. Studies on respiration also revealed regular variations in respiratory activity concomitant with developmental changes in microbial cells. Both endogenous and exogenous glucose respiration rates for synchronized cultures of *Chlorella* 7-11-05 were observed to be lowest in small daughter cells at the end of the dark period (Figure 3). In the initial growth period of illuminated cells, respiratory activity rapidly increased. It soon reached a peak, and then slowly declined until the time of cell division[6,8].

In other investigations, presumably because measurements of respiration were not begun soon enough to catch on initial rising portion of the time-course curve[8], the activity was observed to be highest at the beginning of cell development and to decline during the whole life cycle of cells. NEEB[17] obtained this kind of curve for a naturally synchronized culture of the colonial alga *Hydrodictyon*. He started observations 2 days after the beginning of cell development and continued them over 34 days. Respiration activity was highest in his first measurements and steadily declined until the time of reproduction.

ZEUTHEN and SCHERBAUM[26] placed a single cell of the ciliate protozoan *Tetrahymena pyriformis* in a microrespirometer and followed respiration activity over several generations of cells. The lowest observed respiration rate for the progenies of naturally synchronized cells occurred in the pre-division period. SASA[27] reported a decline in glucose respiration rate over a considerable portion of the life cycle of *Ch. ellipsoidea*. Contrary to this, endogenous respiration was described in SASA's communication, as well as in an earlier report by members of the same group[18], as rising over the part or over the whole life cycle of algal cells.

An increase in endogenous respiration over part or over the whole illumination period observed by the above-mentioned investigators suggests a possibility that, owing to the specificity of the synchronization technique employed by these investigators, cells used to start a synchronized culture had been depleted of much of their respiratory substrate. During starvation, degradation of enzyme systems would not be balanced by rebuilding processes. Naturally, after returning cells to bright light, their respiration rate would be expected to be low and rising with time, the length of the rising portion of the time-course curve depending on the degree of starvation imposed on cells during preculture period. The limitations in using endogenous respiration as an indicator of the metabolic capacity of enzyme systems were discussed in an earlier communication[8]. The inherent capacity of metabolic mechanism is expected to be more adequately expressed if careful precautions are taken against starving cells.

Organic Synthesis in the Course of Cell Development. The overall metabolic activity of a cell focuses on its capacity for organic synthesis. The dependence of synthetic activity of a cell on its developmental status has been studied on synchronized suspensions, on individual cells, and on cell populations separated from non-synchronized suspensions by centrifugation.

Synchronized cells of *Chlorella* 7-11-05 were grown in a complete nutrient medium under optimal conditions. They were harvested at the ages of 0, 2, and 8 h, centrifuged, resuspended in the same medium, and placed back under the same conditions as before centrifugation. In Figure 4, their growth as change in

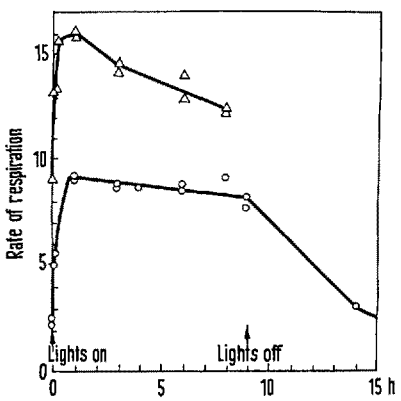

Fig. 3. Rates of endogenous and glucose respiration in mm^3 O_2/mm^3 packed cells and h in the course of cell development of *Chlorella* 7-11-05. Circles, endogenous respiration; triangles, glucose respiration[8].

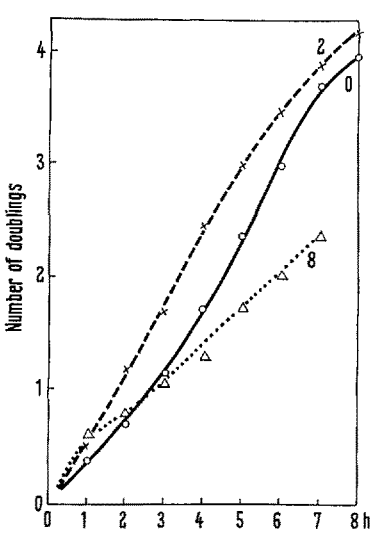

Fig. 4. Time course of organic synthesis in cells of successive developmental stages of *Chlorella* 7-11-05 resuspended in complete nutrient medium. Developmental stages of synchronized cells are indicated on the curves[28].

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optical density is plotted in terms of $\log_2$ against time of observation[28]. The plot indicates number of doublings of cell material in the course of cell growth. At the end of 6 h of observation, there had been 3 doublings of cell material in 0-h cells, $3^1/_2$ doublings in 2-h cells, but only 2 doublings in 8-h cells. The difference would be even more dramatic if the 0- and 2-h cells were compared at the peak of their performance with the 8-h cells at the period of their lowest performance.

As is true also for other metabolic activities, growth is lower at the beginning of the life cycle than at a later developmental stage. For the 0-h cells, maximum growth rate was recorded for the time period from the 3rd to the 7th h; if this rate is calculated per 24-h period, it reaches 17.3 doublings of cell material. In 2-h cells, there was almost no lag phase, and the growth rate for the period of maximal activity, from the 1st to the 5th h, was calculated as 16.2 doublings per 24-h period. The 8-h cells started their growth at a fairly high rate, but this rate declined for the second h of measurements (from the 1st till the 2nd h) to only 4.2 doublings per day. This was the time when most of the cells divided. Then the rate increases to 7.8 doublings per day, for the period from the 3rd to the 7th h. The rate failed to approach that of the 0-h or the 2-h cells because the synchronization in 8-h cells after 6 to 8 h in light was broken, some of the cells remaining in the division phase while others, which divided earlier, had reached relatively old age.

The inherent difference in the synthetic capacity of cells at different development stages was revealed even more clearly when cells, after being harvested and centrifuged from a complete nutrient medium, were resuspended in phosphate buffer (0.02*M*, pH 6.0) (Figure 5). In 0-h and especially in 2-h cells there was an appreciable organic synthesis, and after about 2 h of observation the cell material doubled. In the absence of external nitrogen these cells evidently could draw on their internal pool of nitrogenous intermediates for rebuilding their enzyme systems. The rate of synthesis was, of course, declining with time, and the second doubling of cell material took more than 6 h. In 8-h cells the synthetic activity was weak and even after 7 h of observation there had been only about 0.5 doubling of cell material.

Studies on synchronized cultures of *Scenedesmus obliquus*[29] and of the low-temperature strain of *Chlorella pyrenoidosa*[16,30], like those on synchronized cultures of *Chlorella* 7-11-05, indicated that growth rate declined in the course of cell development. The decline was larger for *Chlorella* cultured in red light[30]. However, it was clearly noticeable also for this strain grown in blue light[30] and for both *Chlorella* and *Scenedesmus* maintained in white light[16,29].

It must be emphasized that in none of the experiments of other investigators just mentioned was the light period long enough to permit organisms to complete their normal life cycle. *Scenedesmus* cultures[29] were kept in light for 14 h and *Chlorella* cultures[16,30] for 16 h. METZNER and LORENZEN[16] stated that under the conditions of their investigations cell division started only after 20 h of light. If cells had been permitted to develop in light over longer periods, the growth rate in older cells would have dropped to a much lower level and the amplitude of differences in growth rates between young and old cells would have been considerably greater.

The decline of growth rate toward the time of cell division has been demonstrated also for synchronized cultures of the protozoan *Tetrahymena*[31] and for single cells of *Amoeba*[32,33]. For bacterial cultures of *Streptococcus faecalis* the decreasing rate of growth over one cell cycle has been shown by MITCHISON[34].

The pertinence of the evidence obtained with synchronized cells to the problem of aging depends upon whether it reflects metabolic events normally taking place in cell development. 'The core of the problem', as SCHERBAUM put it, 'resides in the effect of the synchronizing agent upon the normal metabolism in the cellular life cycle'[35].

The effect of a synchronizing agent on the subsequent performance of cells cannot be denied any more than the effect of any other environmental condition.

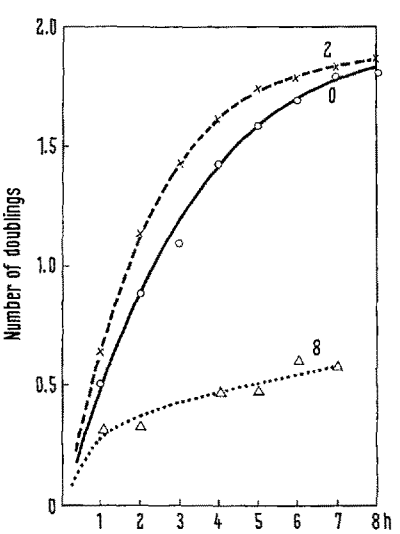

Fig. 5. Time course of organic synthesis in cells of successive developmental stages of *Chlorella* 7-11-05 resuspended in 0.02*M* KH_2PO_4, pH 6.0. Developmental stages of synchronized cells are indicated on the curves[28].

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However, the general decline in metabolic activity during the life cycle of cells observed in synchronized microbial populations has been confirmed on naturally synchronized cells of *Hydrodictyon*[17] and on individual cells of *Amoeba* and *Tetrahymena*[26,32,33] subjected to no synchronization treatment. Even more direct confirmation of the validity of data obtained on synchronized algal cells comes from observations on young and old cells separated from non-synchronized populations by fractional centrifugation.

In the experiment represented in Figure 6, a non-synchronized suspension of *Chlorella* 7-11-05 was grown under optimal conditions and then subjected to fractional centrifugation. Two fractions were obtained, one consisting chiefly of small cells and the other predominantly of large cells. These fractions were representative, respectively, of the young and old cells. Parallel observations on the synthetic activity in terms of optical density, dry weight, and packed volume of cells after these two fractions were resuspended in phosphate buffer at pH 6 and placed in light, in an atmosphere of 4% CO_2-in air-gas mixture, indicated the superior activity of younger cells. Similar measurement of photosynthetic activity in the two kinds of cells revealed that the rate of photosynthetic gas exchange in younger cells was generally higher than in older cells.

In these experiments the cells, before being centrifuged out of a non-synchronized suspension, were subjected to the same environmental conditions. The experiments with non-synchronized cells separated by fractional centrifugation suggest that use of a synchronizing agent, whatever its effect, does not distort the basic trends of metabolic activity in the course of cell development.

Accumulation of Cell Constituents and Changes in Chemical Composition in the Course of Cell Development. Several investigators have reported a decline in the rate of nitrogen accumulation and/or a lower protein/carbohydrate ratio in older cells[24,29,30,36–38]. STANGE et al.[39], using ^{14}C technique, demonstrated an increased incorporation of carbon into sucrose and a decreased incorporation into alanin toward the time of cell division.

A higher protein-carbohydrate ratio in younger cells should not be considered to prove that these cells have a lower capacity for carbohydrate synthesis, but rather to indicate a competition between synthesis of protein and synthesis of carbohydrate. In the presence of external nitrogen, the balance is tipped in favor of protein during the earlier stages of the life cycle. In the absence of external nitrogen, the formation of protein is suppressed and carbohydrate synthesis in younger cells occurs at a rate higher than that in older cells.

In other studies, a decline in nitrogen accumulation or in protein content with age of cells has not been observed[40–43].

Accumulation of ribonucleic acid has been shown to proceed more or less uniformly during the growth period, parallel to protein formation[40–42,44], though LORENZEN and RUPPEL[37] observed a decline in RNA synthesis shortly before cell division.

Most investigators' descriptions of the time course of synthesis of deoxyribonucleic acid, though different in detail, in broad terms reveal one general pattern[19,40,41,44–48]: DNA synthesis usually falls behind protein and RNA synthesis during the initial stages of cell development. Then it increases and reaches a maximum sometimes before cell division. In other studies[49], DNA synthesis was observed to be uniform throughout the growth period. However, in this as well as in previously mentioned investigations, synthesis of DNA was at a minimum or absent during cell division.

Contrary to this, ABBO and PARDEE[42] ascribed earlier observed variations in DNA content, as well as changes

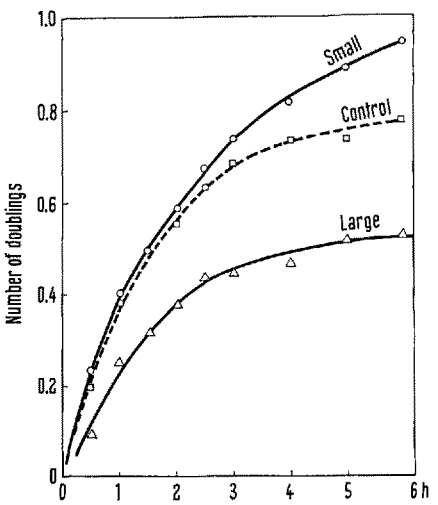

Fig. 6. Time course of organic synthesis in small and large cells of *Chlorella* 7-11-05 separated from a nonsynchronized suspension (control cells) by fractional centrifugation (unpublished).

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in the rate of nitrogen accumulation during the life cycle of cells, to unbalanced growth caused by synchronizing agents and described accumulation of RNA, DNA, and protein in *Escherichia coli* as a continuous process proceeding at a constant rate during both the growth and the division phase of the life cycle. These views contradict the consensus arrived at by different techniques for non-synchronized cells that synthesis of deoxyribonucleic acid in both animal and plant cells is a discontinuous process[50].

Ratio of the nucleic acid bases was shown by several investigators to change in the course of cell development[51–53], though in other studies investigators failed to detect any significant change in nucleic acid composition with age of cells[19,54].

Among all ash constituents, special attention has been paid to uneven accumulation of sulfur during the life cycle of cells. These observations, as well as studies on the effects of sulfur deficiency, led to recognition of a special role of sulfur in cell division[55–57].

An increase in intracellular ATP content was reported for *Tetrahymena pyriformis* prior to synchronized division[58,59] and a sharp decline in phosphorylation activity during the division period[60].

Content of such vitamins as pantothenic acid, folic acid, thiamine, riboflavin, *p*-aminobenzoic acid, and ascorbic acid has been shown to change during the life cycle of *Chlorella* cells[61].

The role of some enzymes such as phosphatase, amylase, and RNA-ase in metabolic changes accompanying the aging process has been discussed[62]. However, the available information on changes in the activities of these enzymes was obtained largely for non-synchronized multicellular tissues and the variations observed may have been due to supracellular effects. The possibility that the observed changes belong in the category of the secondary aging processes must be kept in mind. (See chapter: Primary and Secondary Aging.)

The diversity of observations on chemical composition in the course of cell development has probably been due to the fact that techniques of growing and synchronizing cells used by different investigators for chemical analysis differed even more than those employed in studies of metabolic activity. Chemical composition of microbial cells is even more labile and the effects of external conditions and of hereditary constitution of organisms on their chemical composition are even more pronounced than the effects of those factors on photosynthesis, respiration, or overall organic synthesis.

Nevertheless, the most frequently observed pattern of change in the course of cell development was that of a decline in metabolic activity indicated for phosphorylation activity, nitrogen and nucleic acid metabolism, and probably for most enzymatic processes in general. With greater attention given to obtaining a normally-developing healthy organism, and to eliminating the effects of synchronizing agents, the decline in the level of nitrogen metabolism in the course of cell development will be undoubtedly affirmed with certainty characteristic of observations in which carbon metabolism is predominantly manifested.

Metabolic Activity and Turnover in the Course of Cell Development. The basis for differences in level of metabolic activity in cells of different developmental stages must be sought in changes in pattern of metabolic turnover with the age of the cells. A high level of anabolic activity in younger cells is indicated not so much by usually higher rates of metabolism as by increase in the activity with time in these cells. Even when placed in a nitrogen-free medium, 0-h cells of *Chlorella* 7-11-05 showed an increase in rate of photosynthetic gas exchange in the course of observation (Figure 2).

This increase in photosynthetic activity must be credited to increase in the size and/or the activity of the photosynthetic apparatus. In the absence of external nitrogen, any increase in the size of the photosynthetic apparatus must be at the expense of the internal pool of nitrogenous intermediates. Actually, at this early stage of cell development, the internal nitrogen is more effective in building up the capacity of enzyme systems.

The higher effectiveness of the internal nitrogen in younger cells is reflected in the increased synthetic activity of the cells in media from which nitrogen is absent. As seen in Figure 7, there was more than 0.5 doubling of cell material in 0-h cells resuspended in complete nutrient medium from which nitrogen was absent and also in any other of the nitrogen-free buffers (phosphate, bicarbonate, and Warburg No. 9). Even in distilled water there was more than 0.4 doubling per h of cell material during this early stage of cell development. Compared with this, synthesis of cell material was lagging in complete medium containing urea or nitrate as a nitrogen source. In both media, there was less than 0.4 doubling of cell material during the first hour of observation.

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With the progress in cell development catabolic activity intensifies. In cells of intermediate age, placed in nitrogen-free suspending fluid, photosynthetic activity declines drastically in the course of observation (Figure 2). However, anabolic activity is still strong in these cells, and after some time the rate of photosynthetic gas exchange increases and may reach or even surpass the level of the activity at the beginning of observation. Thus both moieties, catabolic and anabolic, of the turnover are expressed well in cells of intermediate age.

With further progress in cell development, catabolic activity increases and anabolic activity decreases to the extent that the decline in metabolic rates is continuous. Placed in nitrogen-free medium, 8-h cells show no recovery of photosynthetic activity with time (Figure 2). Parallel to this inability of older cells to rebuild their enzyme systems by using nitrogenous intermediates is the increasing dependence on the external nitrogen source. As seen in Figure 8, the rate of organic synthesis for 8-h cells was highest in nitrogen-containing complete media. In nitrogen-free suspending fluids, the synthetic activity of such cells was low even during the first h of observation and in the course of time decreased to a vanishing point. In still older cells the capacity to use external nitrogen also diminished and metabolic activity approached its lowest level.

The decline in the several metabolic activities is not uniform throughout the life cycle of cells. As mentioned earlier, an initial increase in activity may or may not be detected, depending on conditions of observations. However, each particular activity has a characteristic pattern of decline. In *Chlorella* 7-11-05, the first to decrease is respiration. The decline in respiration rate is gradual over the whole growth period. The decline in photosynthetic activity usually begins at a later stage in cell development and the slope of this portion of the curve is generally much steeper than that for respiration.

The last to suffer is overall synthetic capacity. After a short lag phase, growth of a cell continues at a more or less constant rate over the greater part of the growth period. Then, shortly before cell division, the synthetic activity abruptly declines and, in most cases, probably comes to a complete standstill. The fact that respiration, photosynthesis, and organic synthesis are each characterized by an individual time course indicates that enzyme systems involved in each activity have different timetables of their turnover.

A lack of comprehension of changes in the pattern of metabolic activity with the age of the cells, together with possible technical difficulties in measuring protein turnover[63] prevented some investigators[64–67] from recognizing intracellular protein turnover as a universal biochemical reality. True, little degradation of protein was detected in growing cells of bacteria and yeast[68,69]. But in resting cells, prevented from growth by nitrogen or carbon limitation, degradation and resynthesis of protein and nucleic acids were found to proceed at an appreciable rate[69,70].

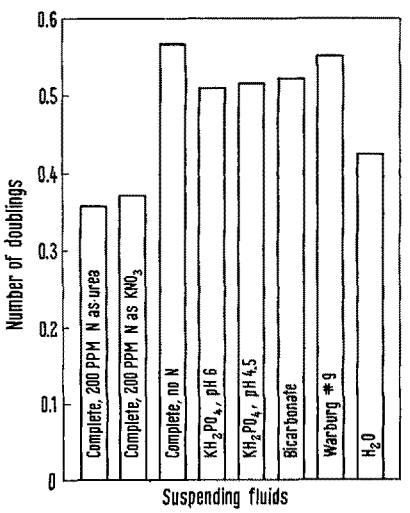

Fig. 7. Organic synthesis in synchronized 0-h cells of *Chlorella* 7-11-05 during the first h after the resuspension of cells in several fluids indicated on the graph[28].

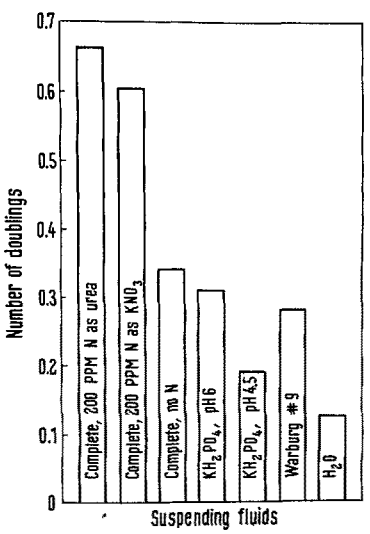

Fig. 8. Organic synthesis in synchronized 8-h cells of *Chlorella* 7-11-05 during the first h after the resuspension of cells in several fluids indicated on the graph[28].

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This disparity is understandable in view of the fact that an actively growing non-synchronized microbial culture consists largely of young cells in which catabolic activity is weak. A healthy suspension of resting cells in which cell division is arrested contains a mixture of both young and old cells as well as a large proportion of cells in intermediate developmental stages. In such cells, both anabolic and catabolic processes are expected to take place. Finally, in an old suspension of resting cells, the anabolic activity may become too low to be detected. Age effects on the rate of renewal of both protein and chlorophyll molecules have been corroborated in studies on metabolic turnover in higher plants[71,72].

As has been mentioned, presence of external nitrogen becomes progressively more important for sustaining metabolic activity in older cells. Increasing dependence on external nitrogen and delay in recovery of metabolic activity in cells of intermediate age, viewed against the background of general decline in metabolic activity with the age of the cells, may be due to several developments possibly taking place during the life cycle of a cell. The nitrogen content, relative to dry weight, declines and catabolic activity intensifies. The biosynthetic enzymes are probably the first to undergo degradation. With biosynthetic enzymes destroyed or inactive, the size of the metabolic mechanism rapidly declines. The products of degradation may change with the age of the cells and possible change in the composition of nitrogenous intermediates may unfavorably affect anabolic activity. These and other possibilities await intensive studies directed at the intricacies of the pattern of metabolic turnover in the course of cell development.

Primary and Secondary Aging. Observations on aging can be referred to any one of several levels of biological organization: a cell, a tissue, an organ, or a multicellular organism as a whole. Aging at higher levels of organization is a sum total of processes taking place at lower levels of organization. Since the cell is the basic biological unit, there is a temptation to try to reduce the complexities of the aging processes at higher levels of organization to the cellular level. However, a simple reduction of the reference unit does not clarify aging processes in multicellular organisms, neither does it lead to comprehension of aging in the course of cell development.

Cells in a multicellular system are influenced by the effects, favorable or unfavorable, of each other and by deterioration in the system with age due to competition between cells and to transportation difficulties that result in local shortages of nutrients and in accumulation of products of metabolism. What have been considered demonstrations of aging in multicellular organisms are, to a large degree, due to overcrowding, malnutrition, and disease. A recognition of the interdependence of cells as a sole source of senescence has led some writers to deny that changes at the cellular level have any role in aging processes[5]. Though other authors have not generally subscribed to this extreme view, doubts regarding the role of intrinsic-determinant properties of cells as a direct cause of senescent change is deeply entrenched in modern writings on aging[2].

Opportunities in studies of aging in multicellular organisms have been also minimized by the commonly accepted definition of aging as a process taking place only in the post-differentiation or even only in the post-reproduction period. As has been shown in this report, changes in cellular metabolism can be discerned at an early stage in cell development. They probably originate at the very moment of the birth of a cell; more precisely, they are probably continuous from the previous generation through the division period and into the growth period of the new generation of cells. Restricting observations on aging to the post-differentiation period has caused the initial and, therefore, basic portion of the aging process of a cell to be ignored.

More than that. Age changes in differentiated cells vary among different kinds of tissue. Some cells are renewed by the organism at short intervals and, therefore, are supposed to age fast. Other cells, such as mammalian nerve cells, persist through the whole life of an individual. For these cells, differentiation does not bring acceleration of the aging process; on the contrary, it makes possible a prolonged existence of these cells.

It is an open question whether the age changes after differentiation are metabolically a continuation of the age processes before differentiation. We saw that in undifferentiated cells aging processes go at a fast gait. It may be suspected that at least in some kinds of differentiated cells these processes are slowed down or even stopped and a new sequence of aging processes is started. For studies of the basic nature of aging as a universal phenomenon, *aging processes in differentiated cells are secondary to the primary aging processes taking place in undifferentiated cells.*

Recognition of a moment in the life history of the cell at which primary aging processes are superseded by secondary processes would be of major physiological interest. This switch may happen before any visual signs of cell differentiation become recognizable. Although secondary aging processes legitimately claim interest, studies of these processes may not lead to comprehension of the basic nature of aging characteristic of any cell in any form of life. A warning of the possibility that changes that occur in cells may be a

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consequence of deteriorating organization at the supracellular level[2] is a constant reminder of the futility of efforts to learn processes at the cellular level in studies on multicellular tissues.

Another limitation affecting studies of aging in multicellular organisms has its origin in the randomness of the age distribution of cells in tissues and organs. Except in a few multicellular systems in which, for a limited time, there is some degree of synchrony of cell divisions[73], a tissue or an organ generally consists of cells in different developmental stages. In populations of microbial cells, age differences can be narrowed through use of synchronization technique. Such cells can be grown, also, under conditions which minimize the effects on cells of other cells and of deterioration in external conditions with time. For these technical reasons, microbial cells seem to be the most suitable object for studies of primary aging processes.

Conclusions and Opinions. Aging is a universal characteristic of life[74]. It is more universal than death, since death is not an inevitable event at the cellular level. It is also more universal than growth or cell division in the sense that growth and cell division occur intermittently. Life can persist for some time without growth or cell division, but life cannot exist without metabolism. In the absence of growth, as long as a cell lives and metabolizes, there is aging. Aging is an aspect of metabolism. More specifically, it is a developmental aspect of metabolism.

The immediate cause of aging of cells is degradation of enzyme systems. Depending on the hereditary constitution of the organism, external factors affecting the organism during and prior to observations, and the developmental stage of a particular cell, the rate of degradation processes differs and can probably be brought under some control. Another factor determining the size and the activity of the enzymatic apparatus is the rate of the rebuilding of the enzyme systems. The relation between degradation and reconstruction processes may temporarily be such that the balance between them will result in increasing activity of a cell over a period of time. In general, retardation of the aging process and rejuvenation of a cell in the process of division are results of an increase in rate of restoration of the enzyme systems.

The general trend of change in the pattern of metabolic turnover in the course of cell development expresses itself in a decline in metabolic activity with advance in the age of the cell. The basis for this decline is thought to be the intensification of catabolic processes with the progress in cell development and the retardation in the recovery of enzyme systems. Both the degradation and the reconstruction of the metabolic mechanism are manifested with particular clearness in cells of intermediate age.

The development of a cell can be subdivided into two periods: one from the origin of the cell to the beginning of cell differentiation, another from the start of differentiation to the death of the cell. In cells which do not differentiate, like microbial cells, the first period extends to the time of cell division. The extension of this part of the life cycle makes the non-differentiating cells especially suitable objects for studies of what are defined here as primary aging processes.

In cells which eventually differentiate, primary aging processes at some moment of cell development are supplemented by secondary aging processes and at a later stage are possibly even replaced by them. The importance of primary aging in studies of secondary aging stems from the fact that in any cell primary aging precedes secondary aging and from doubt that the moment of discontinuation of primary aging processes in cell history and of their replacement by secondary aging processes will ever be ascertained. Secondary aging is, or at least may be, accompanied by primary aging.

Aging of a cell is a result of processes taking place at subcellular and molecular levels. Though aging at the subcellular level would be a most intriguing field of inquiry, nothing dependable is known about the aging of cell organellae. The available information[2,75] is based on homogenation of multicellular tissues and, for the above-mentioned reasons, has only remote bearing on the problem. Decisive progress in this field must wait for the development of a technique similar to the synchronization technique of the populations of microbial cells.

Considerable effort has been directed at comprehension of aging processes at the molecular level. This effort, however, has been largely of a speculative nature. Hypotheses offered in explanation of the nature and mechanism of aging have been counted by MEDVEDEV[76] as over one hundred; this is, in itself, a sign of perplexity. Whatever evidence, on which these hypotheses are based, was obtained through studies of tissues and organs of multicellular organisms. It is, therefore, more pertinent to theories regarding the secondary aging processes. The multitude and diversity of theoretical explanations[76] of aging reflect the diversity in observations on aging in different tissues, organs, and organisms. This diversity gives support to the opinion that a study of senescence in multicellular organisms is a study of the group of processes, different in different organisms[1], and, we believe, also in different tissues of the same organism. The development

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of a unified theory of aging at the molecular level bearing on a group of diverse processes involved in secondary aging seems highly improbable.

The distinction between primary and secondary aging is believed to be fruitful both in experimental design and in theoretical explorations of the problem of aging[77].

Zusammenfassung. Der Begriff des primären Alterns wird neu eingeführt und gegen den des sekundären Alterns abgegrenzt. Wesentliches Kennzeichen des primären Alterns ist eine fortschreitende Abnahme der Stoffwechselkapazität. Experimente belegen die Abnahme der Photosyntheserate, der Atmungsaktivität, der allgemeinen Biosyntheseaktivität und der Anhäufung verschiedener Substanzen im Verlauf der Zellentwicklung. Die unmittelbare Ursache des primären Alterns sind Verschiebungen im Zusammenspiel der Enzymsysteme. Mit dem Altern der Zelle nimmt allgemein die anabolische Aktivität ab, während die katabolische Aktivität zunimmt. Im Zeitpunkt der Zellteilung werden diese Veränderungen rückläufig und die Zelle erhält wieder ihre ursprüngliche Stoffwechselintensität. In reiner Form tritt das primäre Altern bei sich nicht differenzierenden Zellen auf, so z.B. bei Mikroorganismen.

Bei Zellen mit Differenzierungsvermögen wird das primäre Altern von sekundärem Altern zunächst begleitet und dann vielleicht überlagert, was für Gewebe der vielzelligen Organismen typisch ist. Die Kennzeichen des sekundären Alterns sind komplex und können bei verschiedenen Organismen und auch bei verschiedenen Geweben desselben Organismus voneinander ausgesprochen abweichen. Vieles, was über das Altern vielzelliger Organismen bekannt ist, beruht im Grunde auf dem Phänomen einer «Übervölkerung». Daraus resultieren: Konkurrenz, Unterernährung und Schädigung der Zellen. Obgleich das sekundäre Altern auf endogen gesteuerten Eigenschaften der Zellen beruhen dürfte, ist die Situation im vielzelligen Organismus so komplex, dass sich dies schwer beweisen lässt. Bei der Untersuchung des Alterns vielzelliger Organismen erweist sich der Begriff des primären Alterns besonders insofern als nützlich, als er zur folgenden Regel führt: Jedes sekundäre Altern setzt ein primäres Altern voraus oder wird von einem solchen begleitet, jedenfalls aber von ihm beeinflusst.

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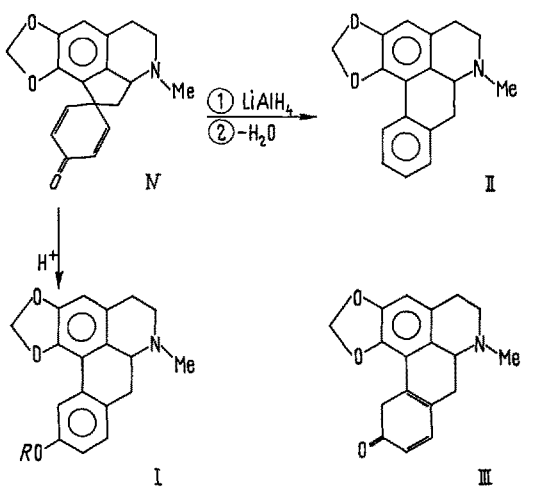
Brèves communications – Kurze Mitteilungen – Brevi comunicazioni – Brief Reports

The Structure of Fugapavine

The alkaloid fugapavine was discovered by YUNUSOV, MNATSAKANYAN and AKRAMOV[1] in various parts of the plant *Papaver fugax* Poir. collected in the Armenian S.S.R. It was shown to have the formula $C_{18}H_{17}O_3N$ with a methylenedioxy group and a tertiary nitrogen to which is attached a methyl group. The remaining oxygen atom was in a carbonyl group since the alkaloid gave a semicarbazone and an IR-absorption peak at 1675 cm^{-1}; the position of the latter also indicated that the carbonyl was conjugated with a double bond[2]. Hydrogenation with platinum black showed two double bonds to be present, and the hexahydro product had alcohol properties[2].

Fugapavine was shown, moreover, to be isomerized with mineral acid to a phenolic compound, isofugapavine (I, $R = H$), which on methylation with diazomethane gave the dextro isomer of the known aporphine alkaloid laureline[2] (I, $R = Me$). Furthermore, fugapavine could be reduced with lithium aluminium hydride to an alcohol which could be dehydrated to isoroemerine[2] (II), evidently a dimorphic form of roemerine[1], and on the basis of this evidence the structure (III) was put forward for fugapavine[2].



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[2] V. A. MNATSAKANYAN and S. YU. YUNUSOV, Doklady Acad. Nauk UzSSR No. 12, 36 (1961).