This is section 5.6. "*The limit of cell division: the key to the mechanism which determines life span?*" from the book:

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in a number of key enzymes belonging to the antioxidant system: catalase and glutathione transferase (Yu et al., 1989). Also observed is an increased rate of protein turnover, which in its turn prevents the accumulation of non-active and low-active forms of partially denatured enzymes (Holehan and Merry, 1986). Reducing the calory content of the diet leads to a reduction in the metabolic load on a number of systems and organs, and to a reduction in the organism's intake of a variety of toxins and xenobiotics contained in the food. As a result, it becomes possible for there to be a reduction in the activity of the microsomal xenobiotic oxidation systems, and consequently, a reduction in the rate at which active forms of carcinogens are generated from the metabolised xenobiotics. For instance, it turns out that the activation of polycyclic hydrocarbons into active mutagenic and carcinogenic epoxides which bind with DNA is significantly reduced in restricted-calory mice (Pashko and Schwartz, 1983). Therefore, the increase in life span which occurs when the calory intake is restricted is in good agreement with wear-and-tear (accumulation of defects) hypothesis, although many of the concrete mechanisms behind the destruction of the organism with age remain to be clarified.

Without doubt, life extension experiments deserve more detailed and careful examination than they have received in this section. For us, however, what is important is that the proposed mechanism underlying the most important methods of life extension is in good agreement with the generalised wear-and-tear hypothesis as the basic cause limiting the duration of life. Readers who wish to acquaint themselves more fully with life extension research may consult the specialised literature (Cutler, 1981; Walford, 1983; 1986; Nikitin, 1984; Obukhova and Emanuel, 1984; Gavrilov, 1985; Holehan and Merry, 1986; Frolkis and Muradian, 1988; Gavrilov, 1990).

5.6. The limit of cell division: the key to the mechanism which determines life span?

One of the favourite arguments of those who support the hypothesis of a genetically programmed life span limit runs as follows: the length of life is fixed, because an organism's cells can only carry out a limited, strictly determined number of divisions, after which they die (see, for example, Fries, 1980).

It must be noted that this idea has deep historical

roots, and many of the contradictions in the publications on the subject can only be understood if its history is taken into account. Therefore, for clarity of exposition, the arguments will be presented in the form of a brief historical excursus. What is more, in order to guarantee the objectivity and authenticity of this excursus, we will be compelled to make abundant citation of the primary sources. We hope that the reader will approve of the exhaustive documentary style in which the arguments are presented.

More than 100 years ago, the famous German biologist August Weismann (1882; 1884) postulated that the limited life span of organisms is determined by the limited capacity of somatic cells for division. Weismann also suggested that the differences in the longevity of different animal species are caused by the different number of generations that the somatic cells of each species can produce (the cells of long-lived species are capable of completing more divisions). In this way, the supposition arose "that life span is connected with the number of somatic cell generations which follow after each other in the course of an individual life, and that this number, like the life span of individual generations of cells, is already determined in the embryonic cell" (Weismann, 1892; 1914). Weismann tried to explain "the different life span of animals by making it dependent on the number of cell generations which was the norm for each different species" (Weismann, 1892; 1914).

Weismann's view that life span is limited because the capability of cells to multiply becomes exhausted appears to have been extremely popular at the turn of the 20th century, and to have been shared by many famous scientists of the day. For example, the Russian scientist Ivan R.Tarkhanov wrote: "... in an embryonic fertilised egg, the total of cell generations which can develop as a result of the creative forces of the embryo is determined in advance, by the laws of heredity" (Tarkhanov, 1891, pp. 551-552). In Tarkhanov's view, "the number of cell generations which can develop from the embryo in the course of an entire life ... determines longevity, the maximal life span which can be achieved by different organisms" (Tarkhanov, 1891, p.542).

Weismann's concept was dominant for 30 years. For this reason, something of a sensation was caused by the experiments of Alexis Carrel (1912) and Albert Ebeling (1913) on the cultivation of chick heart fibroblasts in vitro, which refuted Weismann's ideas. Carrel and Ebeling reported that under appropriate conditions the cells of a multicellular organism can reproduce themselves practically without restriction, like microorganisms (the cell multiplication experiment lasted all of 34 years, and was

terminated only in 1946 after Carrel had himself died). As a result of these experiments, which received general recognition and reached a wide audience, Weismann's concept was abandoned and forgotten, and its place was taken by the diametrically opposed idea of the potential "immortality" of the somatic cells which form a mortal organism. People therefore began to seek the causes of the limited life span of organisms at a supracellular physiological level, in particular at the level of hormonal regulation.

Carrel's concept ruled for more than 40 years, and it seemed to have vanquished finally and irrevocably. Although many investigators repeatedly observed that the capability of cultured cells to multiply became exhausted, it was acceptable to explain these cases as failures of the kind which often accompanied cell cultivation (for example, contamination by viruses, or the toxicity of the particular batch of blood serum used for the culture). The popularity of Carrel's concept was so great that no attention was paid to the many exceptions to the established "rule" (the unlimited growth of animal cell cultures): they were simply considered to be methodological artefacts (see Witkowski, 1987).

H.Earle Swim (1959) seems to have been the first investigator to attempt a radical revision of Carrel's concept. After analysing results from 336 publications, including the results of his own experiments on the serial cultivation of 23 strains of fibroblasts derived from normal tissues of the rabbit and chick embryo (Haff and Swim, 1956), as well as 51 strains of human fibroblasts derived from foreskin, placenta, testicle, uterus and embryonic tissues (Swim and Parker, 1957), Swim came to the following fundamentally important conclusions: "... in most instances where growth occurs the cells eventually undergo nonspecific degeneration" (Swim, 1959, p.145). "The common experience of many investigators indicates that the early cultivation of cells usually follows a characteristic course which can be conveniently divided into three phases. In phase I the cells proliferate rapidly after an initial lag and usually can be transferred serially without difficulty. Phase II is characterized by a decrease in multiplication to a point where it usually ceases and the cells are eventually lost as a result of nonspecific degeneration" (Swim, 1959, p.159). "This was accompanied at first by an increase in the number of granules in the cytoplasm of the cells; later, degenerating cells were observed and their numbers increased progressively until the bottoms of the flasks were covered with a dense layer of cellular debris..." (Haff and Swim, 1956, p.201). The important conclusion was also drawn that

when cells stop multiplying, this is not a methodological artefact caused by such factors as inoculum size, toxic media, or their inability to proliferate on glass (Swim and Parker, 1957). Finally, Swim (1957) notes that "infrequently a third stage is recognized by the appearance of actively proliferating cells in phase II cultures." (p.159). "It should be emphasised that phases I and II represent the usual pattern, while phase III is a relatively rare event." (Swim, 1959, p.160). Swim (1959) also noted that cells in phase III often differ from the original cells both in morphology and growth pattern.

Thus the unlimited capability of animal cells to divide turns out to be the exception rather than the rule, and in many cases the cells which divide without limit little resemble the original normal cells either in morphology or growth pattern (Swim, 1959). It might seem that this conclusion provides every ground for reviewing Carrel's concept and reviving interest in Weismann's concept. However, to carry this through and destroy generally accepted ideas which had been established in the public consciousness for decades would have required exceptionally vigorous efforts by Swim to propagandise his own results and conclusions, and a degree of enterprise which he apparently did not possess. However, other investigators emerged who brilliantly performed this function.

In the 60's and 70's, L.Hayflick published an extended series of academic and popular scientific articles describing the serial cultivation of human fibroblasts (see for example Hayflick and Moorhead, 1961; Hayflick, 1965; 1968; 1974). In fact, these articles confirmed Swim's results and conclusions, as well as Weismann's concept. However most readers, being unaware of the works of these undeservedly forgotten authors, took Hayflick's publications to represent a fundamentally new scientific discovery.* Since Hayflick's publications aroused great interest and reached a wide audience, we shall restrict ourselves to a

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* This reaction to Hayflick's articles was partially caused by the style in which they are written: Weismann's idea that there is a limit to cell division was presented without reference to its originator, and there is only one citation in Hayflick's numerous early papers (Hayflick and Moorhead, 1961), referring to a single work (Swim and Parker, 1957), of the fundamental works of Swim (Haff and Swim, 1956; Swim and Parker, 1957; Swim, 1959). The impression is therefore created that all the results and their interpretation are fundamentally new.

brief exposition of his basic ideas.

In a celebrated article (Hayflick and Moorhead, 1961, p.600), the authors write: "The general history of diploid cell strain may therefore be divided into three distinct phases... Phase I, or early growth phase, constitutes that period when cells have been freed from the intact tissue and are just establishing themselves on glass (primary culture)."* "Phase I ends with the formation of the first confluent sheet, at which time the culture is ready for its first subcultivation and entry into Phase II. Phase II is characterized by rapid cell multiplication... During this phase the diploid strains must be subcultured at least twice a week with split ratios of 2 or 3:1".** "Phase II lasts from 2 to 10 months at the end of which time cell degeneration begins to take place. This degeneration and lessening of mitotic activity heralds the appearance of Phase III or the terminal phase. It is characterized by the appearance of debris..., reduction of mitotic activity and a consequently longer period of time for the development of confluent sheets".*** What is more, the authors note that "an alteration may occur... giving rise to a 'cell line' whose potential life is infinite." (Hayflick and Moorhead, 1961, p.587). It is not difficult to see that this "alteration" corresponds to the transition of the culture into Phase III of Swim's classification (Swim, 1959). Thus, many of the fundamental points in the celebrated and widely cited article of Hayflick and Moorhead (1961) are in fact a repetition of the undeservedly forgotten conclusions of Swim (1959).

On the basis of these results and a number of additional experiments, the conclusion was drawn that human cells are capable of carrying out only a limited, strictly determined number of divisions, after which they perish. According to this cellular version of the "Peau de Chagrin" model, human beings in the course of a lifetime will inevitably exhaust

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* It can easily be observed that Phase I in Hayflick and Moorhead's classification corresponds to the initial lag in Phase I of Swim's classification (Swim, 1959).

** It is clear that Phase II in Hayflick and Moorhead's classification corresponds to Phase I after initial lag in Swim's classification (Swim, 1959).

*** Phase III in Hayflick and Moorhead's classification exactly coincides with Phase II of Swim's classification (Swim, 1959).

the potential for division present in each cell, and "the timing of this loss may well represent the limit of the human life span" (Hayflick, 1980, p.42). Indeed, "it appears that fibroblasts from human embryos will divide in cell culture 50±10 times, those from persons between birth and the age of about 20 will divide 30±10 times and those from donors over 20 will divide 20±10 times" (Hayflick, 1968, p.35). Thus, after almost 100 years, researchers in fact again returned to the once rejected concept of Weismann (1882), forgetting however who originally invented it.

Weismann's idea that the cells of long-lived species are capable of more divisions (Weismann, 1882) was also confirmed. It turns out that the embryonal fibroblasts of mice are capable of doubling their numbers in vitro only 14-28 times. For chicks the figures are 15-35 times, for human beings 40-60 times, and for tortoises 72-114 times (Hayflick, 1974).

Hayflick's numerous publications in the 60's and 70's were considered by many to be a scientific sensation, opening up a fundamentally new approach to explaining the mechanisms which determine life span. Since practically no reference was made to Swim's works, Hayflick's articles were accepted as the first and decisive refutation of Carrel's myth that cells are capable of unlimited division. Since Weismann's concept of limited cell division as the basis of aging was also fundamentally forgotten, Hayflick's articles were considered to represent an essentially new idea, rather than a return to the old hypothesis. Therefore, the limited capability of cells for division became known as the Hayflick limit (see for example Bremermann, 1982; Walford, 1983; Juckett, 1987), and the history of how this phenomenon was discovered is often presented as starting from the work of Hayflick and Moorhead (1961), as if from the year of Our Lord.

The new (or, more precisely, well-forgotten old) concept that cells have a limited capability for division has by now become so well-known and so fully accepted that it is now even to be read in the New Encyclopaedia Britannica (1989, vol.1, p.148): "Laboratory experiments have demonstrated that cells from complex organisms go through only a limited number of cell divisions before they die off, supporting the idea that cellular events can produce senescence". Hayflick's works, which confirmed this idea, came to be treated as an example of a scientific revolution in biology (see Witkowski, 1987). This might seem to be the right point at which to draw this long story to a happy conclusion. However, there are serious grounds for considering that the story is not yet complete.

In 1978, we carried out a revision of the Weismann-Swim-Hayflick (WSH) concept, resulting in the following conclusions (Gavrilov and Gavrilova, 1978; Gavrilov and Yaguzhinsky, 1978):

(1) The mass death of cells in Swim's Phase II and Hayflick's Phase III is a methodological artefact caused by damage to the cells as a result of the replating procedure (treatment of the cells with trypsin and their subsequent suspending and replanting). If the probability of cell death is evaluated excluding the replating periods, it turns out that *the probability of cell death does not increase with the age* of the culture, i.e. there are no grounds for speaking of aging at the cellular level (Gavrilov and Gavrilova, 1978; 1982; Gavrilov and Yaguzhinsky, 1978).

(2) The basic cause of the Swim-Hayflick phenomenon in vitro is the accumulation in the culture of postmitotic cells which are unimpaired from a metabolic point of view (Gavrilov and Gavrilova, 1978; 1982). The process by which these metabolically unimpaired cells are formed resemble *cell differentiation* rather than damage and aging (Gavrilov and Gavrilova, 1978; 1982; Gavrilov and Yaguzhinsky, 1978). Other investigators simultaneously reached the same conclusion (Bell et al., 1978).

(3) When the whole evolving cell population is taken into account, the accumulation of postmitotic cells in the culture is not accompanied by a reduction in the absolute number of dividing cells. In other words, *what occurs is not the disappearance of dividing cells, but only the dilution of the culture with postmitotic cells*. Consequently, there are no grounds for speaking of the limited capability of *all* the cells in the culture for division. On the contrary, in a culture of embryonal human fibroblasts for example, there are cells which complete around 170 (instead of the generally accepted 50) divisions, and have the properties of stem cells with an unlimited capability for division (Gavrilov and Yaguzhinsky, 1978).

Let us now examine these conclusions in somewhat greater detail, undertaking an analysis of the technique of cell cultivation. The following procedures are involved. A tissue sample (skin, for example) is placed in a flask containing a culture medium and left until a portion of the cells from the primary explant migrate to the inner surface of the flask, where they multiply and form a confluent monolayer covering the base. Nobody knows how many cells migrate from

the transplant in this process, or how many divisions they carry out. According to Goldstein et al. (1975), the process corresponds to approximately 10 population doublings. If embryonal tissue is used, it is treated with trypsin, and the suspended cells are strained into a vessel containing a nutrient medium (Hayflick, 1968). When the cell culture forms a monolayer, it is replated by treating it with trypsin, and the required number of cells are suspended and transferred into new vessels containing a nutrient medium. Replating the cells in the ratio 1:2 means that only half the cells which formed the confluent monolayer are transfered into a vessel with the same surface area. When a monolayer is formed in the new vessel as a result of division, doubling of the cell population is considered to have taken place, corresponding in the mean to one division of the cells. This operation is repeated many times (approximately 50 times in the case of embryonal human fibroblasts), until the culture loses the capability of quickly restoring its numbers. We shall now try to estimate the number of divisions which the cells carry out. It is known that one hour after the replating operation only 50-70% of the cells from "young" cultures and only 25% of the cells from "old" cultures remain alive (these latter cells are larger and more easily damaged in suspension) (see Gavrilov and Gavrilova, 1978; 1982).* Thus, 40-60 subcultivations correspond not to 40-60 population doublings, but to 80-120 doublings carried out by the surviving cells (see Gavrilov and Gavrilova, 1978; 1982). Further, one doubling of the cell population corresponds to one cell divison only if all the cells are capable of division. In reality, the proportion of dividing cells drops dramatically during the cultivation process, and by the final subcultivation amounts to only 10-20% (see Gavrilov and Gavrilova, 1978; 1982). It can be shown that if only 10% of cells maintain the ability to divide, they must divide 10 times for the numbers of the whole culture to double (Gavrilov and Yaguzhinsky, 1978; Gavrilov and Gavrilova, 1982). If this fact is taken into account, it turns out that 80-120 doublings of the cell population corresponds to 170±30 divisions (Gavrilov and Yaguzhinsky,

* The mass destruction of cells after replating, in conjunction with the low rates at which the numbers of "old" cultures are restored, has given rise to the illusion of natural mass degeneration and death of cells described in a number of works (Swim, 1959; Hayflick and Moorhead, 1961; Hayflick, 1968).

1978). The result is that 50 subcultivations of the cells corresponds not to 50, but to 170±30 divisions (Gavrilov and Yaguzhinsky, 1978; Gavrilov and Gavrilova, 1982).

The most important point, however, is that this number of divisions is not final. In fact, the death of a cell culture is determined in a highly arbitrary way. It seems that a cell culture is called dead only because in an arbitrary time interval (usually from one to four weeks), its numbers *do not grow* to the required size (the usual target is a number 2-4 times greater than the original)!* What is more, in "dead" cultures 10-20% of cells remain capable of division, and the culture can therefore compensate for natural cell death (which, by the way, does not increase), and even slowly grow (see Gavrilov and Gavrilova, 1978; 1982). When the whole evolving cell mass is taken into account, the absolute number of dividing cells turns out not to decrease, but the dividing cells are simply diluted with non-dividing cells (Gavrilov and Yaguzhinsky, 1978; Gavrilov and Gavrilova, 1978; 1982). As a result, when the proportion of dividing cells approaches 10%, the culture cannot indeed grow quickly enough to be considered alive (Gavrilov and Yaguzhinsky, 1978; Gavrilov and Gavrilova, 1982). Thus, the phenomenon of culture "death" not only cannot be equated with cell death, but does not even imply that all the cells in the culture have a limited capability for division (Gavrilov and Gavrilova, 1982; Gavrilov and Yaguzhinsky, 1978). Our conclusion was that cell differentiation in culture proceeds with the formation of non-dividing differentiated cells, some of the cells having properties of stem cells, which can proliferate indefinitely (Gavrilov and Yaguzhinsky, 1978).

Some of the above-mentioned conclusions we have reached were simultaneously and independently confirmed by other authors (Bell et al., 1978, p.1160): "... one of the least documented features of the aging-in-vitro hypothesis is the accepted conclusion that phase 3 cells are moribund and eventually die. No hard data support this conclusion. ... In our experience, replating itself inevitably results in loss of cells. ... We observed the survival of foreskin fibroblasts (strain 1519) in culture for long periods after cell divisions no longer occured. Our first cultures

* As an example, we cite the following definition: "Cultures were judged to be terminal when they failed to achieve confluent monolayers after 4 weeks with weekly refeeding" (Goldstein and Singal, 1974, p.360).

of 1519 cells were kept alive in Ham's medium... for 22 months, more than a year to 14 months after cell division terminated. ... Biochemical studies have shown that while phase 3 cells differ in some respects from phase 2 cells, they are essentially normal. ... Reported biochemical differences between phase 2 and phase 3 cells reflect differences of differentiation rather than of age".*

Subsequently Hayflick himself, in conjunction with T.Matsumura and Z.Zerrudo (Matsumura et al., 1979), confirmed the absence of mass cell death in Phase III of his classification. The authors of this work maintained "dead" cultures for 6 months, and some of these cultures *increased* their numbers by 16 times over this period (Matsumura et al., 1979)! No signs of imminent mass cell death could be discovered, although the period the "dead" cultures were observed sometimes even exceeded a year (by way of comparison we note that the entire previous procedure of 50 subcultivations usually takes 6-8 months) (Hayflick, 1968).

As a result of these investigations, Hayflick changed his interpretation of his own experiments. Initially, he presented his results in the following manner (see Fig.40): "We found that fibroblasts taken from four-month-old human embryos doubled in this way about 50 times (the limit ranged between 40 and 60 doublings). After reaching the limit of capacity for division the cell population died" (see Hayflick, 1968, p.35). In the 1979 work, another figure is presented (see Fig.41) and everything is expressed differently: "In early Phase III, the culture still proliferates, although the rate of proliferation is decreasing. In late Phase III, cell proliferation is very low. ... *Neither sign of ultimate death* of a culture nor of spontaneous acquisition of infinite proliferation potential was observed during this period." (see Matsumura et al., 1979, p.332; p.333). It must be emphasised that when an investigator changes his position, it is does honour to him as a scientist. The goal of science is not the defence of dogma, but the search for truth. However, it is regrettable that the newly discovered facts became known only to an extremely narrow circle of specialists, while such leading popular science magazines as Scientific American continued to advertise the old myth that "finally the cells undergo a

* Phases 2 and 3 in this article correspond to Hayflick's, rather than Swim's classification. Apparently the authors of the article, like many other investigators, are simply not aware of Swim's works and therefore do not cite any of his articles.

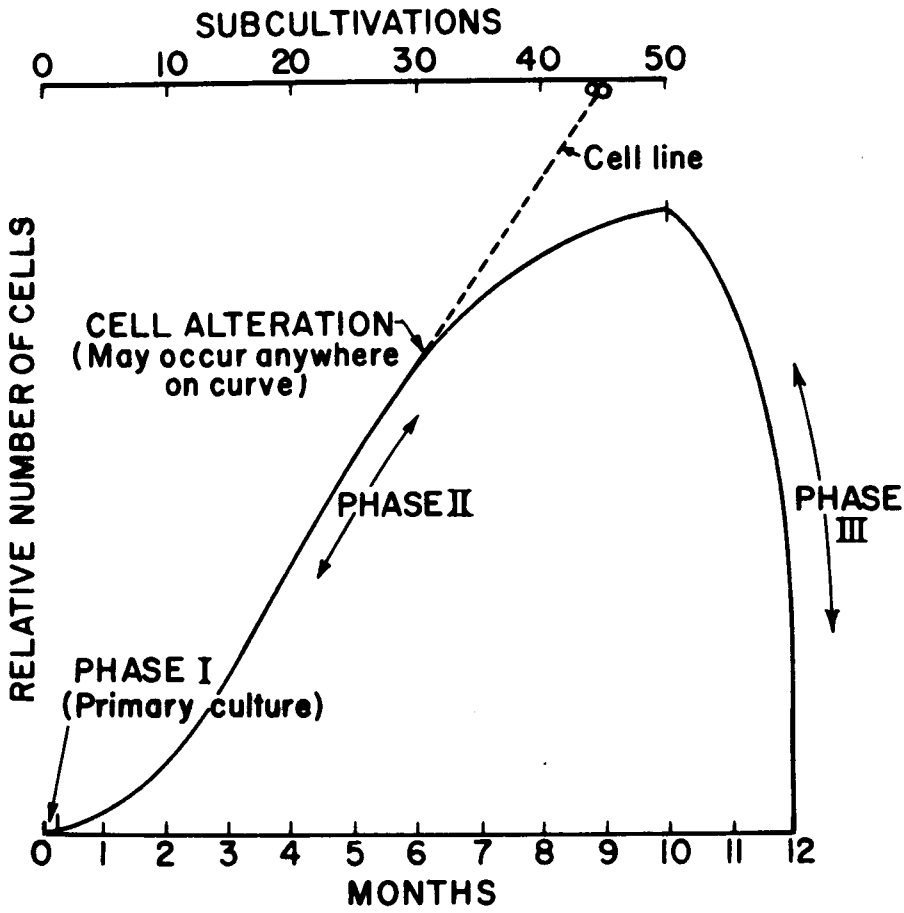

Figure 40 The initial version of the life history of human diploid fibroblasts in culture according to the work of L.Hayflick and P.S.Moorhead (1961). Reproduced with the permission of the copyright owner (Academic Press).

The figure shows an increase in the number of cells as they are cultivated (Phases I and II), followed by a sharp drop in the number of cells (Phase III). This figure, first published in 1961, has been continually reproduced in both scientific and popular scientific publications (see, for example, Hayflick, 1966; 1968; 1977; 1982), and is even adduced in a textbook on the biology of aging (Lamb, 1977).

ordinate - relative number of cells

abscissa (top) - subcultivations

abscissa (bottom) - months

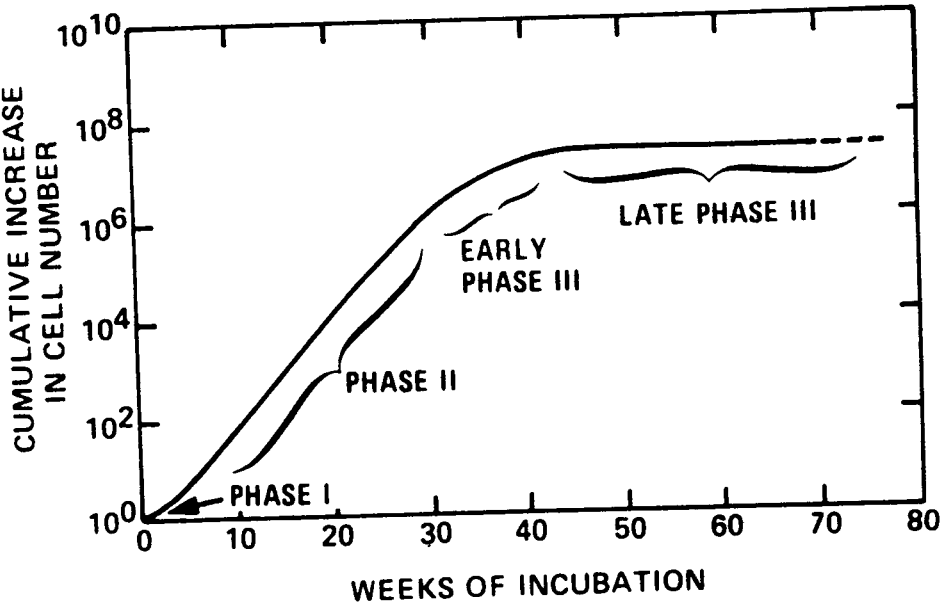

Figure 41 The new revised version of the life history of human diploid fibroblasts in culture according to Matsumura et al. (1979). Reproduced with the permission of the copyright owner (The Gerontological Society of America).

The figure shows an increase in the number of cells as they are cultivated (Phases I and II), followed by a gradual reduction in the rate of growth (early and late Phase III). In distinction to the initial version (see Fig.40), this more correct schema was not advertised and is known only to a narrow circle of specialists.

ordinate - cumulative increase in cell number

abscissa - weeks of incubation

variety of degenerative changes and die" (Hayflick, 1980, p.42). Of course, it is surprising that the propagation of this myth was carried out by the same author who a year before had so well demonstrated its inadequacy.* This riddle however goes beyond the bounds of the present book. At the same time, it becomes comprehensible how antiquated notions about a limit to cell division can become canonised in the latest volumes of such solid publications as the New Encyclopaedia Britannica (1989).

Bearing in mind the new facts, it is easy to see that the Swim-Hayflick phenomenon is no longer so obviously connected with the issue of the limited life span of organisms. While the mass death of cells might understandably lead to the death of the organism, the limited life span of organisms does not directly follow from a reduction in the rate of growth of the cell population. What is more, reductions in mitotic activity are often a normal physiological process connected with the differentiation of cells. For example, when nerve and muscle tissue is being formed, the cells practically lose all capability of division, and under the above-cited definition the cell populations could be called dead. However it is obvious that this definition is not very constructive, since we are dealing with highly specialised and functionally unimpaired cells. In fact, in many cases the performance of highly specialised functions turns out to be incompatible (at least at the same time) with cell division. It is also known that, when cells differentiate, cell division is often essential for their differentiation and for the switching of their metabolism, the critical point in this shift coinciding with the synthesis of DNA (see Gavrilov and Gavrilova, 1978; 1982). Thus, a clear similarity is observed between the counting of the number of cell divisions which occurs in cell differentiation and the counting of the cell divisions which is observed in cell cultures. The hypothesis naturally arises that the phenomenon discovered by Swim and Hayflick is not a fundamentally new process involving aging at the cellular level, but the long known, although insufficiently studied, process of cell differentiation, accompanied by the counting of cell divisions and a reduction in mitotic activity (Bell et al., 1978; Gavrilov

* It is even more surprising that three years later the same author again publishes the out-of-date figure 40 instead of his new amended figure 41, and again writes that "after cessation of mitotic activity, the culture finally undergoes total degeneration" (see Hayflick, 1982, p.64).

and Gavrilova, 1978; 1982; Gavrilov and Yaguzhinsky, 1978). Unfortunately, it is still unclear what particular cell types human fibroblasts differentiate into in vitro. Perhaps a deficiency of certain factors in the culture medium may suppress the expression of the new differentiated phenotype. In principle, it is known that under certain conditions fibroblasts are capable of differentiating in vitro to form non-dividing adipocytes, but the expression of this new phenotype can be held back by deficiencies in a range of factors, for example insulin, biotin, etc. As far as cultured human epidermal keratinocytes are concerned, it has been directly shown that a reduction in the growth rate of these cultures is linked to the differentiation (keratinisation) of the keratinocytes (Rheinwald and Green, 1977).

If we were to accept the differentiation hypothesis, we would not at all expect the number of times the cell population doubles to be a fundamental inherent property of cells, as is supposed by Hayflick, but rather that this number might be relatively easily increased using inhibitors of differentiation (certain growth factors, tumour promotors,* etc.). In fact, it turns out that epidermal growth factor (EGF) increases the number of times the cell population doubles from 50 to 150, with a simultaneous threefold increase in the "lifetime" of the culture (Rheinwald and Green, 1977). At the same time, the increase in the "lifetime" of the culture was accompanied by the delayed appearance of a multitude of signs of terminal keratinocyte differentiation (Rheinwald and Green, 1977).

Another prediction of the differentiation hypothesis is that, contrary to what is claimed by Hayflick, constant active growth of cell cultures may be observed not only in the case of transformed heteroploid cells, but is also possible for normal diploid cells (if these cells do not embark on terminal differentiation). In fact, it turns out that normal diploid mouse embryo cells, which under standard conditions manifest a growth crisis after 7-10 population doublings, may be successfully cultivated without any sign of an approaching growth crisis for at least 200 population doublings. All that is necessary is to change the composition of the culture medium (excluding blood serum and adding a number of ingredients, including the above-mentioned EGF). In this case the cells, which are apparently

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* In this particular case tumour promotors are used not as the cell transformation agents but as the inhibitors of cell differentiation.

capable of unlimited multiplication, remain diploid and nontumorigenic (Loo et al., 1987).

Certain normal diploid cells show a practically inexhaustible capability for proliferation not only in vitro, but also in vivo. Normal drosophila imaginal disc cells have this property if their differentiation inductors are absent (see Finch, 1976), as do haemopoietic stem cells (Harrison, 1984).

Taking all the above cited facts into account, we are forced to recognise that the Weismann-Swim-Hayflick concept must be revised, especially with respect to the additions put forward by Hayflick (1965; 1968). Although this concept enjoys great popularity, especially amongst investigators who are remote from cell biology (Fries, 1980; Benjamin, 1982), it is at present unclear how the results obtained from cell culture research are related to life span. It cannot be ruled out that the limited capability of certain cells for division is only indirectly linked to the limited life span of organisms. Evidence in favour of this conclusion is provided by the fact that organisms which are basically constructed out of postmitotic cells (laboratory drosophila) essentially have the same life span distributions as human beings. On the other hand, it is clear that the transition of a certain proportion of cells into a postmitotic state is the prelude to a reduction in the organism's capabilities for regeneration, and finally to a reduction in the number of functioning cells, as is indeed observed with increasing age (Lamb, 1977; Walton, 1982). Therefore, research into the kinetics of cell populations at different stages in an organism's life may be of great importance in clarifying the mechanisms which determine life span. However, attempts to justify the existence of a genetically programmed limit to life span by alluding to a limit to cell division cannot be considered convincing. The key to the mechanism which determines life span is not an account of the reasons why proliferation rates decrease, but rather an explanation why cells, as well as other structures of the organism, become damaged and die. It is significant that one of the originators of the WSH concept of a limit to cell division himself nevertheless rejects the genetic programme hypothesis, and supports the accumulation of damage (wear and tear) hypothesis (Hayflick, 1988).