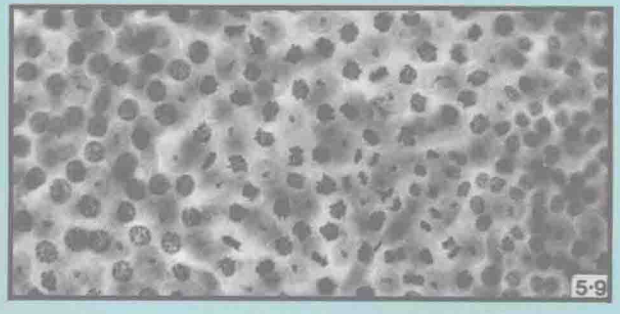
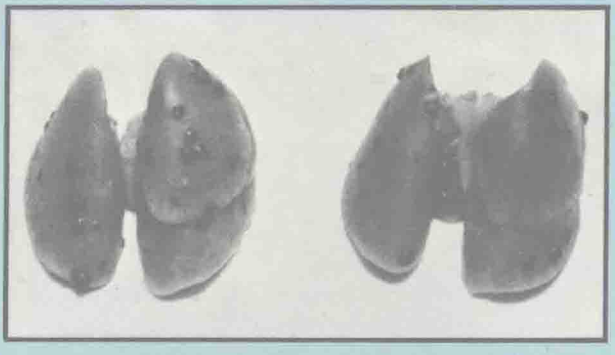
STEM CELLS

THEIR IDENTIFICATION AND CHARACTERISATION

Edited by C.S. POTTEN





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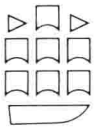

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Introduction

There has been an increasing interest and consequent advance in identification and characterisation of stem cell populations in diverse systems over the past few years. In many instances advances in one system have implications for others. Ten authors have been invited to review the advances in the understanding of stem cell behaviour in a wide range of animal systems. They were asked particularly to point out the features that can be associated with the stem cells that might have general biological relevance.

General questions covered by several authors include: stem cell renewal and its control (or how stem cells are removed to differentiation and how the various differentiation options are selected — pluripotency, bipotency?); how stem cell proliferation is controlled; how the change is achieved in self-renewal probability when the steady state is disturbed (e.g. during clonal regeneration) — also the converse, the change in self-renewal probability from embryonic expansionary growth to the steady-state situation; how the local micro-environment influences the behaviour of stem cells, and finally, the question of potential heterogeneity within the stem cell compartment (age structure).

In the more 'primitive' systems no precise equivalent to the mammalian adult steady state may exist. Hydra grows continuously, if well fed, and contains about 3500 multipotential stem cells which divide with a self-renewal probability of 0.6, the size of the stem cell population being regulated by changes in the self-renewal probability and not by cell cycle duration. The self-renewal probability is apparently controlled by the local stem cell concentration. Stem cell differentiation (to nerve cells and nematocytes) is under morphogenetic control i.e. linked to other features of morphogenesis e.g. a neuropeptide implicated in head morphogenesis also determines differentiation into nerve cells with a trigger point in early S.

Planaria have long been known to possess remarkable powers of regeneration, attributable to the distribution of totipotential stem cells (neoblasts) throughout the body (e.g. 1.4×10^5 in a 10 mm adult with a cranio-caudal differential distribution). These are the adult equivalents of embryonic blastomeres. These cells are also responsible for all tissue replacement. Differentiation here is apparently controlled by the local functional tissue. The hormone determining brain cell differentiation is controlled by a bioelectric field (similar to an embryological developmental field) which may well have a general distribution throughout the animal kingdom. During ageing it appears that the volume of tissue to be re-

placed by each stem cell is increased, which accounts for the reduction in regenerative capacity.

In the small nematodes virtually the entire cell population of the adult can be developmentally traced and this permits the interrelationship between cell division and differentiation to be studied. A characteristic feature of these organisms is that the equivalent of steady cell replacement in the adult is absent. Stem cell-like divisions occur during early development to generate the somatic founder cells and the germ line, and also during later development (post-embryonic) where there is an expansion in cell number in some tissues. During development there cannot be a strict, stepwise, progressive restriction in cell potential associated with cell division since reversions can apparently occur. The determination of functional lines appears to be under some non-nuclear control, possibly through some cytoplasmic gradient. During development dispersed cell types maintain a division rhythm which suggests an inherent cell cycle clock, which again is determined by cytoplasmic factors.

In the mammalian systems covered here, the clonal regenerative capacity of some cells in the tissue has been used extensively as a means of studying stem cells. This raises the question whether stem cells in a steady-state situation can be equated with clonogenic cells observed under artificial conditions of severe cellular depletion. Since this remains a largely unanswerable question it is somewhat academic and the assumption by most of these authors that the two are identical cannot at present be challenged.

In the testis the stem cells are distributed as isolated cells scattered along the basement membrane of the tubules (about $2-3 \times 10^4$ per mouse testis) which can be relatively easily identified. There are about 10 divisions in the transit population but considerable cell loss occurs at some stages so that the stem cells constitute about 1–2% of the proliferative cells in the testis. On division the daughter cells may remain close together as paired or aligned cells. The differentiation of these relatively undifferentiated aligned cells is dependent on the number of cells in the 'clone', while their proliferation rate varies throughout the seminiferous cycle and is believed to be controlled by an inhibitor feedback system. There is some suggestion of a heterogeneity in the stem cell population in radiosensitivity, mutation induction and the number of potential division cycles.

In the bone marrow the stem cells exhibit a considerable degree of pluripotency and the clonogenic cells (CFU-S) can be shown to exhibit extensive self-renewal. There is some evidence for heterogeneity in the stem cell compartment (age structure) though this is open to question. The number of stem cells (CFC-S) is small (about 0.4%, 1 in 250 transit cells). The stem cell turnover is normally very slow with most cells temporarily out of cycle, a phase which may have important implications for DNA repair processes. Rapid turnover does occur if the population is depleted and the control of the cell cycle progression can be studied in some detail. This control is achieved via locally produced inhibitors and stimulators. Techniques are evolving that will permit the study of differentiation control.

In the epithelial systems the movement of functional, maturing and differentiating cells is easily studied and provides a means of identifying, and hence of studying, the stem cells representing the ancestors of these migratory pathways. Thus, in these tissues cell position within the tissue can be used to study

stem cells, but is also likely to have some functional significance for the stem cells by providing a micromilieu that determines self-renewal probability and its inverse differentiation probability. Clonogenic cells can also be studied in some epithelial systems. It has been suggested that stem cells in these tissues may be anchored in some way to prevent their loss into the migratory flow. This anchorage may be related to the micro-environment, impose a polarity (asymmetry) on the stem cell division, and help to determine the differentiation probability of the polarised daughters. In the intestine the best estimate, at present, is that each crypt contains about 16 stem cells while the number of clonogenic cells appears to be greater than this. In the epidermis the number of stem cells and clonogenic cells is comparable. However, at present such estimates can only be approximate. The stem cells in epidermis are most likely to be distributed one per proliferative unit (i.e. about 1400 per mm^2). In both small intestine and epidermis the stem cells appear to be cycling more slowly than the transit cells (by a factor of about two) and it is postulated that control of the cell cycle of the stem cell is again achieved through local control factors. In the tongue epithelium the stem cells, at a specific position in the tissue, are present in low numbers, perhaps 3% of the basal cells, but appear to have cell cycle characteristics similar to some transit cells. In all three epithelial regions the stem cells apparently make up about 10% of the proliferative compartment i.e. there are three to four divisions in the transit population.

In all three epithelial regions there are some characteristics that are exclusive to the cells at the stem cell position and hence these may represent characteristics of stem cells generally. These include a heterogeneity in radiation and drug response, a marked circadian rhythm, selective uptake of thymidine (or a specific pool of thymidine) which permits incorporation into DNA at times beyond the normal thymidine availability period, and finally the probable ability to segregate 'old' and 'new' strands of DNA for selective distribution to the daughter cells at mitosis.

Finally, in tumours the stem cells are inferred from their parallel existence in normal tissue and from clonogenicity studies on cells extracted from tumours. These studies illustrate the heterogeneity of the tumour stem cells and their relatively rapid (but environmental/nutritional determined) proliferation.

It is to be hoped that the bringing together of these 10 chapters will stimulate an exchange of information and ideas across a range of disciplines and result in a better overall understanding of the behaviour of the stem cells that are important in maintaining tissue integrity under normal and disturbed states.

Manchester, 1983 C.S.P.

Contents

Stem cell concepts

The term 'stem cell' is used in a regretfully vague fashion by many authors, in many cases implying an 'ancestor' cell. With the increasing insight into the cellular hierarchies in tissues and cell populations, terms like 'precursor', 'progenitor' and 'stem' are used almost interchangeably, to the detriment of clarity.

A workshop held in 1978, consisting mainly of haematologists (Lajtha 1979a) defined stem cells as 'cells with extensive self-maintaining (self-renewal capacity), extending throughout the whole (or most) of the life-span of the organism. Differentiation potential is a property of some types of stem cells but is not an essential feature of stem-ness.'

This means that any cell type in the body, which can maintain its own numbers by proliferation throughout the life of the organism, in spite of physiological or mildly pathological removal of cells (either by cell death, cell sequestration or differentiation), is defined as a stem cell.

A second confusion arises when stem cells are described as 'undifferentiated' cells. This is clearly a misnomer since, apart from the zygote, every cell in the body *is* differentiated. Some stem cells may undergo *further* differentiation, thereby giving rise to further differentiated descendants. These descendants may complete the initial differentiation step (a genetic reprogramming event) by successive events in time, described as maturation. The further differentiation, mentioned above, is a property of e.g. the haemopoietic stem cell, but not of the hepatocytes which also maintain their numbers by proliferative activity throughout the life of the organism.

These concepts and definitions — as well as the distinction between differentiation (a qualitative determining step), and maturation (its result) — have been discussed in extenso in earlier publications (Lajtha & Schofield 1974, Lajtha 1979b, Potten et al 1979).

While 'stemness' is defined merely as extensive self-replicating capacity — the importance of this will be evident when discussing tumour stem lines — it is recognised that most current work on normal stem cells is primarily concerned with stem cells which have the capacity for further differentiation, i.e. with 'ancestral' stem cells.

THE LESSON FROM THE LIVER: THE G_0 STATE

The hepatocytes maintain their own number by a slow steady proliferation

throughout the life span of the animal. The turnover rate, compensating for the normal cell loss due to physiological attrition in the adult organism, is very low: it is countable in many months, even in the small rodent. However, this low turnover can be speeded up during liver regeneration, e.g. after surgical or chemical partial hepatectomy. A notable feature of this phenomenon is the nearly synchronous entry of hepatocytes into DNA synthesis, after a lag of some 20 hours (Cater et al 1956). The lag is not attributable to an operational artifact (e.g. due to surgical injury), since intensive RNA synthesis starts within a few hours; consequently the regeneration changes in the cycling rate of hepatocytes is not explicable as a mere 'telescoping' of a normally long cell cycle. To explain the above sequence of events, it was proposed that, under normal steady-state conditions, the majority of hepatocytes are not in a state of cell cycle but in a true resting stage (the G_0 state) from which, on demand, they can be triggered into a cell cycle (Lajtha 1963). It was envisaged that in cell populations with low normal turnover, but which are capable of fast cycling on regeneration, the majority of cells in normal steady-state conditions are in a G_0 state.

The same principle has been proposed to operate in the haemopoietic stem cells also (Lajtha et al 1962), and the G_0 state was defined as the state in which the physiological control mechanisms keep the cells which at that time are not called for proliferation. From this state, on demand, the cells may be triggered in a random fashion into cell cycle.

The kinetic aspects of the G_0 concept have been subject to mathematical analyses of increasing sophistication (Lajtha et al 1964, Burns & Tannock 1970, De Maertelaer & Galand 1975, 1977, Radley et al 1976, Svetian 1977) and have been applied also to in vitro cell systems (Smith & Martin 1973, Smith 1977, Shields & Smith 1977), the justification of which, however, is questionable (Pardee 1978, Nedelman & Rubinow 1980, Castor 1980).

Search for biochemical markers, to distinguish a G_0 state from the G_1 phase of the cell cycle, indicated some quantitative differences in synthetic rates (Baserga 1976, 1978, Riddle & Pardee 1980), particularly in normal diploid cells. It should be emphasized that the G_0 concept was for the *normal* proliferation control mechanism, which may not operate in most of the in vitro systems — particularly in those which used transformed cells. The only in vitro system in which normal stem cells can be manipulated by physiological, cell type specific proliferation controlling factors is the long-term bone marrow culture system (Toksöz et al 1980).

CELL POPULATION TYPES: STEM V. TRANSIT

The hepatocytes may be considered an example of the slowly turning over, self-maintaining cell populations. There is no implication in this that *all* hepatocytes are stem cells (i.e. the stem line) of the liver cell population. What is then the situation in tissues with fast cell turnover, such as bone marrow or intestinal epithelium?

In the case of bone marrow, it has been known for a long time that most of the fast proliferating cells, such as the normoblasts and the early myelocytes, are not self-maintaining. These cells are undergoing a maturation sequence, during part of which they are still able to proliferate, but which is essentially a 'suicide'

maturation, resulting in non-proliferating 'end' cells which have a limited life-span in the blood. In analysing cell population types, such cells have been described as 'amplifying transit' populations (Lajtha 1979b), originating from stem cells.

The practical demonstration of stem cells in such tissues came with the clonogenicity tests: the demonstration of cells which can regenerate the tissues in question and do so in a clonal fashion, i.e. the regeneration can be effected by foci of single cells. The first such clonal demonstration of a stem cell population was the spleen colony method of Till & McCulloch (1961) which demonstrated the existence of the pluripotent haemopoietic stem cell. Similar assays for clonogenicity have been developed for intestinal epithelium (Withers & Elkind 1970, Hendry & Potten 1974) and skin epidermis (Withers 1967, Potten & Hendry 1973) demonstrating the respective stem cells in these tissues.

Interestingly, when the cycling rate of stem cells was measured in these tissues, it was found that the stem cell turnover, in each case, is significantly lower than the cycling rate of the amplifying transit cells originating from them. Indeed, from the evidence available at present, the generalisation might be made that if fast cell production is the normal steady state of a tissue, this is achieved by amplifying transit cell populations, while the stem cells (from which the transit populations originate) remain in low turnover state. In the bone marrow erythroid or myeloid transit cells, the amplification involves some 10 cell cycles, i.e. $\times 10^3$ amplification — hence for any 1000 end cells produced, only one stem cell needs to be 'mobilised' into differentiation.

Table 1.1 indicates a schematic situation in which a daily requirement of some 1000 cells can be provided by a stem cell population constituting less than 1% of the total cells in steady-state transit, with a 10 days turnover time (10% per day) of the stem cells and a 24 h cycle time for all proliferation (stem and transit) cells.

While Table 1.1 illustrates the situation as may be applied to the bone marrow, mutatis mutandis, the same principle applies to the skin and the intestinal epithelium (Potten et al 1979). This, of course, implies that, in these fast proliferating tissues, the stem cells are a minority population, the study of which — without good cytological markers — is very difficult.

Table 1.1 Schematic steady-state production of 1024 cells/day from a stem cell population with a 10 day turnover (see text).

10 stem cells	→	5 cycles 'worth' progenitors	5 cycles 'worth' precursors	=	Total in transit
		2 → 4 → 8 → 16 → 32 →	64 → 128 → 256 → 512 → 1024		2047
0.48%		3%	96.9% of the total		

DIFFERENTIATION

It has been mentioned earlier that all stem cells — defined as cells capable of extensive self-maintenance throughout the life of the organism — are differentiated cells relative to the zygote. Some stem cell types, however, are capable of further differentiation, i.e. yielding further differentiated descendants. In such cases the stem cells act as 'ancestor' cells, e.g. for the cells moving up the intestinal villi,

or for the diverse cell populations in haemopoiesis. Depending on whether they are ancestral to one or more lines of descendants, they may be unipotential or pluripotential in their differentiating capacity.

Nothing is known about the stimuli or mechanisms inducing further differentiation in stem cells, but clearly some genetic re-programming is involved as a result of which a stem cell ceases to be a stem cell and is turned into a different (further differentiated) cell. In this respect, differentiation is a process by which stem cells are removed from the stem cell population.

In earlier schemes of stem cell kinetics the concept of an asymmetric cell division has been invoked. According to this, when a stem cell divides, one of the daughter cells remains a stem cell, the other turns into a differentiating cell. This concept implies the unlikely situation that, having received the appropriate stimulus, a stem cell proceeds into a cycle resulting in two different daughter cells; but when there is need for regeneration, e.g. after cytotoxic damage, a different signal induces a different cell cycle, which results in identical daughter cells. The situation is unlikely, at least as a deterministic model, because it implies an inherent asymmetry induced by a proliferation stimulus coupled with a differentiation stimulus. The asymmetry proposed by the Cairns model of 'ancestral' v. 'new' DNA strands segregating in order to preserve the integrity of the genome (Cairns 1975) does not determine differentiation.

A simpler view, more compatible with the current understanding of stem cell proliferation control (Lajtha & Schofield 1974, Lord 1979) and of the Cairns model of genome preservation, is to consider proliferation and differentiation as two distinct processes governed by different mechanisms. Both processes appear to work on stochastic principles, probably modified by the state or environment of the stem cells. Cycling rate differences between spleen or marrow residing stem cells are an example of this, as also are the differences between 'marginally' and 'centrally' located stem cells in the marrow shaft (Lord & Hendry 1972, Lord et al 1975). In epithelial tissues the more clearly defined tissue architecture is likely to influence the differentiation process.

HOW PLURIPOTENCY?

Since the clonal nature of haemopoiesis has been demonstrated (Ford et al 1956, Barnes et al 1959, Becker et al 1963, Wu et al 1967) the haemopoietic stem cells have been proved to be pluripotential in the sense that any one stem cell may give rise to at least three different cell lineages: erythroid, granulocytic, lymphoid. The initial differentiation steps may allow further (more restricted) steps to occur, e.g. granulocyte/monocyte, erythroblast/? megakaryocyte. The monocyte/macrophage line may also provide the osteoclasts (Testa et al 1981); the heterogeneity of the 'lymphoid' cell population is a whole new chapter in immunology.

As a first approximation, it could be imagined that the initial pluripotency means that any one of three possible signals may affect a stem cell: it may turn in any one of three main directions. This concept is challenged by Holtzer (1978) and Holtzer et al (1975) who claim that, at maximum, cells of a given set are only capable of binary decisions, each of which involves a quantal cell cycle. According to this view, which has corroborating evidence in embryogenesis, the early differ-

entiation events in haemopoiesis have to go through a hierarchy of quantal cell cycles: Cell A (? the pluripotent stem) capable of generating B *or* C (? 'myeloid' or 'lymphoid' early progenitors); B (? myeloid) may give rise to C *or* D (erythro-megakaryo or granulo/mono) and so on, stepwise, involving in each step at least one cell cycle.

This concept is attractive and is compatible with existing evidence on the hierarchical interrelationship of the haemopoietic cell lineages.

WHY SLOW CYCLING IN STEM CELLS?

The observation has been referred to earlier that steady-state fast cell production is achieved by amplifying transit populations originating from slowly turning over stem cells. If this is a general rule, the question for its reason (i.e. biological value) may be legitimately asked, but at our present state of understanding, only tentative explanations might be given.

One explanation might be offered by considering the types of cell populations in the mammalian organism (Gilbert & Lajtha 1965). Very roughly speaking, there are three main kinetic types of cell populations: static (no proliferative potential), transit (dividing or non-dividing), and stem (self-maintaining with extensive proliferative potential). If, as is highly probable, the maintenance of the integrity of the genome is biologically important, even in the somatic cells, then the static cell populations might be considered 'low risk' since they do not undergo potentially error-prone DNA duplication, and any error occurring in them, e.g. by extraneous influences, would not be propagated by cell division. Similarly, the transit populations might be considered 'low risk', as their short transit time and eventual elimination would not allow a great amplification of error-containing cells, and they would be mostly eliminated together with their normal counterparts.

The stem cells, however, are 'at risk' because they are truly self-maintaining throughout the life of the organism, and consequently they undergo the potentially error-prone process of DNA replication, and would amplify such errors as might occur in the population.

This being the case, there appears to be an advantage in keeping the cycling rate of such 'at risk' populations as low as possible: this would tend to keep the error accumulation probability low, and would allow longer times between rounds of DNA duplication for biological repair processes. Hence long, non-cycling G_0 states in stem cells may have important biological significance.

Furthermore, since in a stem cell population there will be, at any time, a spread of 'G_0 histories' (i.e. the distribution of immediately preceding times spent in G_0 state), there may be additional mechanisms to discriminate against cells with relatively short G_0 histories in their immediate past. One such mechanism could be to increase the probability of such cells responding to differentiation inducing stimuli, thus effectively removing them from the stem population. Such increased removal probability would manifest itself as a decrease in self-renewal probability and, in the haemopoietic stem cells, there is some evidence that after periods of regeneration (= shortening of the turnover time, shortening of the G_0 'history' of the population) there is a period of decreased self-renewal capacity (Schofield et al 1980).

A molecular model, to offer a mechanism for the preservation of the integrity of the stem cell genome, has been proposed by Cairns (1975). According to the model, the stem cells segregate the 'old' from the 'new' strand of DNA during/following DNA replication. The daughter cell containing the 'new' DNA will tend to be eliminated by having a high probability for entering further differentiation, while the daughter cell containing the 'old' 'ancestral' strands) will tend to remain as a stem cell. Thus errors during DNA replication would tend to be eliminated from the stem cell population. Experimental proof for this model is not easy to obtain until stem cells can be morphologically identified with considerable precision, but evidence compatible with such mechanisms has been published (Potten et al 1978).

TARGET CELLS FOR CARCINO-/LEUKAEMOGENESIS

Two cardinal features of chemical or radiation carcinogenesis or leukaemogenesis are: (a) the long time scale from the administration of known carcinogenic agents and the onset of the disease: months in the small rodent, years in man, and (b) the apparently multi-stage or multi-step nature of the processes following the initial carcinogenic stimulus (Peto 1977), processes which are likely to require several rounds of DNA synthesis and re-arrangements of the genome (Cairns 1981).

The inevitable conclusion from these observations is that the cell which received the initial carcinogenic stimulus must be able to stay in the body as a viable cell for long periods of time, and during that time it must be able to undergo several cell cycles in order to manifest itself eventually as a malignant cell, subsequently undergoing clonal expansion.

Considering the three main kinetic types of cell populations, the static populations (incapable of proliferation, e.g. adult muscle, adult neurons etc.) would be, on the face of it, excluded as potential target cells for carcinogenesis due to their inability to undergo the appropriate number of cell cycles required for the 'steps' of the carcinogenic process. Similarly, most transit populations would be excluded on the basis that the known transit times of most transit populations fall far too short of the time scale of carcinogenic processes; such transit cells, even though proliferating, simply do not stay in the body long enough for that. (A notable exception is presented by some early lymphoid progenitor cells, which have very long life-spans — up to years in man. It is interesting that there is good evidence that most of this long life-span is spent, apparently, in a non-cycling state!) (Buckton et al 1967).

Consequently, the cell population type which is self-maintaining with extensive proliferation capacity and persists in the body long enough to be compatible with the time scale of carcinogenesis, and therefore satisfies the criteria of being the main potential target cell for carcinogens, is the stem cell.

In the foregoing, primarily chemical or radiation carcinogens or leukaemogens were considered. The argument may be raised that the situation may not be quite the same in the case of virally induced malignancy, since it is known that, in experimental systems with certain oncogenic viruses, single-step immediate 'transformation' can be achieved.

While this is certainly true in certain experimental situations, it should be

remembered that most of the known leukaemia viruses (apart from the Abelson and the avian leukaemia virus complexes) require also long 'latency' periods before development of a truly leukaemic self-maintaining clone of cells. For example, the Friend virus will produce an instantaneous erythroid hyperplasia (the Friend disease), but requires long periods for the development of an autonomous Friend cell line. This may well be the result of a requirement for some genetic rearrangements in some of the infected cells to express 'malignancy' (Cairns 1981), and thus the time scale of developments, even in such situations, appears to exclude the transit cells; predominantly the cells proliferating long enough in the body for such rearrangements to be completed are (with the exception of the earlier mentioned lymphoid progenitors) the haemopoietic stem cells.

TUMOUR STEM LINES

The heterogeneity of cells within a tumour or leukaemia has been well demonstrated by histopathological, cytochemical, immunological and cell kinetic techniques. Recently, methods have become available to test the clonogenicity of various malignant cell populations. While the primary plating efficiencies are of questionable significance, a combination of primary and secondary plating effi-

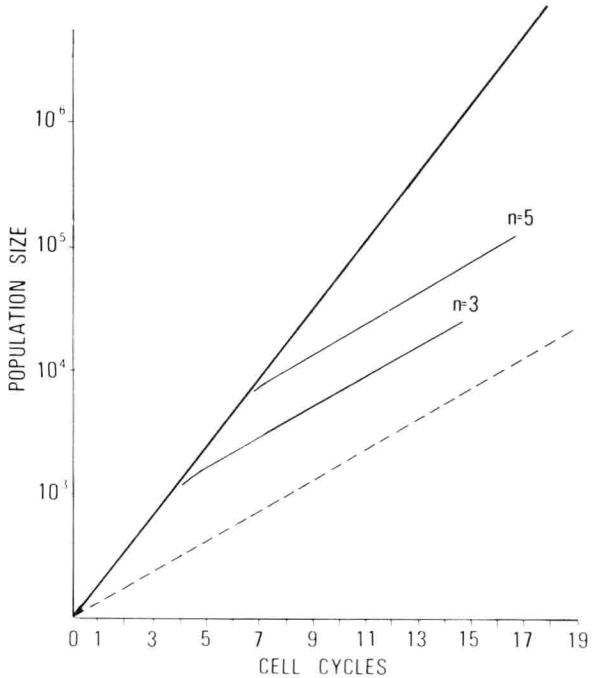

Fig. 1.1 Random cell death in tumours. The solid straight line indicates the theoretical growth as calculated from the measured mean cell cycle time of the tumour cells. The slope of the interrupted line indicates the actual *rate* of tumour cell population growth, and the growth rate of the tumour stem line. The subclones with limited proliferative capacity may augment the actual population size, depending on the number of cell cycles they are able to complete (e.g. $n = 3$, or $n = 5$), but will not affect the rate of the tumour growth. The slope difference between the theoretical (calculated from the cell cycle times) and the actual growth of the stem line is corresponding to about 40% 'loss' of cells per cycle.

ciency indicates that the clonogenic proportion of a leukaemic (AML) cell population is very low: ~ 1–5% (Chang et al 1980). Similarly, the transplantation efficiency of primary tumours, in a syngeneic system, is very low.

Early work, comparing the 'mean cell cycle time' and the population doubling time of tumours, indicated that a significant fraction of the cells in cycle do not contribute significantly to population growth: in tumours (as in leukaemias) there is a considerable degree of (? random) cell death. (Steel 1968, Moore & Dixon 1977, Kerr & Searle 1972, Searle et al 1974, Wyllie 1974).

The consequences of this are schematically illustrated in Figure 1.1 in which the known difference between a theoretical growth curve, calculated from the mean cell cycle time, and an actual population doubling time are shown, assuming an approximate 40% death (removal) of cells per cycle.

The concept that in tumours and leukaemias it is a minority of the cells, the stem line, which is responsible for the sustained growth, has important consequences. It means that most of the biochemical or biological analytical methods used to study properties of the tumours are measuring properties which may be properties of the majority of the tumour cell population (which may be responsible for tumour size, but not tumour growth) but not the properties of the stem line which is the minority population responsible for the tumour growth. The concept helps us to understand some of the therapeutic successes with radiotherapy and chemotherapy in particular, when the sheer mass of the total malignant cells would predict an unacceptably high intensity therapeutic regime to effect cure or even long-term remission (Lajtha 1981).

LONGEVITY V. IMMORTALITY

Malignant cell populations have been described as having been 'immortalised', in the sense of attaining indefinite proliferative capacity. This has been contrasted with the apparently limited life-span of normal diploid cells in vitro (Hayflick 1965). However, a brief consideration of numbers shows that the above statements are somewhat over-simple.

For a human tumour (or leukaemia) to reach a mass of 1 kg — roughly the equivalent of some 10^{12} cells — 40 population doublings from the original malignant cell would be required. With the realisation that malignant growth in vitro is 'inefficient' in the sense of considerable random cell loss during tumour growth, and assuming e. g. a 40% loss per cell cycle, the number of cell cycles to achieve 40 population doublings, against a 40% loss per cycle, would be over 150. A further 2 years' course of the disease with e. g. 48 h cycling rate would add another 360 cycles — a total of over 500 cycles. Solid tumours rarely grow to 1 kg size, but their cell loss rate may well be higher than 40% per cycle; also they may grow for longer periods than leukaemic cell populations, so the number of cycles may well be doubled, to well over 1000, during the course of the disease.

Against this, the normal haemopoietic stem cell population, even assuming a 10-day turnover time in man, will turn over some 2500 times during a 70-year life-span (and can be transplanted to undergo intensive self-renewal). The intestinal crypt stem cells with, say, a 4-day cycle time, will turn over some 6000 times. A tissue culture line of 'immortal' malignant cells would have to be grown for over

16 years, with a continuous 24-h cell cycle, to complete 6000 cycles! It is almost certain that such a line would not remain stable during such a long time: the original starting clone will have disappeared long before that.

In other words, on the evidence available, there is no a priori reason to differentiate normal from malignant stem cell lines on grounds of limited or unlimited proliferation potential, but there is evidence for a significantly greater stability of the normal as opposed to the abnormal stem lines during their life-span.

This stability, coupled with the very extensive self-renewal capacity of the stem cells, reflects in the apparent lack of 'ageing' in those normal stem cells (haemopoietic, intestinal, etc.) which have been so far studied. In the static (incapable of proliferation) cell populations, ageing manifests as a slow decay in numbers. How such decay affects the milieux of the various other cell populations, and thus their function in situ, is a major unsolved problem.

REFERENCES

Barnes D W H, Ford C E, Gray S M, Loutit J F 1959 Spontaneous and induced changes in cell populations in heavily irradiated mice. Progress in Nuclear Energy, series VI, 2. Pergamon Press, London, p 1

Baserga R 1976 Multiplication and division in mammalian cells. Marcel Dekker, New York, p 239

Baserga R 1978 Resting cells and the G_1 phase of the cell cycle. Journal of Cellular Physiology 95: 377

Becker A J, McCulloch E A, Till J E 1963 Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature 197: 452

Buckton K E, Smith P G, Court Brown W M 1967 The estimation of lymphocyte lifespan from studies on males treated with X-rays for ankylosing spondylitis. In: Evans H J, Court Brown W M, McLean A S (eds) Human radiation cytogenetics. North—Holland, Amsterdam, p 106

Burns F J, Tannock I F 1970 On the existence of a G_0-phase in the cell cycle. Cell and Tissue Kinetics 3: 321

Cairns J 1975 Mutation selection and the natural history of cancer. Nature 255: 197

Cairns J 198l The origin of human cancers. Review article. Nature 289: 353

Castor L N 1980 A G_1 rate model accounts for cell-cycle kinetics attributed to 'transition probability'. Nature 287: 857

Cater D B, Holmes B E, Mee L K 1956 Cell division and nucleic acid synthesis in the regenerating liver of the rat. Acta radiologica 46: 655

Chang L J A, Till J E, McCulloch E A 1980 The cellular basis of self-renewal in culture by human acute myeloblastic leukemia blast cell progenitors. Journal of Cell Physiology 102: 217

De Maertelaer V, Galand P 1975 Some properties of a 'G_0' model of the cell cycle. I. Investigation on the possible existence of natural constraints on the theoretical model in steady-state conditions. Cell and Tissue Kinetics 8: 11

De Maertelaer V, Galand P 1977 Some properties of a 'G_0' model of the cell cycle. II. Natural constraints on the theoretical model in exponential growth conditions. Cell and Tissue Kinetics 10: 35

Ford C E, Hamerton J L, Barnes D W H, Loutit J F 1956 Cytological identification of radiation-chimaeras. Nature 177: 452

Gilbert C W, Lajtha L G 1965 The importance of cell population kinetics in determining response to irradiation of normal and malignant tissue. In: Cellular radiation biology. Annual Symposium of Fundamental Cancer Research. University of Texas, M D Anderson Hospital and Tumor Institute, Houston, p 474

Hayflick L 1965 The limited in vitro lifetime of human diploid cell strains. Experimental Cell Research 37: 614

Hendry J H, Potten C S 1974 Cryptogenic cells and proliferative cells in intestinal epithelium International Journal of Radiation Biology 25: 583

Holtzer H 1978 Cell lineages, stem cells and the quantal cell cycle concept. In: Lord B I, Potten C S, Cole R J (eds) Stem cell and tissue homeostatis. Cambridge University Press, Cambridge, p 1

Holtzer H, Rubinstein N, Fellini S, Yeoh G, Chi J, Birnbaum J, Okayama H, 1975 Lineages, quantal cell cycles and the generation of cell diversity. Quarterly Reviews of Biophysics 8: 523