Parathyroid Hormone Assay.—The sensitivity of this assay system varies greatly from laboratory to laboratory. One group of workers have described an assay of great sensitivity and discrimination, demonstrating raised levels of parathyroid hormone in the circulation in all cases of hyperparathyroidism.[15] All other workers have achieved less sensitivity and still find overlap between cases of hyperparathyroidism and the normal range.[4 12 16 17] Selective venous catheterization has been used as an additional diagnostic aid in these circumstances and also for the pre-operative localization of parathyroid tumours.[4]

DOUBLE DIAGNOSIS

Parathyroid tumours sometimes occur in patients with other causes of hypercalcaemia—including non-parathyroid cancers, sarcoidosis, thyrotoxicosis, and Paget's disease. Differential diagnosis is more complex in these cases but the various procedures described above will usually lead to accurate diagnosis of each condition.

Treatment

Hypercalcaemia can usually be corrected and its symptoms relieved, in many cases by removing the cause. In emergencies phosphate loading will lower the plasma calcium whatever the cause.[18] This may be given intravenously as the neutral sodium salt, using doses of one or two grammes of phosphorus in the first 24 hours. Later, phosphate can usually be given orally as disodium hydrogen phosphate, 10 grammes daily (containing about 2 g of phosphorus).

In hyperparathyroidism parathyroidectomy is a standard procedure which we recommend in all patients with symptoms. The Mayo Clinic team on the other hand, have suggested more restricted indications for surgery.[12] Postoperative medical management usually presents problems only in cases with osteitis fibrosa, when treatment with vitamin D, calcium supplements, and aluminium hydroxide gel may be necessary for some weeks or months.

In the conditions grouped under headings (2) and (3) in the Table the treatment is to stop the excess intake of calcium or vitamin D. Hydrocortisone may be a valuable short-term addition to this treatment.

In the hypercalcaemia of cancer, treatment is more palliative, though removal of an operable tumour may be effective in some cases.[19] Local radiotherapy may produce temporary improvement as may antimitotic agents, especially in multiple myeloma and the lymphomas. Hypercalcaemia usually returns when the tumour grows again or when secondary deposits appear.

The administration of corticosteroids produces a good result in some patients with cancer.[20] In others who show only a partial response to corticosteroid administration, satisfactory results can be obtained by adding oral sodium or potassium phosphate. Oestrogens and androgens have been used successfully in the palliative treatment of hypercalcaemic patients with breast cancer and bony metastases.

Disodium ethylenediamine tetraacetic acid given intravenously will lower the plasma calcium level, but its effect is transient and in large doses renal damage may occur. It has contributed little to the management of hypercalcaemia in cancer. The role of other agents which produce hypocalcaemia (calcitonin, glucagon, diphosphonates) is being investigated.

Finally, it should be noted that the early occurrence of hypercalcaemia in patients with cancer does not necessarily mean a bad immediate prognosis. Some patients survive for years after treatment of severe hypercalcaemia, which is actually the cause of all their initial symptoms.

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Scientific Basis of Clinical Practice

Disordered Leucocyte Proliferation in Leukaemia

JOHN STUART

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The application of leucocyte isotope-labelling techniques to the study of acute and chronic leukaemia has provided considerable insight into the disordered leucocyte proliferation which characterizes these diseases. In acute leukaemia a maturation arrest in the leukaemic cell line occurs at the blast-cell stage of development and results in a failure to produce the normal, functional end-cells of that cell line. Extensive replacement with blast cells then leads to a failure to produce the functional units of the other marrow cell lines; this results in the early development of neutropenia, thrombocytopenia, and anaemia, which characterize acute leukaemia. In chronic leukaemia there is

The Children's Hospital, Ladywood, Birmingham
JOHN STUART, M.D., M.R.C.P., Consultant Haematologist

increased proliferation of an entire cell line, without overt maturation arrest, and the increased cell production gives rise to the hepatosplenomegaly or lymphadenopathy of this disease; clinical signs arising from marrow replacement are not a feature of the early stages of chronic leukaemia.

In recent years a more detailed understanding of these disorders of cell proliferation has arisen from isotope-labelling studies of DNA and RNA synthesis in marrow precursor cells by the technique of autoradiography. This knowledge is being applied to the design of clinical trials, making it necessary to have some knowledge of marrow cell kinetics to understand the rationale of combination chemotherapy or reinduction chemotherapy of leukaemia as well as current attempts to increase the proliferative activity of blast cells to enhance their susceptibility to antimitotic agents.

Production-Line Concept of Normal Haemopoiesis

Normal bone marrow may be described as a tissue comprising a series of production lines (for example, myeloid, erythroid, megakaryocytic, and lymphoid). Each line (Fig. 1) consists of precursor cells, which are primarily concerned with proliferation;

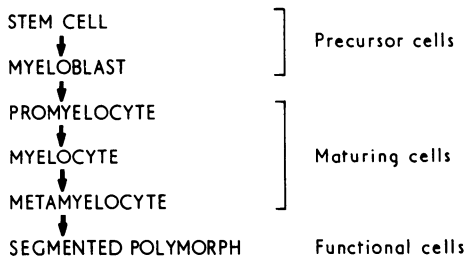

FIG. 1—Bone marrow production line for normal myeloid cells.

maturing cells, which are irreversibly committed to differentiate along a morphologically recognizable pathway; and functional cells (usually end-cells), capable of pursuing a specific metabolic function such as phagocytosis, oxygen transport, or the initiation of haemostasis. It is not clear to what extent the blast cell of acute leukaemia is synonymous with a stem cell, and the term precursor cell is used here in an imprecise sense to describe immature cells concerned largely with self-replication. The precursor cell must provide adequate cells with the potential to differentiate, but once a cell begins to differentiate its intrinsic proliferative capacity becomes limited and subject to the rate-limiting processes which control each particular cell line (for example, erythropoietin in the case of erythroid cells). Eventually the cell loses the capacity for mitotic division (after the myelocyte stage in the case of myeloid cells) and becomes a functional unit with a finite life-span.

Disordered Proliferation in Leukaemia

The gross abnormalities of this normal production-line concept which characterize acute and chronic leukaemia appear initially to be different, though these differences may represent only a varying degree of de-differentiation in a similar malignant process. In acute leukaemia the maturation arrest giving rise to an excess of blast cells occurs at a stage of development when the cell still possesses proliferative potential, at least initially, but has relatively little ability to differentiate. This arrest can probably occur at different stages of blast cell development, as illustrated by the differing cytochemical and ultrastructural features within acute leukaemia. Since the arrested cell does not possess the ability to differentiate, or is unresponsive to initiating factors, its normal pathway of elimination from the marrow is lost and the cell must either continue to proliferate *in situ* or remain in a non-proliferating, suspended state until death occurs. The balance between proliferation, non-proliferation, and cell death will determine the rate of growth of the tumour, the extent of the total leukaemic cell mass in the body, the response to chemotherapy, and the probable duration of subsequent remission.

In chronic leukaemia the maturation arrest is more subtle since the characteristic finding is of considerable hyperplasia of the whole myeloid (or lymphoid) cell line. Thus the leucocytosis consists mainly of morphologically mature, differentiated cells, with no disproportionate increase in blasts. Though the gross morphological appearance of these cells is usually normal, they do show metabolic features of abnormal development. The small lymphocyte in chronic lymphocytic leukaemia shows a loss of immunological responsiveness—characterized clinically by the development of autoimmune disease and by impaired antibody formation and experimentally by a failure to react to antigenic stimulation with plant mitogens such as phytohaemagglutinin.

The neutrophil of chronic myeloid leukaemia is capable of phagocytosis, although this may be suboptimal in most cells,[1] but the cell shows a virtual absence of the lysosomal enzyme alkaline phosphatase. The functional significance of the latter is unknown but since the enzyme normally appears in the secondary granule of myeloid cells at a relatively late stage of development its absence in chronic myeloid leukaemia may represent a maturation arrest in the sense of asynchronous development between nucleus and cytoplasm. The absence of this enzyme is of diagnostic value in differentiating chronic myeloid leukaemia from leukaemoid reactions and the myeloproliferative disorders. Other evidence for cytoplasmic immaturity in the neutrophil includes ultramicroscopical changes[2] and a glycogen content[3] consistent with an early stage of maturation.

The well-differentiated form of neoplasia in chronic myeloid leukaemia shows regression to an undifferentiated type, with the development of blastic crisis, in up to 70% of patients. This represents a failure of the leukaemic myeloid cells to differentiate beyond the blast-cell stage of differentiation and is clinically and morphologically similar to acute myeloblastic leukaemia. These myeloblasts also show poor responsiveness to antimitotic therapy. This example further illustrates that acute and chronic leukaemia may represent different degrees of maturation arrest and therefore cell differentiation within the same leukaemic process.

Application of Autoradiographic Studies

Each marrow cell capable of mitotic division passes through a mitotic or proliferative cycle, in which its DNA content doubles before cell division and the formation of two daughter cells (Fig. 2). In the case of the precursors the resulting cells return to the non-proliferating pool and are then triggered to differentiate or to re-cycle through the proliferative phase, or they die.

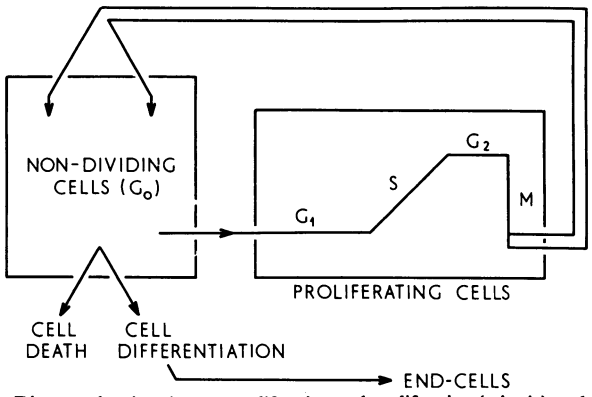

FIG. 2—Diagram showing the non-proliferating and proliferating (mitotic) cycles of bone marrow precursor cells. G_0 = a non-proliferating compartment of cells not participating in the mitotic cycle; G_1 = a phase, difficult to differentiate from G_0, before DNA synthesis in which the nuclear DNA content is diploid; S = the stage of DNA synthesis required to produce a tetraploid nucleus; G_2 = a premitotic resting phase after completion of DNA synthesis; M = mitosis.

The rate-controlling mechanisms operating at this stage are not known, though escape from their control by a stem cell may initiate leukaemic change in a clone of cells.

The technique of autoradiography allows accurate study of this proliferative cycle in haemopoietic cells. Bone marrow cells are ideally suited to the application of autoradiography since the stage of maturation of individual cells can be recognized by standard morphological criteria and leukaemic cells readily identified, at least in the acute form of the disease.

The technique consists of incubating aspirated marrow cells with radio-isotope labelled precursors of nuclear DNA (usually tritiated thymidine) or of cytoplasmic RNA (usually tritiated uridine). If the cell actively synthesizes DNA during the period of exposure to the labelled precursor then the isotope will become incorporated in the nucleus and the resulting isotope emission can be recorded photographically as black grains overlying the nucleus. RNA synthesis is similarly recorded as cytoplasmic grains; the absence of grains indicates that the cell did not undergo either DNA or RNA synthesis during the period of exposure to the isotope.

Using this technique, in conjunction with an estimation of cell DNA content by the Feulgen method, one can determine whether an individual cell was in the DNA synthetic (S) phase of its proliferative cycle, or whether it underwent mitosis (M), or whether it was "resting" in a G_0, G_1, or G_2 phase (Fig. 2). It is also possible to determine the percentage of cells in a population undergoing proliferative activity and this has considerable relevance to the study of leukaemia.

For many years it was believed that neoplastic cells underwent continuous rapid proliferation and outgrew the parent normal tissue. Experimental evidence obtained from the L1210 mouse leukaemia also suggested that leukaemic cells were in an active proliferative phase and increased with an exponential growth pattern. An increase in the size of a cell population may, however, arise by other means: when the number of precursor cells feeding into that line increases to establish accessory production lines; when mitotic activity increases at each stage of cell maturation; or when the cells produced as a consequence of a maturation arrest live for an abnormally long time and progressively accumulate. The use of autoradiographic techniques has shown that in acute lymphoblastic and myeloblastic leukaemia most blast cells are nonproliferating and accumulate in the G_0 phase.[4-6] A small nucleus of more rapidly proliferating cells probably serves to increase the total leukaemic cell mass before these cells also join the non-proliferating compartment. Probably these out-of-cycle blast cells still retain the ability to synthesize DNA[7] and can therefore re-enter the proliferative cycle, though there is no information that they do so.

In contrast to the low uptake of tritiated thymidine by the leukaemic lymphoblast, there is a high uptake of tritiated uridine—indicating active RNA synthesis[8] and revealing that the description "resting" cell applies only to mitotic activity. Actively dividing normal precursor marrow cells show evidence of both high DNA and RNA synthetic activity, in contrast to the low DNA but active RNA synthesis of the lymphoblast.

In chronic myeloid leukaemia there is also evidence that the myeloblast divides somewhat more slowly than the normal marrow myeloblast,[9] with an approximately normal generation time for subsequent polymorph production.[10] The myeloid hyperplasia of this disease is probably a consequence of increased stem cell proliferation giving rise to an expanded compartment of myeloid cells. These may proliferate and differentiate normally but isotope-labelling evidence for this has given conflicting results.

FEED-BACK MECHANISMS

This increase in precursor cell proliferative activity in chronic myeloid leukaemia could represent either loss of a negative feed-back mechanism or loss of responsiveness to it.

In several normal cell systems there is evidence of a negative feed-back mechanism resulting from a substance (termed a chalone) liberated from mature end-cells. This chalone reduces proliferative activity by a suppressant effect on DNA synthesis or mitotic activity of precursor cells. This mechanism is believed to reduce to normal the increased mitotic activity of epidermal cells after wound repair (epidermal chalone).[11] A similar substance has been elaborated from mature granulocytes (granulocytic chalone)[12] and possibly a decrease in its activity in chronic myeloid leukaemia granulocytes accounts for the myeloid hyperplasia of that disease.

A cell line producing an end-cell of finite life need not, however, have a feed-back mechanism dependent on the release of a substance by end-cells. Equally good regulation could be achieved by an extrinsic factor which regulates the number of stem cells triggered to differentiate along that cell line. This type of mechanism, with erythropoietin as the extrinsic factor, is believed to control day-to-day marrow erythropoietic activity. Thus the myeloid hyperplasia of chronic leukaemia could instead represent an abnormal response to such an agent.

It is well established that most blood lymphocytes in chronic lymphocytic leukaemia are also non-proliferating and long-lived. Thus the leukaemic process in general cannot be regarded as a disease of continuous, rapid leucocyte proliferation and this has profound implications in respect of the effectiveness of antimitotic chemotherapy, particularly in acute leukaemia.

Therapeutic Significance of Decreased Proliferative Activity

Most antimitotic agents are active only within the proliferative phase of the cell cycle, and usually within selective portions of it (see Table). Thus it is surprising that these agents are able to show their marginal selectivity for leukaemic cells since the more frequently dividing normal haemopoietic cells might be expected to provide the major target organ. Despite the low proliferative activity of most lymphoblasts in childhood acute leukaemia, complete haematological remission can be obtained in about 90% of patients with the combination of prednisolone and vincristine. This is the most successful combination of two drugs available for this disease and comprises one agent which acts during mitosis and another which acts on out-of-cycle cells as part of the well known lymphocytotoxic action of steroids.

Probable Site of Action, in Relation to the Mitotic Cycle, of Some Chemotherapeutic Agents Used in Treating Leukaemia

Drug	Site of Action	
	In-cycle	Out-of-cycle
Prednisone	Prolongs late G_1 Blocks entry to S	General lymphocytotoxic effect
Daunorubicin	S	Complexes with preformed DNA
Alkylating agents		
Cyclophosphamide	Non-specific	Non-specific
Busulphan		
Chlorambucil		
Vincristine	M	—
6-Mercaptopurine	S	—
Methotrexate	S	—
Hydroxyurea	S	—
Cytosine Arabinoside	S	—

The failure to achieve equally rapid remission in adult acute leukaemia may reflect the lack of an agent effective against the out-of-cycle myeloblast, though the recent success in inducing remissions with the combination of daunorubicin and cytosine arabinoside[13] may reflect the possible action of daunorubicin on preformed DNA in out-of-cycle cells.[14] When additional drugs are added to these combinations then they should be chosen from agents active in other parts of the proliferative cycle—for example, the addition of daunorubicin to prednisolone and vincristine in the treatment of acute lymphoblastic leukaemia.

TISSUE TOLERANCE

There is evidence from recent chemotherapeutic trials of intensive combination chemotherapy that the limit of drug tolerance of normal tissues has been reached; further increases in drug dosage are likely only to increase toxic effects. Further therapeutic advantage could be obtained, however, if the blast cells were rendered more susceptible to the action of currently available drug combinations. This might be achieved if out-of-cycle blasts could be triggered to re-enter the proliferative cycle, but this re-entry would probably require to occur in a synchronous manner. It is already known that more blast cells are in a proliferative phase of their cell cycle in the relapse state of acute lymphoblastic leukaemia compared with the time of initial diagnosis,[8] and some of these cells are resistant to antimitotics. This may, in part, represent the emergence of resistant cells but an asynchronous progression through the mitotic cycle may also be responsible.

If more synchronous entry into, and progression through, the mitotic cycle could be achieved then the timing and duration of antimitotics acting in the S and M phases could be tailored to anticipate the arrival of stimulated blast cells. Higher drug doses could be given for shorter periods, allowing normal marrow cells to regenerate between courses. There is already evidence that some degree of stimulation of out-of-cycle cells can be achieved after the administration of methotrexate[15] and after extracorporeal irradiation.[16] Partial synchronization of mitotic activity has also been reported after an infusion of cytosine arabinoside.[17]

TIMING OF ADMINISTRATION

Experimental studies of this nature have not yet been effectively applied to chemotherapeutic trials, but probably some improvement in the use of single drugs and drug combinations can still be achieved by more appropriate timing of administration. For example, the administration of maintenance oral methotrexate in acute leukaemia in childhood has been shown to be more effective in prolonging remission duration when given twice weekly compared with daily oral administration.[18] Periodic reinduction courses of prednisolone and vincristine against this background maintenance therapy also effectively further prolong the duration of remission.[18]

It is theoretically possible that the administration of vincristine against a background of continuous prednisolone therapy, or as reinduction immediately after methotrexate maintenance, limits the degree of cytotoxity achieved by this vinca alkaloid. Since vincristine is believed to act only during mitotic division (M) itself, the prior use of prednisolone to hold-up cells in late G_1, and of methotrexate to prevent cells passing beyond the S phase, may delay the arrival of an appreciable number of cells at the M stage until some of the vincristine effect has passed. A short delay before the administration of the latter may enhance its effectiveness and simultaneously reduce its toxicity to normal myeloid precursors.[19]

Curability of Leukaemia

Out-of-cycle marrow blast cells in acute leukaemia may mutate, develop resistance to maintenance antimitotic chemotherapy, and then, since they are capable of DNA repair replication,[7] return to a phase of proliferative activity.[17] The blood blast cell, which has even lower proliferative activity than the marrow blast, may also be capable of returning to the marrow compartment.[20] Leukaemic cure cannot, therefore, be expected until all non-proliferating leukaemic cells are destroyed as well as the more actively proliferating nucleus of blast cells. This necessitates either an antimitotic active against non-proliferating cells, a means of inducing mitotic activity in resting cells, or an alternative mode of therapy such as immunotherapy.

For clinical recovery the complete elimination of a leukaemic cell line must be followed by repopulation of the marrow by a normal cell line; this assumes that in leukaemia both normal and leukaemic stem cells co-exist and that the former can resume normal proliferative activity. In acute leukaemia the return to a normal chromosome number and morphology in remission does suggest that a normal stem cell population remains. The failure to achieve this in the so-called "remission" state of chronic myeloid leukaemia, where the Philadelphia chromosome persists in the myeloid and other cell lines, implies instead that the inadequately suppressed leukaemic cell line may be the only one present. The increased leucocyte alkaline phosphatase activity in "remission" cannot be cited as evidence for a co-existing normal cell line since this alteration in lysosomal enzyme content may reflect merely improved cytoplasmic maturation, an altered marrow transit time for myeloid precursors, or, alternatively, a more mature population of polymorphs produced in the spleen.[21]

Long survivors among patients with acute lymphoblastic leukaemia, and also myeloblastic leukaemia, probably include some patients in whom a leukaemic cell line has been successfully eliminated. The question remains whether current intensive combination chemotherapy will increase the small number of long survivors or whether improved forms of immunotherapy or cell cycle manipulation will be necessary to eliminate those out-of-cycle blast cells which defy destruction by currently available drug schedules.

Conclusion

Cell kinetic studies have greatly increased our understanding of the nature of the disordered leucocyte proliferation which characterizes leukaemia. More detailed knowledge of the decreased proliferative capacity of the leukaemic blast cell is required to devise techniques to increase its susceptibility to antimitotic therapy. Failure to completely eradicate non-proliferating blast cells which possess the ability to return to active proliferation may explain the low success rate in attempts to cure acute leukaemia.

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