Future Possibilities for the Development of Treatments of Leukemia¹

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SUMMARY
To assess future possibilities of treatments of leukemia, biochemical and biologic characterizations of the disease under discussion have been reviewed. These characterizations have been used as base lines for speculations and have been summarized in Table 1. A distinction has been made between drugs that destroy leukemic cells and homeostatic regulators that might achieve palliation or cure by redressing imbalances in the leukopoietic systems.

Someone wise has said: “It is always better to speak after the event as an historian than before the event as a prophet.” It is therefore a formidable task even to attempt crystal gazing at a time when so much work directed toward a solution of the problem under discussion is in progress all over the world. A good prophet is a person who has taken in all the relevant and established facts of a case and has digested them properly. Then from his computing mind may jump, like Pallas Athene out of the head of her father Zeus, a well-armed idea or ideas, which may prove to represent reliable forecasts for the future. This contribution to this symposium is based on the authors’ restricted knowledge of this field, and in speculating about the future developments of leukemia treatments, they must stick out their necks and be prepared to have their heads chopped off even by their kindest colleagues.

As far as we are aware, the gulf between chronic and acute forms of leukemia may not be as wide or deep as textbooks convey. The situation is complex; furthermore, it seems that the dividing line between the various forms of acute leukemia—myelogenous, lymphoid, monocytic—is sometimes not so sharply defined as the clinician or hematologist may wish for his diagnostic and prognostic considerations or for his choice of therapy. It is outside our competence to take sides, and an attempt is made 1st to assess the past, present, and future status of the biochemical and biologic base lines of acute leukemias, extrapolating where necessary from considerations of the chronic disorders. In this way it may be possible to find leads to potentially more effective therapies of this group of diseases. Although the causative or etiologic factors ought to play an important part in assisting the search for improved methods of treatment, we are refraining from any detailed discussion on such leukemogenic studies in the framework of this paper. The same applies to the physiologic role of leukocytes. For information on these aspects of the over-all problem, readers should consult extensive reviews such as those of Hayhoe (30) and Burdette (13).

BIOCHEMICAL BASIS
The biochemist has tended to look for metabolic differences at the molecular level between leukemia and corresponding normal cells, as has been done with the cells of other cancerous diseases, hoping to discover some unique aspect of metabolism, which may be exploited therapeutically. As yet, however, no such qualitative differences have been identified; only quantitative manifestations have become apparent.

A further difficulty confronting the biochemist studying the acute leukemias is the absence of a suitable experimental system in animals that mimics satisfactorily the situation in man. This makes it very difficult to study the progressive biochemical changes taking place during leukemogenesis. Owing to their insidious onset, the chronic, and sometimes acute, leukemias are well established before a diagnosis is made, and at this stage one may be observing metabolic abnormalities that are attributable to a new autonomous population of abnormal leukocytes. Thus it remains important to distinguish between metabolic changes that are significant to the etiology of the disease and changes that are significant to its therapeutic control. Naturally, one cannot in the framework of this paper mention all the metabolic studies in this field, and consequently a somewhat arbitrary selection of comparative data has been made.

To begin with energy metabolism, Beck (3, 4) made a quantitative study of the aerobic glycolysis of circulating leukocytes from patients with chronic myelogenous and lymphatic, but unfortunately not with acute, leukemia.

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He attributed the low rate of aerobic glycolysis in these cells to a deficiency in the rate-limiting enzyme hexokinase. This also accounted indirectly for the increased oxidation of glucose by way of the pentose shunt. The same author (5) has measured lactic acid dehydrogenase (LDH) activity at the cellular level, whereas other workers (53) have examined the levels of this enzyme in the sera of leukemic patients. All these results need reappraisal in the light of the more recently discovered isoenzymatic properties of LDH. The pattern of isoenzyme distribution may be an important factor in controlling leukocyte metabolism.

Investigations by Cahn et al. (14) have shown that there are 2 "pure" modifications of LDH, characteristic of heart (HHHH) and muscle (MMMM) tissue, respectively; binomial combination of the 4 constituent polypeptide units results in the formation of 5 independent modifications of LDH, which can be separated and distinguished by their different enzymic behavior. Abnormalities in the distribution of LDH isoenzymes in leukemic leukocytes have been recorded by Goldman and Kaplan (20) and by Dioguardi et al. (20). The deviations from the distribution pattern present in healthy leukocytes are most marked in cases of acute leukemia; less extreme variation is found in the leukocytes of acute leukemia in remission and of chronic myeloid leukemia. In cases of leukocytosis, the isoenzyme patterns are similar to those in normal leukocytes. In continuing studies of this phenomenon and extending them to other isoenzyme populations, it may be possible to account for many of the quantitative differences in the biochemistry of normal and leukemic leukocytes and at the same time to provide a more reliable basis for future chemotherapy.

The presence of low levels of alkaline phosphatase in leukocytes from chronic myeloid leukemia or myeloblastic leukemia is well known (30). In normal individuals administration of ACTH or cortisol elicits an increase in granulocyte alkaline phosphatase activity (49) (cf. Ref. 39). In contrast, the same drugs given to patients with myelogenous leukemia do not stimulate synthesis of this enzyme, which indicates the possible failure of control mechanisms in this disorder.

One of the metabolic patterns that appears to be significantly altered, at least in myelogenous leukemia, is that of the sulfur amino acid derivatives. Thus Contopoulos and Anderson (17) claimed to have detected abnormally high levels of glutathione in leukocytes from patients with acute myelogenous leukemia, and Weisberger and Levine (51) noted that these cells appear to have an elevated avidity for the sulfur amino acids, cystine, cysteine, and methionine, compared with normal leukocytes. Following these observations and provoked by the problem of resistance to busulfan (Myleran) in patients with chronic myelogenous leukemia, we studied levels of soluble sulfhydril and disulfide compounds in the blood fractions from a number of leukemic patients (29). In summarizing the results, Chart 1, it was found that the leukocytes of all patients with chronic myeloid leukemia contained oxidized glutathione, the gross concentration being dependent on the state of the disease and the course of therapy: e.g., busulfan lowered the soluble thiol and disulfide levels by a factor of approximately 10. No such disulfide was detected in normal leukocytes. In 2 patients who had been maintained on busulfan during the chronic phase of myeloid leukemia until they became resistant, surprisingly, the presence of oxidized glutathione in their erythrocytes was demonstrated before the onset of an acute blast-cell transformation. This observation makes it desirable to extend such investigation to acute leukemia cases. Furthermore, there is an indication from this work that the leukocyte population in well-controlled cases of chronic myeloid leukemia, although apparently morphologically normal, is biochemically still abnormal, since soluble disulfide remains in these cells.

It is interesting to note that the coenzyme closely connected with sulfur metabolism—pyridoxal phosphate—is low in the leukocytes and plasma of patients with chronic and acute myeloid leukemia (50). This prompted us to examine the conversion of pyridoxine (vitamin B6) to the coenzyme in leukemic patients. The results (Harrap, K. R., unpublished results) indicate that in patients with chronic and acute granulocytic leukemias there is a diminished capacity to convert pyridoxine into pyridoxal phosphate.

Although DNA and RNA metabolism is important for the development of a rationale for the treatment of acute leukemia, space does not permit a consideration in any detail. The topic will be treated briefly further on.

**BIOLOGIC BASIS**

Suitable leads derived from the experimental biologic investigations may be found among cytologic studies, assisted by tissue culture and transplantation techniques; genetic research into the possible prevalence of chromosomal aberrations; and immunologic and virus studies.

It is well known that the study of leukemic and normal leukocytes and related cells (apart from those in blood samples directly transferred to the microscope slide) was not a great success when they were grown in culture media.
or transplanted, for instance, into the pouch of the cortisonetreated hamster. Human material proved to be intrac-
table in the latter case, and the application of various tech-
niques of “tissue culture” with blood or bone marrow
cells (cf. Ref. 38) showed definite limitations and did
not produce clear-cut results. Morphologic changes fre-
quently occurred during the period of successful growth.
Nevertheless, it is suggested that further efforts in this
field would be worth while, perhaps by using bone marrow
in the form of “organ cultures” (cf. Ref. 1), checking the
morphology after short periods in comparison with normal
material, and exposing both to old and new drugs. It
remains to be seen whether the transplantation of human
leukemic bone marrow into animals is feasible, once a
better knowledge of immunologic tolerance has been
achieved. It would certainly assist in the assessment of
any novel remedies if such heterologous bone marrow
could be grown in the animal as a test system, thus eli-
minating preliminary clinical risks. The problem of the
relationship between the foreign host and the leukemic
cell will have to be resolved.

The discovery of chromosomal irregularities—e.g., the
Philadelphia chromosome in cases of chronic granulocytic
leukemia—has opened up new approaches to cytogenetic
aspects of a small number of diseases. It has not yet
been established whether acute leukemias, where frequent
chromosome alterations have been noted, come into this
category. Obviously, more detailed investigations of the
chromosomal DNA of leukemic cells (15) have to be
carried out during the coming years to place cytogenetic
aberrations on a safer basis. This aspect has a bearing
on the proposals to be made below, where the possibility of
the effects of extraneous nucleic acids will be discussed.

This brings us to the role of immunology in the field of
leukemias and, arising out of it, the possible treatment
by antisera. Specific surface antigens have been dis-
covered in normal leukocytes, and antisera against the
latter and a variety of leukemic cells have been prepared;
such sera show at least some in vitro effects (30). The
existence of specifically leukemic antigens of leukocytes is
uncertain; their presence or absence is still to be proved.
The possibility of loss of antigens during leukemogenesis
has to be considered as well. The situation is reminiscent
of that prevailing in other neoplastic diseases. It seems

TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Substances</th>
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<tbody>
<tr>
<td>I. “Killer” drugs</td>
<td>Improvements of existing drugs and elimination of their resistance</td>
</tr>
<tr>
<td></td>
<td>Discovery of novel types, either organic molecules or antisera, vaccines</td>
</tr>
<tr>
<td></td>
<td>Hormones (steroids, adrenocorticotropin, and others)</td>
</tr>
<tr>
<td>II. Homeostatic regulators</td>
<td>Catabolic and anabolic: functional proteins, enzymes, metal activators</td>
</tr>
<tr>
<td></td>
<td>Transforming factors, nucleic acids, nucleoproteins, polyelectrolytes</td>
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<td></td>
<td>Differentiation inductors, maturation agents</td>
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</table>

advisable to pursue this immunologic aspect to ascertain
whether there is any foundation for claims by workers
such as de Carvalho (19) for specific hyperimmune gamma
globulins in animal serum (for instance, the horse and
donkey received a preparation from leukemic spleens
purified by precipitation of the “normal antigens” with
antibodies against normal tissues). It is not improbable
that refined techniques and the application of procedures
enhancing antigenicity may lead to more tangible results.

In contrast to certain animal leukemias produced by
filtrable agents (24, 25, 28), the virus or subcellular particle
tiology of human types has not been proved. Induction
experiments in animals with cell-free material from human
leukemic patients have sometimes shown positive results,
but the nature of the causative agent or the exact type of
the disease, for instance in mice, is far from clear. For
many years Dmochowski and his school have demonstrat-
ed the presence of virus-like material in samples of
malignant tumors; recently a claim has been made by
Melnick et al. (34) for the presence of minovirus-like par-
icles in the plasma of 80% of children with acute leuke-
mia. Evidence that these particles are the cause of the
disease is still missing, especially since these “viruses”
have also been observed in children with infectious mono-
nucleosis. Consequently, it is too early to speculate on
the possibility of producing vaccines.

FUTURE TREATMENTS

In this section the emphasis is on speculation rather
than on logical deduction from established facts.

We propose that improvements of existing treatments
(whether these are designed to improve selectivity or to
eliminate drug resistance) and any novel forms of therapy
should be treated under the headings of (a) Group I:
“killer drugs” and (b) Group II: homeostatic regulators
(restitutive agents) (6, 21). Group I aims at the destruc-
tion or total elimination of a pathologic cell type or types,
which gives the formation site of normal cells (normal in
proliferation and maturation) a chance to recover suffi-
ciently so that only healthy leukocytes are produced at a
controlled rate. Group I also includes antisera (as far as
they exist or will be discovered) and hormonal substances,
though only in part, since the action mechanism of the
latter could be regulatory. Group II exists now to a
large extent as a concept; it includes those compounds,
materials, and extracts, mainly of natural origin, which
may be capable of healing lesions of a biochemical or bio-
logic character, which are fundamentally connected with
the etiology of the blood disorders under discussion. Such
remedies could achieve their curative effects by altering
the proliferation rate of leukopoietic systems and by
transforming the cell types arising therefrom into more
mature, normal forms. Although the practical outcome
is uncertain, there is evidence for the existence of an ex-
perimental basis (chemistry, biochemistry, and biology of
large molecules and their role in cellular events (see Bergel
[7]) (Table 1).

In Group I, one could deal first with improvements of
existing drug types, such as anti-metabolites, alkylating
agents, and, in part, hormonal substances. The elimina-
tion of drug resistance is closely connected with the prob-
lems of achieving greater selectivity of anti-leukemic action. In all this work greater attention should be paid to biochemical abnormalities of leukemic tissue and cells, and more intense exploitation of even present-day knowledge of metabolic lesions could be attempted (see "Biochemical Basis"). With the assumption that past and current research by many laboratories in the field of purine and pyrimidine analogs, of anti-folies, and of alkylating agents is generally known (see Ref. 16), it should be pointed out that further improvements will originate more readily from an increased exploration of the action mechanism of some of these drugs than from random synthetic programs. Considerable progress has been made in this respect with 6-mercaptopurine and its derivatives and related catabolic inhibitors (2, 22, 33, 36, 44), with thioguanine (32), with aza-uracil (39), with anti-folies (10, 31, 52), and others and, although not applicable to acute leukemia, with busulfan (29, 41). However, it is really sufficient to attack the obstacles to the control of acute leukemia with ingenious modifications, such as the bis(thioinosine)-5',5''-phosphate (36) and nicotinamide 6-mercaptopurine dinucleotide (2)? Of course other changes will be rung, and variants of anti-folies will be made, such as tetrahydrohomofolate (27), which might circumvent amethopterin resistance. Yet doubts linger in one's mind about the final clinical success. Perhaps novel organic molecules will suddenly appear (45), our friends the serologists will produce purified hyperimmune sera (19), or if the "hunches" about virus leukemogenesis (34) solidify into proofs, they will provide vaccines for preventive treatment.

However, it is felt that more attractive possibilities may exist with hormonal substances of the steroid and ACTH types. If their action and resistance mechanism could be fully elucidated and structures synthesized that would carry, on the basis of these elucidations, more specific therapeutic features, then considerable advances could be predicted.

A portion of this hormonal activity must be due to the influence of the compounds on homeostatic regulation. It is not known whether this happens via a favorable change of cell-membrane penetration, allowing for an improved trade between the inside and the outside of the cell, or whether enzymatic patterns are altered, perhaps because of the effect of the steroids themselves or other intercellular constituents on the allosteric properties of functional proteins (35). Whatever the nature of the receptor with which they interact, we are convinced that studies of possible means to obtain homeostatic regulation, which aims, as mentioned before, at the complete or at least partial restoration of normality, should be carried out without delay.

In our opinion this could be done in many ways, whereby the simultaneous application of a number of procedures may be necessary. Apart from the hormonal studies suggested above, the following may represent working hypotheses for future treatments: changes in the levels or character of functional proteins by various means; restitution of cofactors and metal activator deficiencies; alterations of genetic controls, directed not only toward operative but also toward regulatory ones; and introduction to the diseased tissues of maturation factors or redifferentiation agents.

The imbalance of biocatalysts in leukemias could be corrected directly by extraneous application of purified enzymes, preferably of enzymimetic systems (which are much less efficacious than holoenzymes but do not carry immunologic hazards) and of coenzymes. It could be corrected indirectly by utilization of feedback mechanisms with substrates, products, or compounds, which according to Monod et al. (35), would influence the conformational properties or tertiary and quaternary structures of endogenous enzymes, including isoenzymes. Manipulation of the inducer-repressor controls, if they should prove accessible to extraneous methods, could also be used.

The administration of exogenous enzymes or enzymimetics, which catalyze degrading processes, could deprive the diseased sites of leukopoiesis or the circulating abnormal cells of metabolites on which they depend more than their healthy counterparts. If such enzymatically active substances promote cellular syntheses that are found to be suppressed or missing, the use of exogenous enzymes could repair such deficiencies. Examples of the catabolic type, though not yet fully and convincingly demonstrated (6), are purified xanthine oxidase, crystalline ribonuclease, a model cysteine desulfhydrase, and aspiraginase.

In the case of the model cysteine desulfhydrase, consisting of pyridoxal phosphate and vanadyl salts (pyrvaln), Bergel et al. (8, 9) have shown that it degrades cysteine in vitro and, in collaboration with their clinical colleagues, that a mixture of pyridoxine and sodium vanadate can cause a fall in the total leukocyte count in acute myeloid leukemia. In view of the disturbance of the pyridoxine-pyridoxal phosphate biosynthesis in leukemic patients (see above), it seems desirable to study further the effects of a complex of vanadium and the preformed coenzyme or of an appropriate cell-penetrating derivative. Such investigations could be extended to other enzymimetic systems, whether known now or still to be discovered.

Wherever a malignant tissue proved to be asparagine dependent, Broome (12) had succeeded in controlling, for instance, experimental lymphomas by the administration of purified asparaginase from guinea pig serum. This observation should also be given more attention, especially if the diseased bone marrow of patients with acute leukemias depends on asparagine for its proliferative activities.

Whether catabolic or anabolic processes are involved, it may prove easier to introduce the appropriate coenzymes, especially where the deficiency is not attributable to the apoenzymes. Thus, the low levels of NAD/NADH in many growing tissues (37) could be replenished by administration of lipid-soluble derivatives of NAD, which have an increased chance to enter the target cells. Unfortunately, whenever the levels of cofactors are below normal, the biosynthetic processes leading to them are deficient as well, and it is consequently no use to offer the organism more nicotinamide or, as mentioned before, pyridoxine. A wide field of work with synthetics presents itself to the medicinal chemist who wishes to follow the
footsteps of Montgomery et al. (36) with mercaptopurine nucleotides or Atkinson et al. with NAD analogs (2). Should the observation by Harrap and Speed (29) concerning the presence of oxidized glutathione in blood fractions be confirmed in acute leukemias, the use of NADPH (a cofactor of glutathione reductase) could be explored.

Assuming that the appearance of altered isoenzyme patterns of LDH and possibly other enzymes has any bearing on the etiology of acute leukemia, changing them back to normal would not be easy unless their site of production is treated. This could be achieved only by interference with the control mechanism, operative or regulatory. Cases of altering protein biosynthesis by extraneous means in mammalian cells are as yet rare. Some (48) have claimed that human culture cells (1 in 10^4) were transformed from a deficient type to an enzymatically complete type with the DNA of the latter. Others (43) have reported that levels of interferon in virus-infected cultures were increased by the addition of "foreign RNA," or even by polyadenylic acid. Taking into consideration the infectivity of some isolated virus RNA's and the claim of a number of authors (see Ref. 7) of having affected tumorigenesis and carcinolysis by administration of nucleic acids, either DNA or mRNA, one wonders whether one of the most important future developments for the treatment of acute leukemia will be found in this direction. The question may sound absurd at this moment: could not some of the favorable effects of blood transfusions (18) be caused in part by the presence of such polynucleotide or oligonucleotide fractions in the cells suspended in the transfused fluid? Having been asked specifically to do some crystal-gazing, however, we venture to recommend the studies of material from such fluids, then of purified some crystal-gazing, however, we venture to recommend the studies of material from such fluids, then of purified

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