Nucleic Acids and Protein Metabolism in Acute Leukemia Cells

By Felice Gavosto, Giovanni Maraini and Alessandro Pileri

IT IS generally agreed that the genetic information in the cell which defines the specific sequence of the amino acids of protein chains is transmitted from DNA (desoxyribosenucleic acid) through the nucleotide sequence of RNA.[1] On the basis of this important role of nucleic acids, considerable efforts have been made during the past years to detect peculiarities in the DNA and RNA of leukemic cells. Studies on the content,[2-7] base composition,[3,8,9] chromatographic elution profiles,[10-12] molecular weight and other physico-chemical characteristics[13,14] have yielded unspecific and sometimes contradictory results.

More dynamic information has been obtained by studying the incorporation of isotopically labelled nucleic acid precursors:[15-18] a high degree of incorporation has been detected in all leukemic cells except in the lymphocytes of chronic lymphatic leukemia. However, the extreme cellular pleomorphism resulting in different percentages of cellular types at various stages of maturation in normal and leukemic bone marrows decreases the intrinsic value of these results obtained by chemical methods.

More reliable data may be obtained by comparing the same cellular types in normal and leukemic bone marrows. Autoradiography with tritium labelled compounds is a very useful tool in this regard. With this technic, DNA metabolism has been studied utilizing the most specific DNA precursor, H^3-thymidine, and very useful information on the kinetics of cellular proliferation, both in normal and leukemic bone marrows, has been obtained.[19-24] With the same technic, RNA and protein metabolism has also been studied utilizing H^3-cytidine[25] and H^3-DL-leucine[26] as precursors.

The present report deals with a comparative investigation of DNA, RNA and protein metabolism in acute leukemic and in normal blast cells. A radio-autographic technic utilizing tritium labelled precursors was employed.

Materials and Methods

The bone marrows of 6 normal donors and of 8 patients with untreated acute leukemia were investigated. H^3-thymidine (s.a. 890 mc./mM), H^3-uridine (s.a. 62 mc./mM), H^3-DL-leucine (s.a. 29.1 mc./mM) and H^3-DL-β-phenylalanine (s.a. 126 mc./mM) were used to study DNA, RNA and protein metabolism. Since uridine will be incorporated into RNA as well as DNA, cells not in the phase of DNA synthesis must be selected for the study of RNA metabolism. For this purpose H^3-thymidine was added to the sample of the incubation mixture containing tritium labelled uridine. Cells in the phase of DNA synthesis could be easily recognized by their heavy labelling (fig. 1 a & b).

Bone marrow was obtained by sternal puncture and aspirated into a heparinized

Fig. 1.—Nuclear labelling after H^3-Uridine incorporation in acute leukemia cells (cells 1,2,3,4,5,6). The cell No. 7 has incorporated also H^3-Thymidine: its degree of labelling is about sevenfold that detected in the other cells.

syringe. It was immediately diluted with an equal volume of Eagle's Basal Essential Medium and transferred into siliconized test tubes. H^3-thymidine was then added to a final concentration of 2.5 μc./ml. and H^3-DL-leucine and H^3-DL-β-phenylalanine were added to a concentration of 10 μc./ml. Incubation was carried out in a rotating system at 37 C. Smears were made on gelatine-coated slides after one hour of incubation for thymidine and after 1, 2 and 3 hours for uridine, leucine and phenylalanine. After fixation in Carnoy's solution, autoradiographic preparations were made by the usual stripping technic, using AR 10 Kodak plates. After processing, the smears were stained by the May-Grünwald-Giemsa method. The grains over the cells were counted and the grain count of the back-

ground was subtracted. The degree of isotope incorporation was estimated in at least 40 myeloblasts in each normal bone marrow and in 200 cells from each case of acute leukemia.

In one normal and one acute leukemia marrow, incubation with H^3-uridine was discontinued after one hour, the cells were washed three times with Eagle's Medium and resuspended in a medium containing unlabelled uridine (2 mM/ml.), after which incubation was continued for seven hours.

RESULTS

Thymidine Incorporation.—Table 1 shows the percentage of cells which incorporated tritiated thymidine. A strikingly lower percentage of labelling is evident in acute leukemia cells as compared with normal myeloblasts. On the other hand, the values for the mean grain counts were not significantly different in normal and leukemic labelled blast cells.

Uridine Incorporation.—The values for the mean grain counts for Uridine are shown in table 1; these data were obtained after one hour of incubation. Incorporation was present in all cells, both normal and leukemic, and occurred almost exclusively in the nuclei. Statistical analysis did not reveal significant differences among the myeloblasts of the various normal bone marrows.

The uptake detected in the cells from cases of acute leukemia was constantly and significantly lower than that in myeloblasts from normal bone marrows (P < 0.001). Striking differences in the degree of uridine uptake were observed in the different cases of acute leukemia. These differences bore no relationship to the different cytologic types of acute leukemia.

Table 1

Normal bone marrows	Leucine mean grain count/cell	Phenylalanine mean grain count/cell	Uridine mean grain count/cell	Thymidine % labelled cells
Case 1	49.4 ± 34	36.7 ± 13	33.9 ± 15	50
Case 2	62.3 ± 33	49.5 ± 25	52.8 ± 34	53
Case 3	62.7 ± 37	40.6 ± 13	——	40
Case 4	52.5 ± 17	46.2 ± 9	44.2 ± 20	—
Case 5	49.9 ± 13	39.5 ± 15	49.7 ± 26	35
Case 6	57.3 ± 18	41.9 ± 10	38.9 ± 12	35
Mean values[*]	54.4 ± 28	40.7 ± 18	43.4 ± 22	42
Acute Leukemias				
Stem cell leuk.	12.3 ± 6.5	——	——	0.2
" " "	10.3 ± 7.8	——	18.3 ± 14.6	1
" " "	14.2 ± 5	1.4 ± 1.4	5.3 ± 4.5	1.5
" " "	1.7 ± 2.5	5.0 ± 6.3	13.1 ± 12	9.1
Micromyeloblastic	1.6 ± 1.8	——	20.4 ± 8.7	1
"	1.7 ± 2.1	4.4 ± 5	6.4 ± 5.3	1.5
Myeloblastic	30.8 ± 19.7	25.8 ± 16.8	15.2 ± 16.4	6.6
"	——	10.6 ± 8.2	13.2 ± 13	4

The values for the mean grain counts for leucine, phenylalanine and uridine are referred to the first hour of incubation and to an exposure of 20 days.

[*]Statistical analysis did not show significant differences between the myeloblasts of the different normal bone marrows.

When incubation was prolonged for 5 to 7 hours, progressive labelling of the cytoplasm was evident. When, after one hour of incubation, the labelled cells were washed and resuspended in a medium containing unlabelled uridine the progressive increase in the cytoplasmic labelling was still present; at the same time a diminution in nuclear labelling occurred. This was observed both in normal and in leukemic myeloblasts.

Amino acid incorporation.—The values for the mean grain counts for leucine and phenylalanine are shown in table 1. These data refer to the first hour of incubation; incorporation occurred in all the cells, both in the nucleus and in the cytoplasm (fig. 2), and was shown to increase linearly with time during the three hours of incubation. Statistical analysis did not show significant differences among the myeloblasts of the various normal bone marrows.

The incorporation of both amino acids was constantly and significantly lower (P < 0.001) in the cells from cases of acute leukemia than in normal myeloblasts.

Table 2 shows the values for incorporation corrected for the same specific activity of the precursors. In normal myeloblasts the uptake of leucine was about six times greater that of phenylalanine and this ratio appears to be remarkably constant. On the other hand, the relative degree of incorporation of the two amino acids varied greatly in the different cases of acute leukemia.

DISCUSSION

In acute leukemia the decrease in the percentage of cells labelled with thymidine, as already shown by previous investigations,[23] has been taken as evidence of a decreased proliferative capacity of these cells as compared to normal myeloblasts. This is in good agreement with the results previously obtained by other investigators[27] utilizing the statmokinetic method on bone
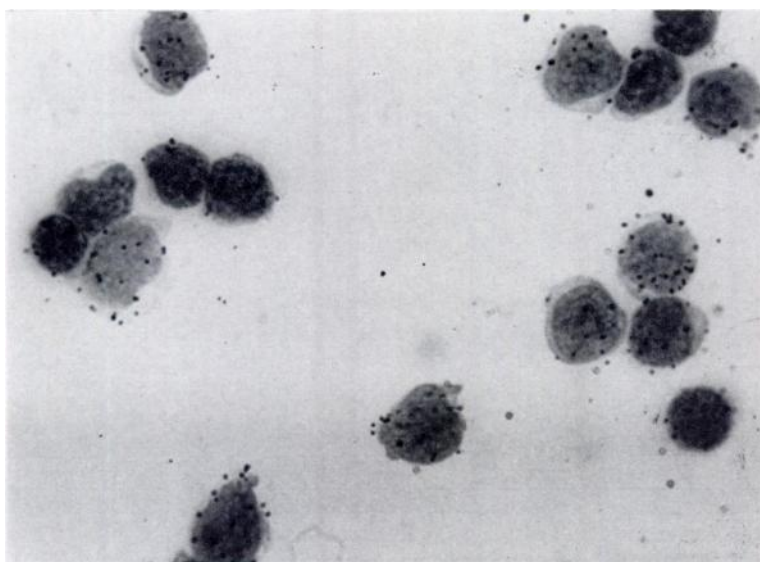

Fig. 2.—Leucine incorporation in acute leukemia cells.

Table 2

Normal bone marrows	Leucine mean grain count/cell	Phenylalanine mean grain count/cell	Uridine mean grain count/cell	U/L ratio	U/Ph ratio
Case 1	214 ± 147	36.7 ± 13	67.8 ± 31	0.31	1.84
Case 2	270 ± 143	49.5 ± 25	105.6 ± 68	0.38	2.13
Case 3	272 ± 176	40.6 ± 13	——	—	——
Case 4	228 ± 73	46.2 ± 9	88.4 ± 40	0.38	1.91
Case 5	216 ± 56	39.5 ± 15	99.4 ± 53	0.45	2.51
Case 6	248 ± 78	41.9 ± 10	77.8 ± 24	0.31	1.85
Acute Leukemias					
Stem cell leuk.	53.3 ± 28	——	—— —	—	—
″ ″ ″	44.7 ± 34	——	36.6 ± 29	0.81	—
″ ″ ″	61.6 ± 22	1.4 ± 1.4	10.6 ± 9	0.17	7.57
″ ″ ″	7.4 ± 11	5.0 ± 6.0	26.2 ± 24	3.55	5.24
Micromyeloblastic	6.9 ± 8	——	40.8 ± 17	5.87	—
″	7.4 ± 9	4.4 ± 5	12.8 ± 10	1.73	2.90
Myeloblastic	133.0 ± 85	25.8 ± 17	30.4 ± 33	0.22	1.17
″	——	10.6 ± 8	26.4 ± 26	——	2.49

The values are corrected for the same specific activity.

marrow cultures in vitro. The absence of significant differences in the mean grain counts of labelled cells in normal and leukemic myeloblasts would seem to indicate that the rate of DNA synthesis is essentially the same; this confirms the interpretation of a decreased mitotic capacity of acute leukemia cells.

Our data show also that the incorporation of RNA and protein precursors, although different from case to case, is constantly and significantly lower in acute leukemia cells. Two possibilities may account for these results: a decreased RNA and protein content or a lower turnover of these compounds in acute leukemia cells. The only investigations made so far at the cellular level are those of Thorell,[7] which demonstrated a normal or even increased RNA and protein content in acute leukemia cells as compared with normal myeloblasts. This seems to support the interpretation of a decreased RNA and protein turnover in these cells.

Although intracellular RNA appears to be mainly a cytoplasmic constituent, recent investigations have demonstrated that it is synthesized almost exclusively in the nucleus and subsequently transferred to the cytoplasm.[28,29] Our experiments show that in human bone marrow cells (normal and leukemic) uridine is also initially incorporated exclusively in the nucleus and that the cytoplasm becomes labelled only in a later period. The same phenomenon has been observed for H[3]-cytidine incorporation by Feinendegen et al.[25] in human leukemic cells (Osgood J-96) cultured in vitro. Since in the experiments in which the cells were resuspended in unlabelled medium the increase in cytoplasmic labelling was coincident with a decrease in nuclear radioactivity, we may reasonably assume that a real transfer of RNA from the nucleus to the cytoplasm occurs. Investigations designed to quantitate this phenomenon as well as to determine RNA turnover in normal and leukemic cells are now in progress in this laboratory.

The existence of a strict interrelationship between RNA and protein metab-
olism was established by the pioneer investigations of Brachet and Caspersson.
RNA is directly involved in the process of protein synthesis, both in the trans-
fer of activated aminoacids to the microsomes[30,31] and as a template in the
intimate mechanism of the synthesis of specific proteins.[1] Thus, RNA is the
carrier of nuclear information to the cytoplasm and determines the specificity
of the synthetic processes. This interrelationship has been demonstrated in
mammalian tissues by several authors,[32-34] both in the case of resting and
in the case of growing and differentiating cells. In normal bone marrow we[35]
were able to demonstrate that the existence of such a strict interrelationship
between RNA and protein metabolism holds for all the different cellular
types at various degrees of maturation (fig. 3). From the present investigation
it is evident that the ratio between uridine and amino acid incorporation is
constantly altered in acute leukemia cells as compared with normal myelo-
blasts (table 2).

The existence of an interrelationship between RNA and protein metabolism
can be considered as evidence of a well-defined metabolic finality of the cell
related to its specific functions (growth, synthesis of specific proteins, matura-
tion, differentiation, etc.). The dissociation of RNA and protein metabolism
in the cells of acute leukemia could possibly be related to the well known
incapacity of these elements to differentiate and to mature; this, together
with their lower proliferative capacity, seems to constitute the most important
functional defect of these elements.

SUMMARY

DNA, RNA and protein metabolism was investigated by means of a high
resolution autoradiographic technic in normal and acute leukemia blast cells
by studying the incorporation of tritiated thymidine, uridine, leucine and
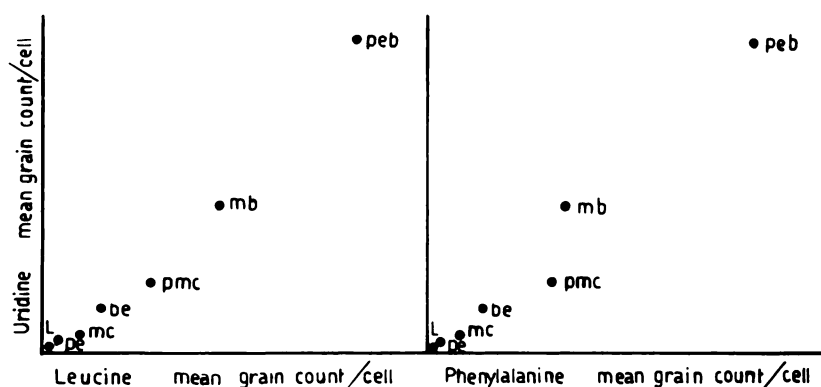phenylalanine. A strikingly lower percentage of cells labelled with thymidine

Fig. 3.—Interrelationship between RNA and protein metabolism in normal bone
marrow cells. PEB = proerythroblasts; MB = myeloblasts; PMC = promyelocytes;
MC = myelocytes; BE = basophilic erythroblasts; PE = polychromatic erythro-
blasts; L = lymphocytes.

was demonstrated in acute leukemia and was interpreted as evidence of a decreased proliferative capacity. A very significantly lower uptake of uridine, leucine and phenylalanine was detected in acute leukemia cells.

In normal and leukemic cells, amino acid incorporation occurred both in the nucleus and in the cytoplasm; uridine was incorporated exclusively in the nucleus during the first hour of incubation and the cytoplasm became labelled only in a later period.

The constant ratio between uridine and amino acid incorporation detected in normal myeloblasts was always altered in acute leukemia cells.

The lower RNA and protein metabolism and its dissociation in acute leukemia cells was discussed as related to the well-known maturation defect of these cells.

SUMMARIO IN INTERLINGUA

Le metabolismo de acido disoxyribonucleic. acido ribonucleic, e proteina esseva investigate per medio de un technica autoradiographic a alte resolution in blastocytos normal e de leucemia acute, determinante le incorporation de tritiate thymidina, uridina, leucina, e phenylalanina. Un frappantemente plus basse procentage de cellulas marcate con thymidina esseva demonstrate in leucemia acute, lo que esseva interpretate como indication de un reducite capacitate proliferative. Un multo significative reduction del fixation de uridina, leucina, e phenylalanina esseva detegite in cellulas de leucemia acute.

In cellulas normal e in cellulas leucemic, le incorporation de amino-acidos occurreva tanto in le nucleo como etiam in le cytoplasma. Uridina esseva incorporate exclusivemente in le nucleo durante le prime hora del incubation, e le cytoplasma acceptava le marcation solmente in periodos subsequente.

Le proportion constante que esseva detegite in myeloblastos normal inter le incorporation de uridina e illo de amino-acido se monstrava invariabilemente alterate in cellulas de leucemia acute.

Le reducite metabolismo de acido ribonucleic e de proteina e su dissociation in cellulas de leucemia acute es discutite in relation al ben-cognoscite defecto de maturation de iste cellulas.

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ACQUIRED APLASTIC ANEMIA: AN ANALYSIS OF 31 CASES AND REVIEW OF THE PERTINENT LITERATURE. *J. L. Scott, G. E. Cartwright and M. M. Wintrobe.* From the University of Utah College of Medicine, Salt Lake City, Utah. Medicine 38:119, 1959.

The authors define acquired aplastic anemia as a syndrome in which pancytopenia is caused by marrow destruction or marrow inhibition without evidence of infiltrative disease of the marrow or increased blood destruction. In a scholarly review this syndrome is separated from other pancytopenias, and 31 thoroughly studied cases are analyzed. Bone marrow examination shows a reduced generalized cellularity in almost all cases, but it is em- phasized that needle aspirations tend to show a more severe reduction in cellularity than marrow specimens obtained by surgical biopsy or at autopsy. Foci of lymphocytes in the marrow are frequently seen, although absolute lymphopenia is not uncommon. In 12 of the 39 cases chemicals were thought to be of etiologic significance, with chloramphenicol considered to be re- sponsible in 5 cases. Complete or partial recovery was observed in 14 cases, and the place of corticosteroids and splenectomy in the treatment was discussed.—A. E.