one of which is chondroitin sulfuric acid(9). The absence of activity in cartilage is of further interest because of the recent demonstration of the uptake of S^{35} by epiphyseal cartilage(10) and its incorporation into chondroitin sulfate(11).

Summary. Using p-nitrophenyl sulfate as substrate no sulfatase activity could be demonstrated in costal cartilage at varying stages of differentiation or in bone providing no red blood cells or marrow elements were present.

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Preferential Incorporation of Formate Carbon into Leukemic Blood Cells as Indicated by Autoradiography.* (18946)

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Demonstration of the incorporation of C^{14} into individual blood cells was first accomplished by Boyd, *et al.*(1) who showed by means of autoradiograms that the alpha carbon atom of glycine is taken up most rapidly by rat lymphocytes. The polymorphonuclear leukocytes incorporated C^{14} from glycine less rapidly than the lymphocytes and the circulating erythrocytes were considerably less avid in the utilization of the glycine carbon. The most obvious explanation for this difference in C^{14} content of the different cell types at 25 hours after injection appears to be the difference in their rate of formation (synthesis employing the alpha carbon of glycine as a precursor) and disappearance from the peripheral blood.

In the light of these results, it appeared of interest to study hematopoiesis in leukemic animals using the autoradiographic technic. If we assume that precursors of the various types of blood cells and the mature blood cells are exposed to the same order of concentration of a labeled compound, preferential incorporation by neoplastic cells suggests (1) a difference in the rate of synthesis (if the labeled compound or a degradation product is a metabolite) or (2) qualitative metabolic differences between the formed normal or neoplastic cells.

As a first approach to the study of hematopoiesis in experimental leukemia utilizing the autoradiographic technic, C^{14}-labeled sodium formate has been employed. Formate has been reported to be a precursor of the 2- and 8-carbon atoms of desoxyribose nucleic acid (DNA) and ribose nucleic acid (RNA) guanine and adenine and the 5-methyl carbon of DNA thymine(2). Also, formate carbon has been shown to appear in the alpha carbon of serine and in glycogen(3), in the methyl group of methionine, and the methyl groups of choline(4).

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Experimental. Transplanted Ak-4 leukemia in Akm strain mice has been used in the present experiments. In this leukemia the number of abnormal forms in the differential counts parallels the rise in the total leukocyte count(5,6). "The immature cells characteristic of Ak-4 leukemia were unlike the lymphocytes of normal mice. The young forms most closely resembling prolymphocytes were the largest cells observed and were often two or three times the size of neutrophils. The shape of the prolymphocytes varied from round to oval." With Wright's stain, the cytoplasm ranged from grey-blue to a darker intense blue and was usually coarse, uneven, and vacuolated. The appearance of the chromatin varied, sometimes slightly clumped, more typically very clumped and pyknotic(5). Burchenal(5) further observed in the advance stages of this disease an occasional elevation of mature polymorphonuclear cells. However, because of the staining reactions, general morphology, and complete lack of intermediate myeloid forms, the term "prolymphocyte" was believed most applicable to these leukemic cells.

In our first experiments, 4 leukemic mice of about 2 to 3 months of age were injected intraperitoneally with 4.0 μc each of $HC^{14}OONa$. These mice were in the sixth day of the disease (transplanted Ak-4 leukemia) and had total leukocyte counts of the order of 100,000. Blood was taken directly from the heart of injected mice at 6 hours and the packed cells were washed twice with inactive heparinized mouse plasma. This washing was necessary to remove active sera which would cause confusing background in the final autoradiogram. The cells were then diluted with inactive mouse plasma (5:1) prior to making smears in a darkroom. Smears were made directly on Eastman NTB plates, emulsion thickness 10 μ, air dried, and exposed for 6 weeks(1). After exposure, the cells were fixed with methyl alcohol and the NTB plates were developed 2 or 20 minutes (the latter to bring out beta tracks) in full strength D-19 at 20°C. The plates were then cleared in 10% sodium thiosulfate and the cells stained with Wright's stain.

In this first study, the autoradiograms which resulted from injection of 4.0 μc per mouse were faint even after 6 weeks' exposure. These autoradiograms were examined by an arbitrary technic which entailed counting silver grains immediately surrounding the individual cells and comparing with the background. An eyepiece reticle which divided a given field at 970X into 10 μ squares was placed in the microscope. Then, centering a given cell at the intersection of a 4-square area, the silver grains in the 4 squares (20 μ x 20 μ) were counted while focusing up and down in order to observe grains in different planes. The background was counted in 26 different fields where there were no cells and the average number of grains per mm^2 determined. This average was subtracted from the values obtained around the cells examined giving a net count in the area immediately around the respective cells. The difference between background and prolymphocyte grain counts was statistically significant, (Table I). These counts showed the prolymphocytes to have on an average about 7 times as many silver grains (net particles per mm^2) in the area immediately around the cell as did the small lymphocytes. The small lymphocytes and polymorphonuclears had grain counts slightly but not significantly above background.

In a second series of experiments, mice with advanced Ak-4 leukemia were injected I.P. with 100 microcuries each of C^{14}-formate and blood smears made at one hour. After varying periods of exposure, the NTB plates were developed, stained, and examined. With this level of carbon 14, the autoradiograms were so pronounced at one week after development that it was relatively simple to classify cells as grossly positive or negative with regards to their radioactivity. In NTB plates exposed for a period greater than 2 weeks, there were so many silver grains associated with the cells that they interfered with the morphologic classification. In view of this, it was decided to use cell diameter in addition to morphology in classification. The polymor-

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TABLE I. Grain Counts Immediately Around Blood Cells from a Mouse Injected with $HC^{14}OONa$ (4 μc).

	No. of fields counted	Range of counts	Avg count per mm²	σ	Avg net count per mm²*
Background	26	2,500–27,400	13,400	5,000	
Prolymphs	12	24,900–90,000	48,000	15,500	+35,400
Lymphs	17	2,700–20,600	18,800	7,000	+5,400
Polys	13	5,400–31,400	19,000	8,300	+5,600
Erythrocytes	12	2,700–18,400	11,600	4,000	—1,800

* Average net count per mm² = average counts—background.

TABLE II. Per Cent Positive Blood Cell Autoradiograms Observed in Mice with Advanced Ak-4 Leukemia at One Hour after Injection of C^{14}-Formate (100 μc).

				Differential count				% positive autoradiograms			
Mouse No.	Observer	Cell origin	Total WBC	Polys	Prolymphs	"Large" lymphs	Lymphs	Polys	Prolymphs	"Large" lymphs	Lymphs
2	1	Blood	45,000	20	18	11	51	0	89	36	2
	2			14	13	9	53*	0	100	50	5*
	3			18	20	10	52	0	94	50	1
	4			19	21	10	52	0	90	35	2
	5			—	—	—	—	0	82	78	6
3	1 and 4	Blood	64,000	16.5	19	14	78	0	82	78	6

* This value included 10 "unidentified" negative cells in the range of 6-9 μ diameter.

Note: All observations are on random fields and include counts of 100 cells or more. The classification "'large' lymphs" includes lymphoid cells of 10-12 μ (difficult to classify morphologically because of the presence of silver grains in the nuclear track emulsion). A large percentage of these cells are believed to be leukemic.

phonuclear cells were easily classified since they gave uniformly negative autoradiograms. Non-radioactive leukemic blood smears on NTB plates carried through the development and staining procedure showed that normal lymphocytes ranged from about 6 to 9 μ in diameter. Prolymphocytes (leukemic cells) ranged in size from about 10-18 μ in diameter. There were a small percentage of cells in the lymphocytic series which were intermediate in size (10-12 μ) and which could not be unequivocally classified as normal large lymphocytes or leukemic cells. It was therefore considered most practical for this initial work to report these autoradiograms on the following basis:

Type	Criterion
Polymorphonuclears	Identifiable by morphology
Lymphocytes	6-9 μ diameter
'Large' lymphocytes	10-12 μ "
Prolymphocytes	12-16 μ "

It is believed that on the basis of the morphology of these "large" lymphocytes, a goodly percentage may be leukemic cells. In any event, under this classification, little opportunity prevails for mistaking the prolymphocytes (leukemic cells) and the normal lymphocytes.

Five observers have now studied a large number of autoradiograms of leukemic mouse blood taken at one hour after I.P. injection of 100 μc of $HC^{14}OONa$. The agreement on the per cent of active cells of various types has been good. The results of these observations are given in Table II. Seven additional smears on NTB emulsion made from blood taken from mouse No. 2 were examined by two different observers as were duplicate smears from four additional leukemic mice. The additional results obtained were in good agreement with those reported in Table II. These autoradiograms were progressively darker with increased exposure and on 20-minute development in full strength D-19, beta tracks were in evidence. Typical autoradiograms are shown in Fig. 1 and 2.

The possibility of chemical fogging in these

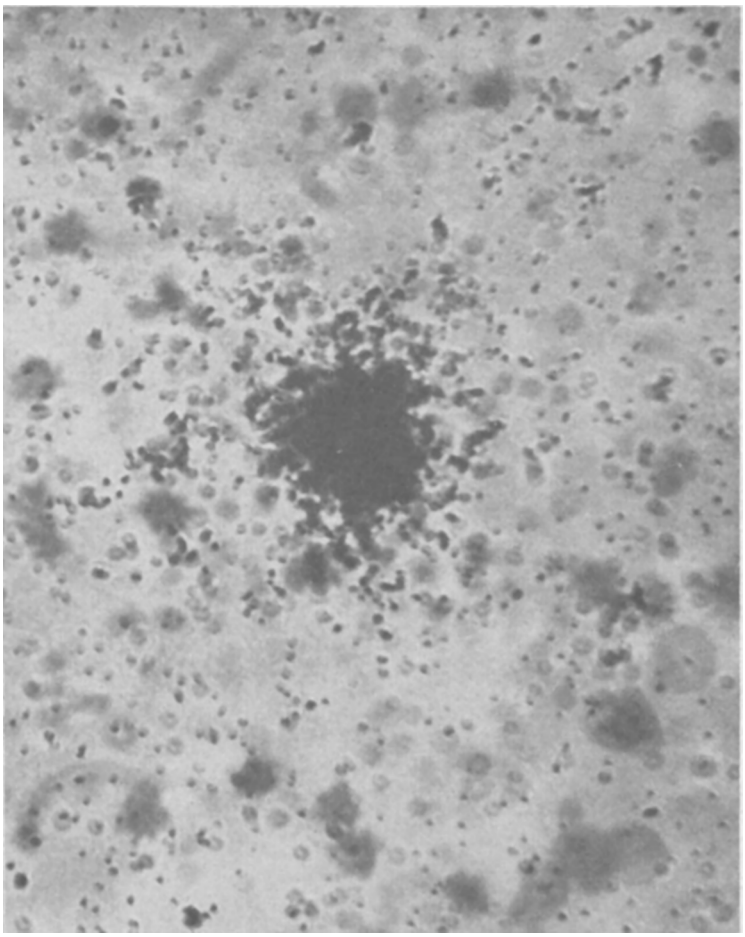

FIG. 1. A positive autoradiogram of a leukemic prolymphocyte showing the peripheral concentration of silver grains obliterating the outline of the cell. Blood taken from a leukemic mouse 1 hr after injection of 100 μc of C^{14}-formate.

studies has received considerable attention. In no instance have we observed chemical fogging in control smears, from normal or leukemic mice, made in the same manner and exposed and developed under the same conditions. To date 65 control smears have been made on blood from 18 leukemic mice and 49 control smears have been made on blood from 11 normal mice.

Discussion. From the results obtained, it appears that at one hour after injection of C^{14}-formate into mice with Ak-4 leukemia most (approximately 90%) of the prolymphocytes (leukemic cells) contain considerable amounts of the active atom, while the more mature cells are relatively inactive. A small percentage of the cells classed as normal lymphocytes were active though generally much less so than the prolymphocytes. In no instance in these one-hour experiments have we seen positive polymorphonuclear leukocytes, erythrocytes, or thrombocytes. These observations could be explained on the basis of the very rapid mitotic division and accompanying synthetic activity which must be existent in the leukemic hematopoietic tissue. Such an assumption would require the accompanying conclusion that there has been an almost complete turnover of leukemic cells within the period of these experiments while very few normal lymphocytes, polymorphs, or erythrocytes have been produced. A second and perhaps more reasonable conclusion which might be drawn from these data is the exist-

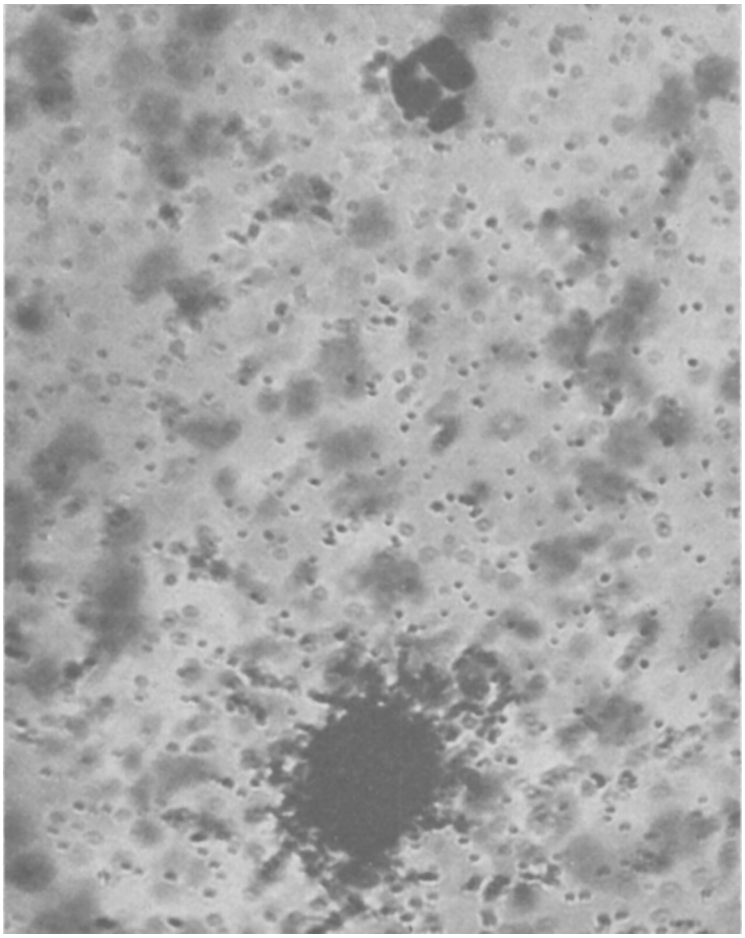

FIG. 2. A positive autoradiogram of a leukemic prolymphocyte and a negative polymorphonuclear leukocyte. Negative erythrocytes appear as shadows in the background. Blood from a leukemic mouse 1 hr after injection of 100 μc of C^{14}-formate.

ence of a profoundly different rate of formate metabolism in formed leukemic and normal cells.

Any attempt to explain the present data on a biochemical level must take into consideration the level and activity of citrovorum factor coenzyme (thought to be involved in formate transfer) in normal and leukemic cells. Bethell (7) has observed that leukemic cells are considerably higher in citrovorum factor than are normal white blood cells. In view of the fact that: (a) formate is a precursor of nucleic acid moieties(2) and carbon atoms of certain amino acids(3) and other important biochemicals(3,4), (b) folic acid antagonists inhibit the conversion of folic acid to citrovorum factor (8); cause the build-up of 4-amino-5-carboxamido-imidazole in bacteria(9); and inhibit incorporation of formate into nucleic acids(10), (c) folic acid deficiencies inhibit incorporation of formate into proteins(11), (d) folic acid inhibitors preferentially affect

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production of leukemic cells(5,6) and increase the life span of leukemic mice(12,13), (e) the anti-leukemic action of folic acid antagonists may be reversed by folic acid or citrovorum factor(14,15), and partially reversed by DNA or B_{12}(16,17), certain possibilities pertinent to the present discussion would seem to exist. Certainly the present data indicate that leukemic cells are geared to a more active formate metabolism than are normal white blood cells. Since inhibition of formate metabolism via the citrovorum factor coenzyme preferentially inhibits proliferation of leukemic cells, it might be suggested that these malignant cells are utilizing their supplies of this coenzyme to capacity while normal blood elements possess a marginal reserve of this formylating system. If this were true, folic acid antagonists might provide specific anti-leukemic action *in vivo* even though the antagonist concentration in the malignant and normal cell was of the same order because of differential demands on the formylating system.

Further studies are under way in an attempt to determine the significance of this difference in formate incorporation by normal and leukemic cells.

Summary. At one hour after injection of 100 μc of C^{14}-formate into mice with advanced Ak-4 leukemia, it has been shown by means of blood smear autoradiograms that about 90% of the leukemic cells are highly active as compared to about 5% of the more mature lymphoid elements. No active polymorphonuclears, erythrocytes, or thrombocytes were observed within one hour.

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Measurement of Total Body Chloride.* (18947)

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The chloride ion has been considered to be confined for the most part to the extracellular fluid. It has been accepted, nevertheless, that the chloride ion penetrates certain cell walls, in particular the wall of the erythrocyte, the gastric mucosa, the small intestine, the colon mucosa, the liver cell to be excreted in the bile, the cells of sweat and salivary glands, and of the kidney tubule. Precisely how much of the chloride is included in the intracellular water has not been appreciated until it was shown recently that inulin was distributed through a volume of fluid which more closely approximated the anatomical extracellular volume than did the larger "chloride space"(1). When the volume of fluid in which inulin is distributed is taken to be the measure of extracellular fluid, the difference then between the chloride contained in this body of fluid and the total chloride in the body represents intracellular chloride.

Bromide is distributed in tissues in much the same manner as chloride(2-4) and has,

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