in view of the small amounts of starting material (0.01 to 0.07 mmole).

The polymerization of O^2,5′-cyclothymidine 3′-phosphate is of immediate interest because of the novelty of the approach and its theoretical aspects. In all polymerizations of deoxyribonucleotides, the underlying principle has been phosphate activation to phosphoric anhydrides. We have introduced a different principle of polymerization, that of displacement on carbon of an intramolecular leaving group by a phosphomonoester as a nucleophile. A related approach that led to the formation of unnatural polynucleotides has been described (*13, 14*). We have suggested (*14*) that this principle might have relevance to the prebiotic formation of polynucleotides, although the nature of the prebiotic leaving groups is an enigma. Research on the behavior of cyclonucleotides should include such practical aspects as catalysis by acid or metal ions or irradiation as possible means of providing activation energy.

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Anemic Stress as a Trigger of Myelogenous Leukemia in the Unirradiated RF Mouse

Abstract. *Ninety-six percent of mice that were bled of 50 percent of their blood volume when they were 9 weeks old succumbed to myelogenous leukemia by 15 months after phlebotomy, the majority of them dying between 7 and 10 months after this treatment. These results suggest that (i) anemia is an effective stress for triggering myelogenous leukemia in animals that are predisposed to the disease, (ii) the RF mouse is "naturally" prone to the development of myelogenous leukemia, and (iii) the concept of two-step de novo induction of myelogenous leukemia appears to be applicable in this animal.*

Myelogenous leukemia (ML) can be induced in rats that apparently are not predisposed to spontaneous development of the disease (*1*). The induction process requires that the animal be first rendered prone to leukemia by x-irradiation and, second, that the leukemia be triggered by an acute anemia such as that induced by phlebotomy. If this two-step mechanism of leukemogenesis is generally valid, then (i) any particular population showing a tendency for the development of ML is bound to have more members prone to leukemia than members manifesting the overt disease; (ii) the tendency could be artificially induced (for example, by x-irradiation in the rat) or it could be spontaneously or "naturally" occurring (in other words, not dependent on any prior experimental conditioning); and (iii) the actual incidence of frank myelogenous leukemia in a population that is prone to leukemia would then be a function of the probability that individual animals would develop an anemia of sufficient severity to trigger the disease. Because of the relatively high incidence of spontaneous ML in the RF mouse, up to 4 percent (*2*), it was thought that this strain might be naturally prone and would thus be a suitable subject for testing these hypotheses. We report here that essentially all RF mice developed ML after half of their blood volume was removed. Since no other treatment was used, it would appear that this strain was indeed spontaneously prone to the development of ML.

Thirty female RF mice (*3*), 9 weeks old, were bled of 50 percent of their total blood volume (6.6 percent of body weight) from the posterior orbital sinus. Ten animals were nonbled controls. The two groups were kept in separate plastic cages lined with wood shavings but were otherwise treated in identical fashion; they were given free access to water and Purina chow and were weighed and examined daily. Hematocrit values and counts of total white blood cells and of percentages of different cell types were made at various intervals in the majority of the mice. All animals survived the acute effects of the phlebotomy; the first death occurred 17 weeks after bleeding. By 65 weeks after this treatment, all bled mice and three of the controls had died. (Three of the bled animals could not be analyzed.) The average survival time of the experimental mice was 39 weeks after venesection (50 percent had died after 37 weeks) (*4*). In all

Table 1. Summary of blood and marrow values found in animals with myelogenous leukemia. Blood films obtained before death from only 16 mice and marrow smears obtained after death from 23 animals were available for examination. Blood or marrow samples, or both, were obtained from each of 26 bled mice ($\bar{X} \pm$ S.E.M., mean $\pm$ standard error of the mean; *N*, number of animals; WBC, white blood cells; RBC, red blood cells; Neut., neutrophilic).

Test	$\bar{X} \pm$ S.E.M.
Blood values (N = *16*)	
WBC per cubic millimeter	32,000 ± 1,400
Differential count (%)	
Myeloblasts*	7.4 ± 1.1
Basophils*	4.1 ± 0.6
Eosinophils	2.7 ± 0.5
Neutrophils	62.4 ± 4.3
Monocytes	4.0 ± 0.5
Lymphocytes	21.0 ± 0.6
Marrow values (N = *23*)	
WBC : nucleated RBC (modal)	20 : 1
Differential count (%)	
Myeloblasts*	8.8 ± 0.8
Neut. promyelocytes and myelocytes	20.0 ± 1.1
Neut. metamyelocytes	31.8 ± 1.5
Neut. stab cells	13.1 ± 0.5
Mature neutrophils	6.6 ± 1.1
Basophils (all ages)*	4.8 ± 0.5
Eosinophils (all ages)	5.2 ± 0.5
Nucleated RBC	5.8 ± 0.4
Lymphocytes	6.5 ± 0.3

* Myeloblast values in seven nonleukemic (nonbled) mice were 0.4 ± 0.2 percent in the blood and 1.0 ± 0.4 percent in the marrow; basophil values in these mice were 0.4 ± 0.1 percent in the blood and 0.5 ± 0.1 percent in the marrow. Ranges of values in the blood and marrow samples of the leukemic mice were 2.5 to 12.0 percent basophils and 3.0 to 18.5 percent myeloblasts; and in the nonleukemic animals, 0.0 to 1.0 percent basophils and 0.0 to 1.5 percent myeloblasts.

cases, death appeared to be due to complications resulting from infection (found in all bled mice examined), anemia, and inanition (seen in all bled mice). Surviving control mice were killed after 65 weeks. Dead mice were necropsied whenever possible, and representative tissues were fixed in buffered formalin for histologic sectioning. In addition, imprints of marrow, liver, and spleen, as well as marrow smears, were prepared and stained with Wright-Giemsa stain.

Before death, all animals succumbing to ML showed a weight loss ranging from 10 to 50 percent of their maximum weight (median, 26 percent) and a drop in hematocrit ranging from 20 to 55 percent of the packed red cell volume before relapse. In addition, ruffling of the fur, cutaneous ulcerations, epistaxes, conjunctival hemorrhages, ecchymoses of the regions of the paws, and blood-stained genital regions were common. The peripheral blood (Table 1) showed a leukocytosis as high as 77,000 cells per cubic millimeter (32,000 ± 1,400, mean ± standard error of the mean). An alteration of the relative number of lymphocytes in the blood was also noted; these cells represented 21.0 ± 0.6 percent of the circulating leukocytes in the experimental animals as compared with the 66.0 ± 1.4 percent in the seven controls. "Doughnut" or "ring" neutrophilic metamyelocytes were frequently seen in the bled animals (Fig. 1C). In addition, small numbers of normoblasts were generally observed in blood smears, and polychromasia, poikilocytosis, anisocytosis, and the presence of target cells were also noted. A variable number of thrombocytes were noted, as were shortened or prolonged clotting times. Slight to marked splenomegaly, three to twenty times the normal organ weight (0.24 ± 0.01 percent of body weight), and occasional hepatomegaly, up to five times the normal weight (2.70 ± 0.22 percent of body weight) were seen. The thymuses and other lymph nodes were not enlarged. Histologically, the marrow was hyperplastic and devoid of fat cells. The spleen showed a reduction in white pulp; imprints of both this organ and bone marrow demonstrated increased numbers of metamyelocytes, myelocytes, promyelocytes, and myeloblasts. Mitotic figures,

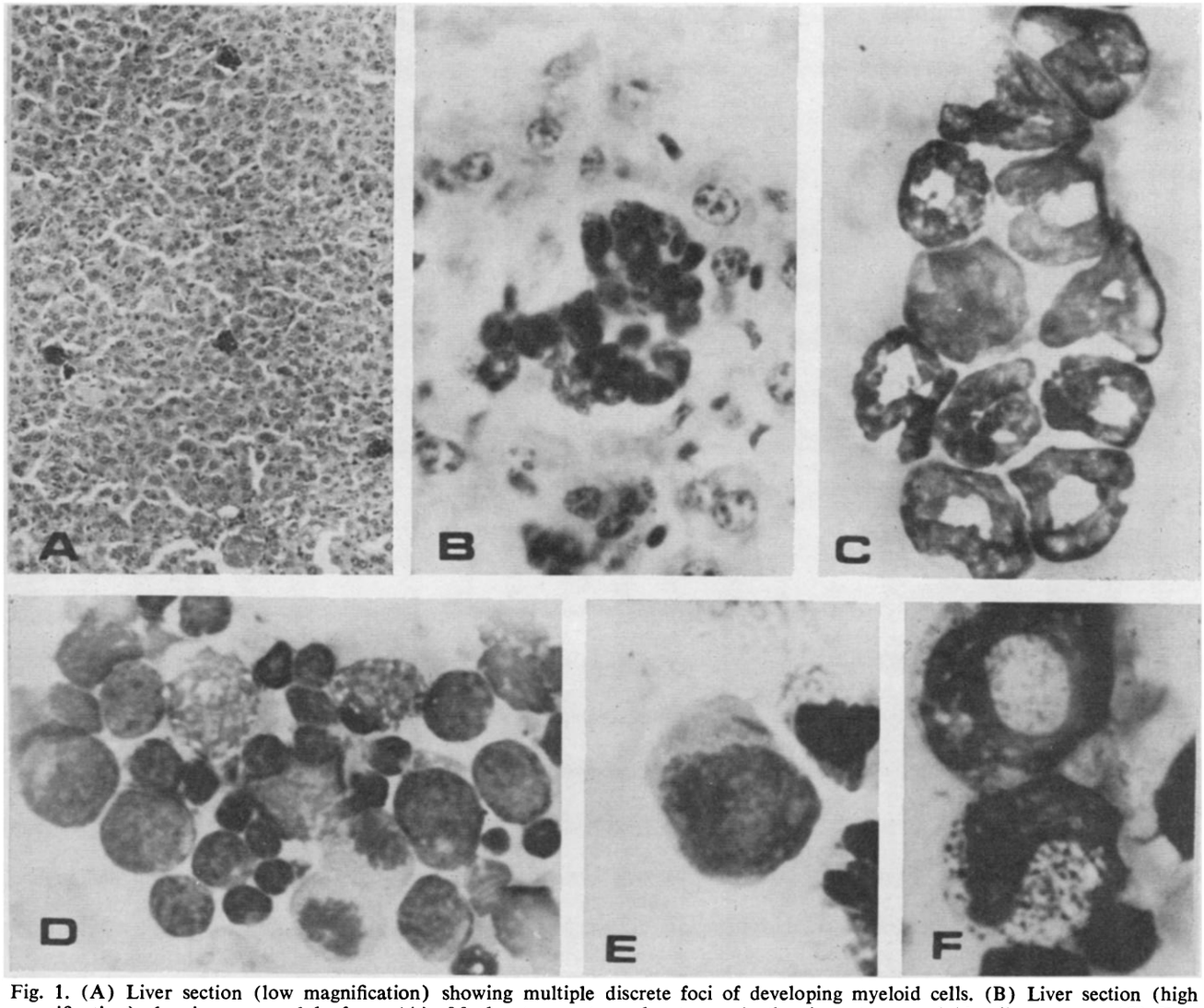

Fig. 1. (A) Liver section (low magnification) showing multiple discrete foci of developing myeloid cells. (B) Liver section (high magnification) showing one nodule from (A). Myelocytes, metamyelocytes, and other immature myeloid cells are seen. (C) Dry-film blood smear demonstating a considerable number of mature and immature neutrophils and a myeloblast. (D) Dry-film smear from subcutaneous cervical chloroma (*9*). Several myeloblasts with prominent nucleoli are identifiable. One mitotic figure is present. (E) Marrow smear showing a myeloblast with an Auer rod. (F) Dry-film blood smear showing a basophil adjacent to a neutrophil.

megakaryocytes, and normoblasts were observed in greater than normal numbers in splenic imprints. The marrow smears revealed high ratios of myeloid to erythroid cells, frequently in the order of 20 : 1. A few of the liver sections showed distinct foci of immature granulocytic elements in the space of Disse (Fig. 1, A and B), as well as Kupffer cells containing hemosiderin. Overall, these features conformed with those described in the RF mice (*2*).

Other features seen in only a few of the bled mice were (i) chloroma tumors (Fig. 1D) in the liver, the area of the mandible, the thoracic cavity, and subcutaneous regions; (ii) myeloblasts containing Auer rods (Fig. 1E); and (iii) neutrophils with the "pseudo-Pelger" type nuclei. The latter two cell types, which were also noted in the rat (*1*), would lend additional support for the contention (*5*) that murine and human granulocytic leukemias have many features in common. In addition to all the features of ML detailed above, all mice with the disease consistently had elevated percentages in basophils (Fig. 1F) and myeloblasts in both blood and marrow (Table 1). Similar increases in basophil and myeloblast counts were also observed in rats induced to develop ML by phlebotomy after x-irradiation.

The minimal criteria for diagnosing ML in this study included premature death due to complications resulting from a combination of anemia, infection (generally pneumonia), and inanition, plus elevated amounts (at least 2.5 percent) of basophils or myeloblasts in either the blood or marrow. Three ML cases were diagnosed on the basis of blood values (one of these animals had chloroma), ten diagnoses were based on marrow values (two animals with chloroma), and thirteen diagnoses were based on both blood and marrow values (four animals with chloroma). All but one of the experimental mice developed ML; the exception developed thymic lymphoma (TL). Among the ten controls, one case of ML and two cases of TL were observed. The apparently preferential development of ML after bleeding in a strain known to be predisposed to development of ML and TL indicates that anemia is an effective triggering stress for the induction of ML (*6*) and also implies that a different stress is needed for inducing TL (*7*, *8*).

In summary, all members of the RF mouse population studied were naturally prone to ML development, and the anemia resulting from the removal of 50 percent of the blood volume of these animals was sufficient to trigger the overt manifestation of the disease. Overall, the results would tend to support the concept of a two-step mechanism of de novo induction of ML.

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4. Earlier reports (*2*) showed that various groups of RF mice succumbing to ML after exposure to large single doses of x-rays (150 to 450 r) had average ages at death of 10.5 to 15.7 months. However, the survival time after irradiation of these leukemic populations (regardless of the dose of x-rays used or the age of the animals when they were irradiated) ranged from 9.3 to 11.7 months, or 9.9 ± 0.9 months (mean $\pm$ standard deviation). This time is similar to the average survival time of 9 months after phlebotomy found in our anemia-induced leukemic mice. It is thus possible to imagine that the primary leukemogenic effect of x-irradiation in the RF mice involved the triggering of the disease, possibly by an induced anemia. That most of the irradiated mice did not develop the disease could then be due to the absence of such an anemia in most of the exposed animals.
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6. It could be argued that anemia not severe enough to trigger ML could induce the development of TL. However, since the severity of radiation-induced anemia is dose-dependent, and increases in the incidence of TL in RF mice (*2*) occurred at an x-ray dose range higher than that needed to increase the incidence of ML, this argument would not appear valid.
7. Although the exact mechanism for TL induction is yet to be described, there is little doubt that the triggering of TL can be elicited by x-irradiation in the appropriate dose range, since incidence of both ML and TL was increased in RF mice that had been irradiated (*2*). That ML was found in nearly all of the bled RF mice, a strain in which many animals can develop TL, would indicate that proneness to both ML and TL probably coexisted in at least some individual animals. However, the finding of only TL among irradiated germ-free RF mice (*8*)—that is, when stresses for triggering both ML and TL were simultaneously applied—would suggest that proneness to both was not necessarily based on a single pathological entity. This observation on germ-free animals would also suggest that the "natural" proneness to ML seen in conventional mice was related to some exogenous factor.
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10. We thank Mary Byers for excellent technical assistance. This work was supported by grants FR-5330 and DE-001-67 from the U.S. Public Health Service.

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Terminal Saccharides on Sperm Plasma Membranes: Identification by Specific Agglutinins

Abstract. *Six specific agglutinins were used to identify the terminal sugar residues in the surface oligosaccharides of rabbit and hamster spermatozoa by specific agglutination. Species differences in epididymal sperm were found in the terminal residues, resembling* α-D-*mannose,* D-*galactose,* N-*acetyl*-D-*glucosamine, and* N-*acetyl*-D-*galactosamine. Species similarities were found in terminal residues, resembling* L-*fucose and* N-*acetylneuraminic acid. When ejaculated rabbit sperm were compared to epididymal sperm, the latter were more agglutinable with a specific agglutinin recognizing* N-*acetyl*-D-*glucosamine.*

Mammalian spermatozoa are complex haploid cells that are surrounded by a highly specialized continuous plasma membrane. Although no distinct morphological differences have been found among areas of the plasma membrane surrounding the sperm acrosomal, postacrosomal, midpiece, and tail regions (*1*), differences have been found in the distribution of charges on the membrane surface by the orientation of sperm in electric fields (*2*), the distribution of surface antigens (*3*), and the differential binding of colloidal iron to the plasma membrane surrounding the various sperm components (*4*). Also, the sperm plasma membrane undergoes ultrastructural changes in specific regions as the spermatozoon passes through the female genital tract and upon its penetration of the egg investments (*5*). These changes suggest that