LEUKEMIA EVOKED WITH 7, 8, 12-TRIMETHYLBENZ(A)ANTHRACENE IN RAT

I. Changes in Spleen and Thymus*

By COLIN BIRD,‡ M.B., and CHARLES HUGGINS, M.D.

(From The Ben May Laboratory for Cancer Research, The University of Chicago, Chicago, Illinois 60637)

(Received for publication 23 July 1971)

A stem-cell leukemia associated usually with erythroblastosis can be induced rapidly (< 100 days) and in high incidence in rats with a set of pulse-doses of homogenates of 7, 12-dimethylbenz(a)anthracene (1) and 7, 8, 12-trimethylbenz(a)anthracene (TMBA)[1] (2). It is characteristic of this type of stem-cell leukemia that exuberant growth of leukemic cells occurs in the sinusoids of the liver and there is terminal hepatic insufficiency. The thymus, however, is notably free of leukemic involvement. Specific chromosome abnormalities occur in bone marrow cells of leukemic rats (3–5).

In the present study a sequence of morphologic and biochemical events was followed during the evolution of leukemia in Long-Evans (L-E) rats. In addition to structural changes, the concentrations of phosphatases and dehydrogenases were determined. The earliest sign of leukemia was found in the spleen.

Alkaline phosphatase is a characteristic enzyme of the polymorphonuclear leukocyte series of most mammalian species (6). This enzyme occurs in myelocytes and mature polymorphonuclear leukocytes; it is not present in lymphocytes or cells of the erythroid series. Alterations in alkaline phosphatase activity have been demonstrated in some human leukemias (7–10).

Acid phosphatase occurs in high concentration in erythrocytes (11, 12). Changes in acid phosphatase activity in erythrocytes have been described in certain hematological disorders (13).

A high rate of aerobic and anaerobic glycolysis is a metabolic characteristic of

* This work was supported by grants from the American Cancer Society, the Jane Coffin Childs Memorial Fund for Medical Research, and U. S. Public Helath Service, National Institutes of Health (No. CA 11603).

‡ Goldsmiths Travelling Fellow, Medical Research Council of Great Britain.

[1] *Abbreviations used in paper*: LDH, lactate dehydrogenase; MDH, malate dehydrogenase; NAD and NADH, oxidized and reduced forms of nicotinamide adenine dinucleotide; Q $\frac{\text{LDH}}{\text{MDH}}$, quotient $\frac{\text{lactate dehydrogenase}}{\text{malate dehydrogenase}}$; TMBA, trimethylbenz(a)anthracene; ±, standard deviation of mean.

rapidly growing malignant tumors (14). In tumors of rodents, Meister (15) found that the concentration of lactate dehydrogenase (LDH) much exceeded that found in the normal tissue of origin. In the hyperplasias and neoplasia of the mammary gland of rodents, Rees and Huggins (16) found concentrations of both LDH and malate dehydrogenase (MDH) were rather similar and both exceeded by an order of magnitude that of other pyridine nucleotide-linked enzymes which they measured: in the mammary hyperplasia of advanced pregnancy and lactation MDH > LDH; in mammary cancer LDH > MDH.

Materials and Methods

Male L-E rats were used for all experiments. The animals have been bred at random *inter se* for 12 yr in our closed colony. They were housed in metal cages in air-conditioned rooms at 25° ± 2°C, fed a commercial ration (Rockland Mouse/Rat Diet, Teklad, Inc., Monmouth, Ill.), and given water *ad libitum*. Hepatic biopsy was performed under ether anesthesia with aseptic precautions.

A lipid emulsion[2] containing 7, 8, 12-TMBA, 0.5% (w/v), was prepared by the method of Schurr (17). The emulsion was injected in a caudal vein; the day of first injection is designated day 0.

Heparinized blood for hematological studies was obtained by cardiac puncture. Leukocytes and erythrocytes were counted electronically (Coulter Counter Model Z, Coulter Electronics, Inc., Hialeah, Fla.). Hemoglobin content was measured spectrophotometrically after the method of Drabkin and Austin (18).

Tissues for conventional morphological studies were preserved in Bouin's fixative and paraffin sections stained with hematoxylin and eosin. Tissues for alkaline phosphatase histochemistry were fixed in ice-cold (4°C) absolute acetone. Paraffin sections were stained by Gomori technique (19); one-half of the section was counterstained with eosin and the other half with hematoxylin and eosin.

For enzyme studies rats were decapitated and tissues excised rapidly and weighed. Tissues were homogenized in an ice-cold solution (2 ml) of 0.15 N NaCl containing 0.003 M $NaHCO_3$ and kept at 4°C thereafter. Homogenization was performed with Willems Polytron Homogenizer (Brinkmann Instruments, Inc., Westbury, N. Y.). The homogenate was centrifuged at 13,000 *g* for 20 min in a refrigerated centrifuge and the supernatant used for enzyme assay. Spectrophotometric determinations were made with a Beckman Model DU spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) using optical cells with 1 cm light path.

Alkaline phosphatase (orthophosphoric monoester phosphohydrolase, E.C.3.1.3.1.) activity was measured in barbital buffer, pH 9.3, using *p*-nitrophenyl phosphate as substrate as previously described (20). Acid phosphatase (orthophosphoric monoester phosphohydrolase, E.C.3.1.3.2) activity was similarly measured using 0.1 M sodium acetate buffer, pH 5.1. One unit of enzyme is defined as the enzyme activity which liberated 1 μmole of *p*-nitrophenol in 30 min at 38°C.

Lactate dehydrogenase (L-lactate: nicotinamide adenine dinucleotide [NAD] oxidoreductase, E.C.1.1.1.27) and malate dehydrogenase (L-malate: NAD oxidoreductase, E.C.1.1.1.37) activities were measured concurrently (16). The initial velocity of the reaction was measured under conditions which yielded zero order kinetics. One unit of LDH or MDH is defined as the enzyme activity which resulted in oxidation of 1 μmole of NADH in 1 min at 25°C. All of the enzyme units are expressed in terms of 1 g wet weight of tissue.

[2] We thank: John Pataki, Ben May Laboratory for Cancer Research, The University of Chicago, Chicago, Ill., for synthesis of 7, 8, 12-TMBA; and Paul E. Schurr, The Upjohn Co., Kalamazoo, Mich., for lipid emulsions.

Protein content was measured by the method of Lowry et al. (21). The results are expressed as g/100 g wet weight of tissue. The results were subjected to statistical evaluation and significant differences between the means ($P < 0.05$) are given.

RESULTS

Characteristics of Leukemia in L-E Rat.—Leukemia was evoked in a group of male L-E rats with four or five intravenous pulse-doses of 7, 8, 12-TMBA, 30–35 mg/kg, at 10-day intervals, beginning at age 28 days. Hepatic biopsy was performed to confirm leukemia at least 10 days before enzyme assay.

TABLE I

*Hematological and Anatomical Findings in Male Rats With Leukemia**

	Leukemia	Control	P
Body weight g	154 ± 38	294 ± 26	<0.01
Hemoglobin g/100 cc	11.2 ± 3.1	14.7 ± 1.5	<0.01
Leukocytes × 10^3/mm^3	19.40 ± 11.27	7.94 ± 2.68	<0.01
Erythrocytes × 10^6/mm^3	5.02 ± 1.43	7.18 ± 1.27	<0.01
Liver g/100 g	6.84 ± 3.28	3.22 ± 0.14	<0.01
Spleen g/100 g	0.370 ± 0.211	0.184 ± 0.023	<0.05
Thymus g/100 g	0.031 ± 0.012	0.065 ± 0.019	<0.01
Lymph nodes (3) g/100 g	0.014 ± 0.005	0.005 ± 0.001	<0.01
Adrenal glands (2) g/100 g	0.033 ± 0.016	0.013 ± 0.003	<0.05

* Leukemia was evoked in 12 male rats with four or five intravenous pulse-doses of 7, 8, 12-TMBA, 30–35 mg/kg, at 10-day intervals beginning at age 28 days. Autopsy was performed at age 97 ± 20 days. 10 untreated control male rats were autopsied at age 110 ± 11 days. Mean values with standard deviation are given.

Anatomical and hematological findings: In early leukemia the rats were active and appeared healthy although somewhat reduced in size compared with untreated controls. When leukemia was advanced, there was marked reduction in body weight, the animals became hunched, and their coats lacked normal luster. Progressive anemia occurred with advancing leukemia and there was reduction in numbers of circulating erythrocytes (Table I). In most animals advanced leukemia was associated with leukocytosis, usually of moderate grade (Table I). But in early leukemia the number of circulating leukocytes in many instances was in normal range or leukopenia was present. With leukocytosis

there appeared in the peripheral blood large atypical mononuclear cells, 12–20 μ in diameter, and many erythroblasts some of which were atypical. Plasma alkaline phosphatase activity in leukemic rats was reduced from the normal control value of 4.9 $\pm$ 0.7 units/ml plasma to 2.0 $\pm$ 0.6 units ($P < 0.01$). Weil and Russell (22) have shown that plasma alkaline phosphatase activity in rats can be greatly reduced by fasting, only the ingestion of unsaturated fatty acids of certain types will restore plasma phosphatase activity to normal.

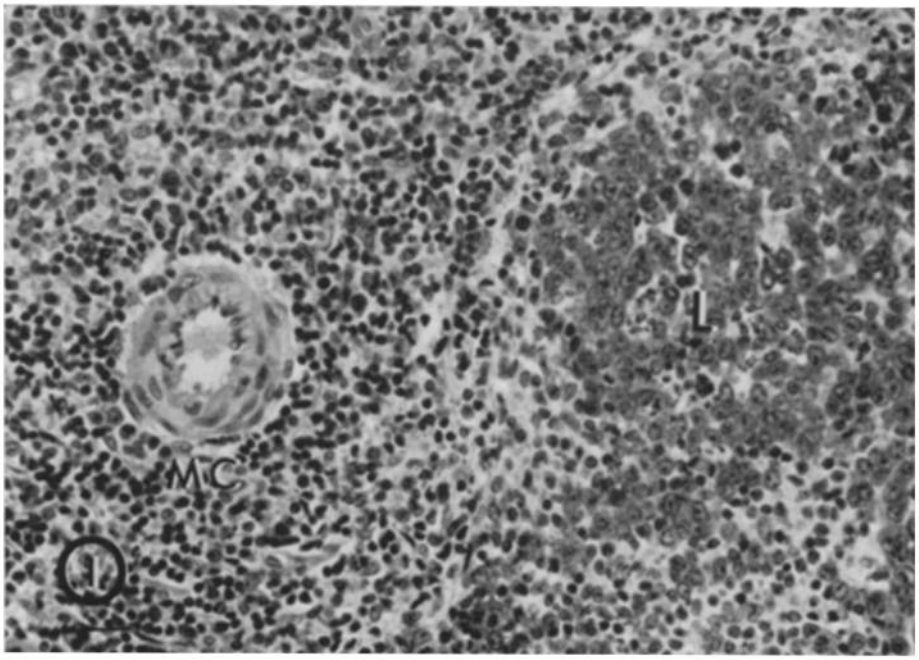

FIG. 1. Leukemic stem cell infiltration of splenic red pulp (*L*). Malpighian corpuscle (*MC*) is not involved. Hematoxylin and eosin. × 320.

Leukemic rats showed moderate enlargement of the spleen (Table I) which developed a smooth capsular surface and was frequently studded with one or more irregular pale nodules 1–8 mm in diameter. In the terminal stages the liver was greatly enlarged, granular, and friable; the thymus was atrophied but lymph nodes and adrenal glands were somewhat increased in size.

Histologically, the hepatic sinusoids and red pulp of spleen were massively infiltrated with leukemic stem cells but malpighian corpuscles were not involved (Fig. 1). Leukemic stem cells consisted of large mononuclear cells with vesicular nuclei, one or several prominent dark nucleoli, and variable amounts

of basophilic cytoplasm; they were admixed usually with erythroblasts. Similar small leukemic deposits were found in lymph node sinuses and adrenal cortex. The thymus, however, was notably free of leukemic infiltration.

Biochemical findings: Enzyme levels were determined in the spleens of 12 leukemic male rats age 82–145 days and 10 untreated male controls of comparable age. The results obtained have been repeatedly confirmed in other groups of animals.

As previously described (23) it has been found useful to relate the activity of LDH and MDH measured concurrently as a quotient: $Q\frac{LDH}{MDH}$. In the spleen of the normal adult male rat, the activity of MDH exceeded that of LDH, thus $Q\frac{LDH}{MDH} = < 1$. In leukemia LDH activity in the spleen was increased while MDH activity was reduced, thus $Q\frac{LDH}{MDH} = > 1$ (Table II).

TABLE II

*Phosphatase and Dehydrogenase Enzymes in Spleen of Male Rats With Leukemia**

Group	Phosphatase		Dehydrogenase			Protein
	pH 5.1	pH 9.3	LDH	MDH	$Q\frac{LDH}{MDH}$	
Leukemia	196.3 ± 31.4‡	15.3 ± 11.5§	107.9 ± 24.4‡	91.1 ± 13.2‡	**1.20 ± 0.28**‡	10.1 ± 0.9
Control	233.0 ± 21.3	6.0 ± 2.6	83.2 ± 4.3	124.2 ± 9.1	**0.67 ± 0.06**	10.5 ± 1.8

* Leukemia was evoked in 12 male rats with four or five intravenous pulse-doses of 7, 8, 12-TMBA, 30–35 mg/kg, at 10-day intervals beginning at age 28 days. Autopsy was performed at age 97 ± 20 days. 10 untreated control male rats were autopsied at age 110 ± 11 days. Enzyme activity is expressed in units per gram wet weight of tissue as defined. Protein levels are expressed as grams per 100 gram wet weight of tissue. Mean values with standard deviation are given.

‡ $P < 0.01$.

§ $P < 0.05$.

Acid phosphatase activity in the spleen was significantly reduced in leukemia whereas alkaline phosphatase was increased. Histochemically there was a modest increase in nonleukemic alkaline phosphatase-positive myelopoietic foci in the red pulp but leukemic stem cells were completely devoid of alkaline phosphatase activity. By contrast, in myelogenous leukemia, the spleen contains numerous alkaline phosphatase-positive leukemic cells (Fig. 2). Normal adult rats rarely contain substantial numbers of myelopoietic foci in the spleen.

There were no significant differences in levels of protein in the spleens of leukemic rats compared with controls (Table II), so that enzyme activity expressed as specific activity (units per milligram of protein) showed no significant differences from enzyme activity expressed in terms of wet weight of tissue.

Initial Effects of Pulse-Doses of 7, 8, 12-TMBA.—Groups of male rats were

given a series of intravenous pulse-doses of 7, 8, 12-TMBA, 30–35 mg/kg, at 10-day intervals, beginning at age 28 days. A group was killed 3 days after each pulse-dose of hydrocarbon. For comparison, groups of untreated male rats were sacrificed at intervals of 10 days.

Anatomical and hematological effects: After the first dose of hydrocarbon, there was no decline in body weight, but retardation of growth occurred after the second and subsequent doses. After each injection of hydrocarbon there was a

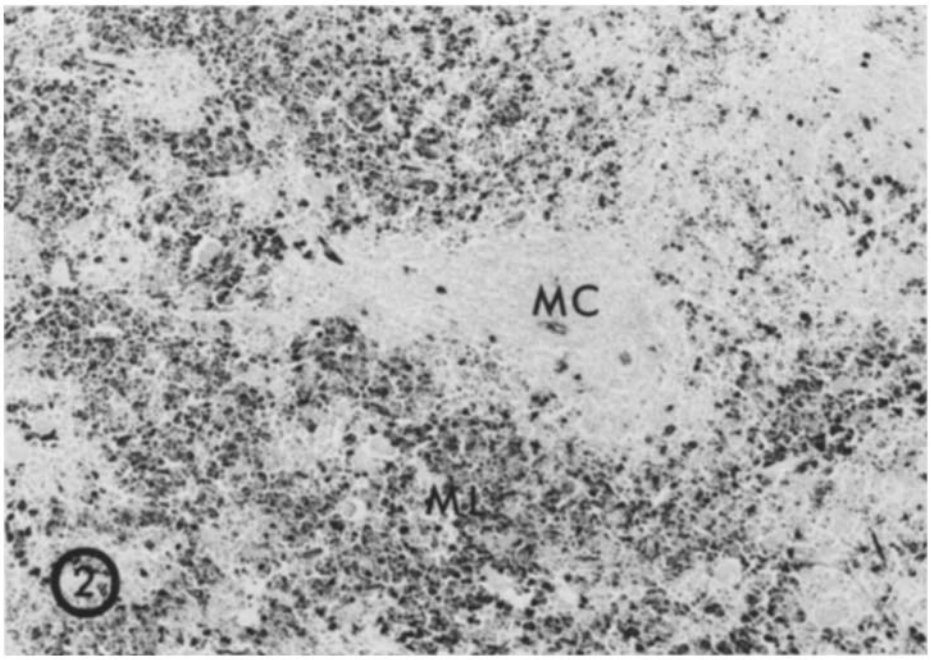

FIG. 2. Myelogenous leukemia in rat spleen Numerous alkaline phosphatase-positive leukemic cells are present in red pulp (*ML*). Malpighian corpuscle (*MC*) is not involved. Gomori's alkaline phosphatase reaction counterstained with eosin. × 150.

characteristic decline in circulating leukocytes affecting both polymorphonuclear leukocytes and lymphocytes; recovery to preinjection levels before the next pulse-dose of hydrocarbon has been previously described (2). In the normal weanling rat, moderate anemia was a normal physiological occurrence and hemoglobin content progressively increased towards adult levels concurrent with normal sexual maturation (Table III). A series of pulse-doses of hydrocarbon prevented normal hematological development and moderate anemia persisted (Table III). Reiterated pulse-doses of 7, 8, 12-TMBA also prevented normal growth of the thymus in which there was depletion of lymphocytes,

TABLE III

*Hematological and Anatomical Findings in Male Rats Receiving Series of Pulse-Doses of 7, 8, 12-TMBA**

Group	Day	Mean body weight	Hemoglobin g/100 cc	Leukocytes × 10^3/mm^3	Liver g/100 g	Spleen g/100 g	Thymus g/100 g
		g					
Control	0	53	9.5 ± 1.0	5.0 ± 1.0	4.13 ± 0.26	0.41 ± 0.07	0.30 ± 0.05
7, 8, 12-TMBA	3	66	10.3 ± 0.8	4.0 ± 0.7	4.10 ± 0.21	0.31 ± 0.05‡	0.19 ± 0.04‡
Control	10	86	11.9 ± 1.0	8.0 ± 1.4	4.08 ± 0.12	0.44 ± 0.08	0.28 ± 0.07
7, 8, 12-TMBA	13	99	10.4 ± 1.2§	3.7 ± 1.0‡	4.32 ± 0.33	0.24 ± 0.03‡	0.19 ± 0.04‡
Control	20	143	12.1 ± 0.7	7.1 ± 1.7	4.13 ± 0.24	0.34 ± 0.09	0.24 ± 0.05
7, 8, 12-TMBA	23	120§	11.9 ± 1.5	2.4 ± 1.0‡	4.29 ± 0.31	0.21 ± 0.02‡	0.16 ± 0.07§
Control	30	163	13.4 ± 0.3	7.1 ± 2.0	3.55 ± 0.24	0.31 ± 0.04	0.20 ± 0.04
7, 8, 12-TMBA	34	140§	10.4 ± 1.2‡	2.1 ± 0.8‡	3.94 ± 0.20‡	0.18 ± 0.03‡	0.13 ± 0.02‡

* A group of 32 male rats received pulse-doses of 7, 8, 12-TMBA, 30–35 mg/kg, at intervals of 10 days beginning at age 28 days (day 0). Eight rats were sacrificed 3 days after each pulse-dose of hydrocarbon. Controls consisted of groups of eight untreated male littermates sacrificed on each of the days hydrocarbon was injected. Mean values with standard deviation are given.

‡ $P < 0.01$.

§ $P < 0.05$.

FIG. 3. Hyperplastic stem-cell nodules (*N*) in subcapsular zone of spleen, containing erythroblastic elements. Hematoxylin and eosin. × 50.

but growth of the liver was virtually unaffected except for a slight increase in its relative weight.

Initially, atrophy of the spleen followed successive doses of 7, 8, 12-TMBA. Lymphoid elements were especially affected with reduction in size of malpighian corpuscles and mantle zones. The earliest morphological evidence of incipient leukemia in spleen comprised focal aggregation of large mononuclear stem cells in the red pulp. These cells either formed a collar around malpighian corpuscles

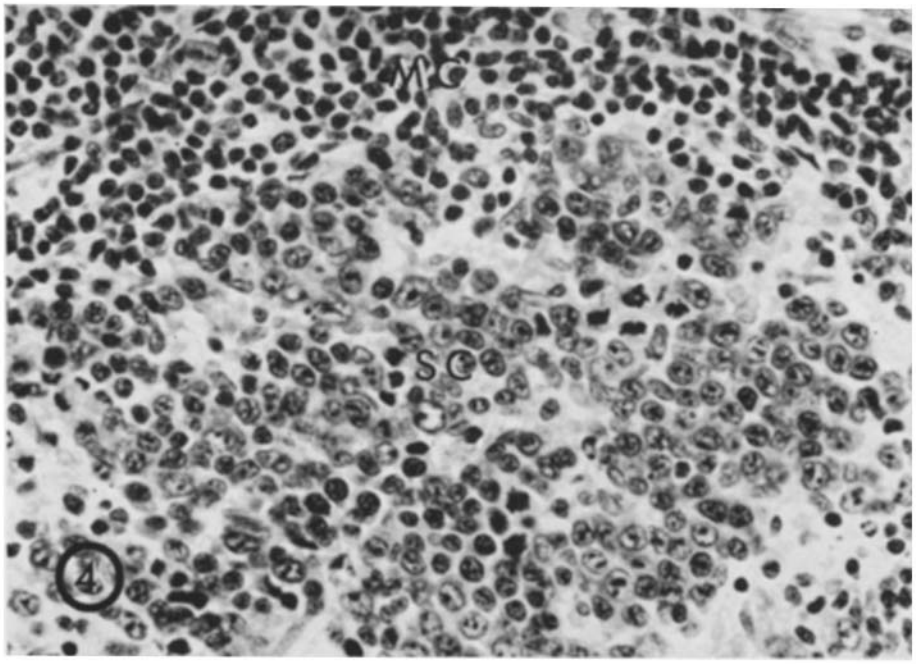

FIG. 4. Proliferating stem cells (*SC*) in splenic red pulp. Note numerous mitoses. Lymphoid elements of malpighian corpuscle (*MC*) adjacent. Hematoxylin and eosin. × 510.

or when situated in the subcapsular areas of spleen caused formation of wedge-shaped nodules (Fig. 3). Variable numbers of such nodules, 1–4 mm in diameter, were found in half of the spleens after the third and fourth injections of hydrocarbon. The proliferating stem cells were identical morphologically with leukemic stem cells and were admixed with variable numbers of erythroblasts (Fig. 4). Alkaline phosphatase staining showed that stem cells were devoid of enzyme activity and only an occasional small positive myelocytic focus was found in the adjacent areas of red pulp. No stem-cell foci were found in liver at this stage and splenic changes preceded hepatic changes.

Biochemical effects: In the spleens of normal rats, acid phosphatase activity

greatly exceeded alkaline phosphatase activity (Tables II and IV). Further, in the normal weanling rat, alkaline phosphatase activity in the spleen exceeded by severalfold that of the adult animal; with increasing maturity a progressive reduction of enzyme occurred (Table IV). The spleen of the immature rat exhibits considerable histological evidence of hemopoietic activity in the red pulp (erythro- and myelopoiesis); during puberty this activity diminishes concurrent with the disappearance of "physiological anemia." Alkaline phosphatase activity in the spleen is restricted to the endothelium of small blood vessels together with myeloid elements (myelocytes and polymorphonuclear leukocytes) in the red pulp (Fig. 5). Thus, disappearance of myeloid elements from

TABLE IV

*Phosphatase and Dehydrogenase Enzymes in Spleen of Male Rats Receiving Series of Pulse-Doses of 7, 8, 12-TMBA**

Group	Day	Phosphatase		Dehydrogenase		
		pH 5.1	pH 9.3	LDH	MDH	Q $\frac{LDH}{MDH}$
Control	0	173.9 ± 15.2	19.7 ± 10.2	88.0 ± 7.2	109.1 ± 9.5	0.81 ± 0.04
7, 8, 12-TMBA	3	190.7 ± 14.2§	21.5 ± 9.0	98.1 ± 11.2§	127.4 ± 12.9‡	0.77 ± 0.07
Control	10	154.8 ± 9.2	17.0 ± 8.1	85.8 ± 10.9	110.7 ± 13.9	0.78 ± 0.04
7, 8, 12-TMBA	13	191.6 ± 9.3‡	11.2 ± 3.7	94.7 ± 6.7	132.6 ± 11.6‡	0.72 ± 0.07
Control	20	163.0 ± 13.3	13.4 ± 4.3	94.0 ± 15.4	127.6 ± 18.3	0.74 ± 0.02
7, 8, 12-TMBA	23	208.8 ± 27.4‡	14.9 ± 3.8	100.3 ± 7.1	139.5 ± 8.3	0.72 ± 0.05
Control	30	175.4 ± 13.6	9.8 ± 2.4	100.2 ± 9.9	139.5 ± 18.0	0.72 ± 0.04
7, 8, 12-TMBA	34	221.4 ± 14.7‡	11.7 ± 1.4	101.6 ± 5.2	148.7 ± 18.2	0.69 ± 0.06

* Groups of eight male rats were sacrificed 3 days after receiving pulse-doses of 7, 8, 12-TMBA, 30–35 mg/kg, at 10-day intervals beginning at age 28 days (day 0). A group of eight untreated control male littermates were sacrificed at each of the days hydrocarbon was given. Enzyme activity is expressed as units per gram wet weight of tissue as defined. Mean values with standard deviation are given.

‡ $P < 0.01$.

§ $P < 0.05$.

the red pulp of the spleen parallels the quantitative reduction in content of splenic alkaline phosphatase activity. Thus it is possible to use measurement of splenic alkaline phosphatase activity as an index of myelopoietic activity of the spleen.

Repeated doses of 7, 8, 12-TMBA did not affect the initial levels of alkaline phosphatase activity or myelopoiesis in the spleen. By contrast, significant increases in acid phosphatase activity followed each dose of hydrocarbon (Table IV).

LDH and MDH activity in the spleens of hydrocarbon-treated rats always exceeded that of untreated controls, but no significant changes in relative proportion of either enzyme occurred (Table IV).

In normal rat thymus acid phosphatase activity exceeded that of alkaline phosphatase by severalfold; and, the activity of MDH normally exceeded that

of LDH ($Q\frac{LDH}{MDH} = < 1$). Repeated pulse-doses of 7, 8, 12-TMBA did not significantly change the level of phosphatase or dehydrogenase enzymes in rat thymus.

DISCUSSION

In our experiments the earliest morphological evidence of leukemia elicited with 7, 8, 12-TMBA was found in the spleen. Hyperplastic foci of cells indis-

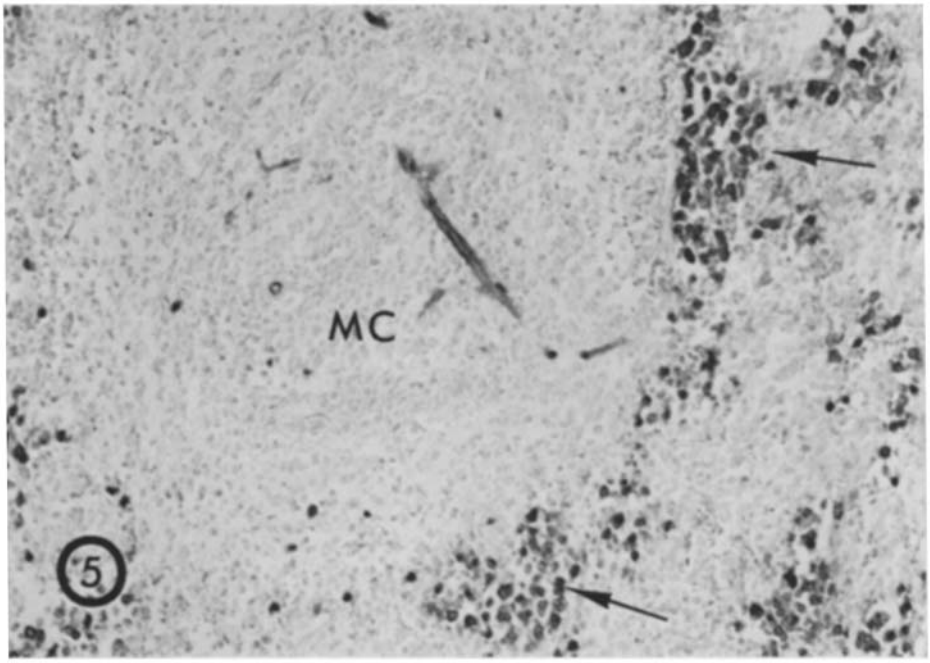

FIG. 5. Spleen of untreated control rat age 28 days. Alkaline phosphatase activity is present in endothelium of small blood vessels in malpighian corpuscle (*MC*) and in myelopoietic foci (*arrows*) in red pulp. Gomori's alkaline phosphatase reaction counterstained with eosin. × 200.

tinguishable from leukemic stem cells, together with erythroblastic elements, were found in the red pulp especially after the fourth pulse-dose of hydrocarbon. These proliferating stem cells formed a collar around malpighian corpuscles or when situated in subcapsular areas of the spleen, caused nodule formation in the gross. These hyperplastic nodules bear some resemblance to the undifferentiated erythropoietic colonies in spleens of lethally irradiated mice injected with isologous bone marrow and subjected to various procedures to augment or suppress erythropoiesis (24). The normal immature rat spleen exhibits con-

siderable hemopoietic activity at a time when physiological anemia exists; as the physiological anemia regresses hemopoietic activity in the spleen diminishes. In immature rats which received a series of pulse-doses of 7, 8, 12-TMBA, normal hemopoietic development was disrupted and physiological anemia persisted. It is postulated that this anemia may act as a stimulus for hyperplasia of nonleukemic erythroblastic stem cells in the spleen which, under hydrocarbon influence, may undergo aberrant differentiation with ultimate development of stem-cell leukemia. The relationship between the early splenic changes and subsequent development of leukemia in other tissues requires further elucidation.

A consistent attribute of rapidly growing cancers, discovered by Warburg (14), is a high rate of glycolysis both in the presence and absence of oxygen. In glycolysis, conversion of pyruvate to lactate depends upon the catalytic actions of LDH; considerable evidence has now been adduced which suggests that in many cancerous tissues the isoenzyme component of LDH which is best adapted functionally for anaerobic metabolism is increased (25).

Measurement of LDH and MDH activity concurrently was useful. In normal rat spleen MDH predominated and $Q\frac{LDH}{MDH} \lessapprox 1$, whereas in leukemic spleen the reverse was true, $Q\frac{LDH}{MDH} \gtrapprox 1$. The increased levels of LDH activity found in leukemic spleen are consistent with the anticipated higher glycolytic rate of a rapidly growing cancer.

Pulse-doses of hydrocarbon markedly affected the normal growth of the spleen and thymus. It is remarkable, however, that only minimal changes occurred in the activity of the critical enzymes measured after each dose of hydrocarbon.

SUMMARY

An early sign of erythroblastic leukemia in rat was nodule formation in the spleen. Hyperplastic foci of stem cells, indistinguishable histologically from leukemic stem cells, were found in the red pulp whereas the malpighian corpuscles were uninvolved.

Anemia is a normal phenomenon in immature rats and the spleen of the prepubertal rat possesses considerable hemopoietic potential. Pulse-doses of 7, 8, 12-trimethylbenz(a)anthracene prevented the physiologic hematological development of maturing rats and was associated with subsequent development of leukemic stem cells in the red pulp of the spleen.

Significant enzyme changes were observed in leukemic spleens. Compared with the spleens of normal littermates, the concentration of lactate dehydrogenase rose while that of malate dehydrogenase fell; the content of alkaline phosphatase rose whereas acid phosphatase fell. Increased alkaline phosphatase activity in leukemic spleen was attributed to nonleukemic foci of myelopoiesis.

BIBLIOGRAPHY

1. Huggins, C., and T. Sugiyama. 1966. Induction of leukemia in rat by pulse-doses of 7, 12-dimethylbenz(a)anthracene. *Proc. Nat. Acad. Sci. U.S.A.* **55:**74.
2. Huggins, C., L. Grand, and H. Oka. 1970. Hundred day leukemia: preferential induction in rat by pulse-doses of 7, 8, 12-trimethylbenz(a)anthracene. *J. Exp. Med.* **131:**321.
3. Sugiyama, T., Y. Kurita, and Y. Nishizuka. 1967. Chromosomal abnormality in rat leukemia induced by 7, 12-dimethylbenz(a)anthracene. *Science (Washington).* **158:**1058.
4. Sugiyama, T., and F. P. Brillantes. 1970. Cytogenetic studies of leukemia induced by 6, 8, 12- and 7, 8, 12-trimethylbenz(a)anthracene. *J. Exp. Med.* **131:**331.
5. Rees, E. D., S. K. Majumdar, and A. Shuck. 1970. Changes in chromosomes of bone marrow after intravenous injections of 7, 12-dimethylbenz(a)anthracene and related compounds. *Proc. Nat. Acad. Sci. U. S. A.* **66:**1228.
6. Maloney, W. C. 1958. Leukocyte alkaline phosphatase activity in the rat. *Ann. N. Y. Acad. Sci.* **75:**31.
7. Wachenstein, M. 1946. Alkaline phosphatase activity in normal and abnormal human blood and bone marrow cells. *J. Lab. Clin. Med.* **31:**1.
8. Valentine, W. N., and W. S. Beck. 1951. Biochemical studies on leukocytes. I. Phosphatase activity in health, leukocytosis and myelocytic leukemia. *J. Lab. Clin. Med.* **38:**39.
9. Robinson, J. C., J. E. Pierce, and D. P. Goldstein. 1965. Leukocyte alkaline phosphatase: electrophoretic variants associated with chronic myelogenous leukemia. *Science (Washington).* **150:**58.
10. Block, J. B., P. P. Carbone, J. J. Oppenheim, and E. Frei. 1963. The effect of treatment in patients with chronic myelogenous leukemia. *Ann. Intern. Med.* **59:**629.
11. Martland, M., F. S. Hansman, and R. Robison. 1924. The phosphoric esterase of blood. *Biochem. J.* **18:**1152.
12. Roche, J. 1931. Blood phosphatases. *Biochem. J.* **25:**1724.
13. Hopkinson, D. A., N. Spencer, and H. Harris. 1964. Genetical studies on human red cell acid phosphatase. *Amer. J. Hum. Genet.* **16:**141.
14. Warburg, O. 1930. Metabolism of Tumours. Constable & Co., Ltd., London.
15. Meister, A. 1950. Lactic dehydrogenase activity of certain tumors and normal tissues. *J. Nat. Cancer Inst.* **10:**1263.
16. Rees, E. D., and C. Huggins. 1960. Steroid influences on respiration, glycolysis, and levels of pyridine nucleotide-linked dehydrogenases of experimental mammary cancers. *Cancer Res.* **20:**963.
17. Schurr, P. E. 1969. Composition and preparation of experimental intravenous fat emulsions. *Cancer Res.* **29:**258.
18. Drabkin, D. L., and J. H. Austin. 1935–36. Spectrophotometric studies. II. Preparations from washed blood cells; nitric oxide hemoglobin and sulphhemoglobin. *J. Biol. Chem.* **112:**51.
19. Gomori, G. 1941. The distribution of phosphatase in normal organs and tissues. *J. Cell Comp. Physiol.* **17:**71.
20. Huggins, C., and S. Morii. 1961. Selective adrenal necrosis and apoplexy induced by 7, 12-dimethylbenz(a)anthracene. *J. Exp. Med.* **114:**741.

21. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:**265.
22. Weil, L., and M. A. Russell. 1940. Studies on plasma phosphatase activity in relation to fat metabolism in rats. *J. Biol. Chem.* **136:**9.
23. Reddi, A. H., and C. Huggins. 1971. Lactic/malic dehydrogenase quotients during transformation of fibroblasts into cartilage and bone. *Proc. Soc. Exp. Biol. Med.* **137:**127.
24. Curry, J. L., J. J. Trentin, and N. Wolf. 1967. Hemopoietic spleen colony studies. II. Erythropoiesis. *J. Exp. Med.* **125:**703.
25. Goldman, R. D., N. O. Kaplan, and T. C. Hall. 1964. Lactic dehydrogenase in human neoplastic tissues. *Cancer Res.* **24:**389.