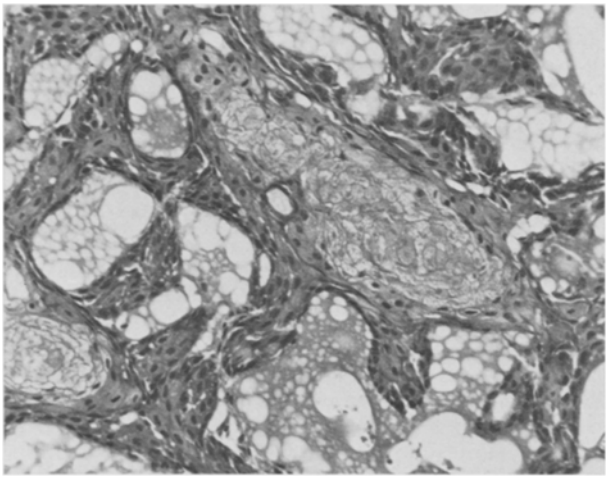
When female rats were injected with 2 mg of 7,12-DMBA 3 times at intervals of 3 days, the majority of them developed mammary tumors; the incidence was higher than 90%. At a dosage of 4 mg/rat at weekly intervals (besides mammary tumors) about 10% of the rats also developed leukemia. Tumors arising in the cheek area were observed in both groups regardless of the dosage, the length of the intervals of injections and developed 2–7 months after the last injection. These neoplasms were histologically benign, and were probably either of adnexal or salivary gland origin, and architecturally resembled sebaceous adenomas to some extent. Histologic morphology was characterized by interconnecting cords of uniform epithelial cells associated with a multifocal central clustering of sebaceous cells (Figure).



Histo-architecture of the tumors from the cheek area most closely resembled sebaceous adenomas. Interconnecting cords of uniform epithelial cells were interspersed by aggregates of foamy sebaceous cells (center). Epithelial cords were further subdivided by small irregular cystic spaces. Hematoxylin and eosin. ×240.

It appeared that the incidence of these neoplasms was higher with male than female rats (Table). Mammary tumors and leukemia rarely developed in the same rat, but simultaneous occurrence of these benign tumors with mammary tumors or with leukemia was not uncommon. The affinity of 7,12-DMBA for certain specific tissues in the induction of tumors has been speculated insofar as its interaction with the nuclear DNA of that particular tissue is concerned[2,3,5]. The method employed in this laboratory in the preparation of the 7,12-DMBA emulsion may facilitate the affinity of this oncogenic material for another region, namely, the salivary gland or adnexa in the cheek area of the rat. The possibility that these benign tumors are unrelated to DMBA cannot be excluded. Spontaneous growth of these tumors, however, is extremely rare. The relative high incidence of these tumors occurring in this study suggests a causal relationship to the administration of DMBA.

Zusammenfassung. Die Injektion von 7,12-Dimethylbenz(a)Anthrazol in die Schwanzvene von Ratten führte zu Bildung von Brusttumoren und in einigen Fällen zu Leukämie, dabei wurde auch, mit grösserer Häufigkeit bei männlichen Ratten, ein neuer Tumortypus im Gebiet der Ohrspeicheldrüse festgestellt.

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The Blood as an Erythropoietic Organ in Anaemic *Xenopus*

The process of erythropoiesis (blood cell formation) is of general interest since the blood is a rapidly turning-over tissue with a tightly controlled cell population size and a versatile supply system of stem cells. In most vertebrates so far studied the blood cells are replenished by release of more or less well differentiated red blood cells from concentrations of erythropoietic tissue resident in certain body organs. The organ sites of the blood forming tissues vary with the particular animal group and the age of the individual, liver, spleen, kidney and bone marrow being the most frequent locations of erythropoietic tissue[1].

A useful way to study blood cell formation is to accelerate the process by the induction of anaemia although, of course, the animals' response to anaemia may not follow precisely the same pattern as normal erythropoiesis. We are here reporting our observations on the response of the amphibian *Xenopus laevis* to phenylhydrazine-induced anaemia, our main conclusion being that under these conditions most of the process of erythropoiesis is a circulatory phenomenon.

Adult *Xenopus* of both sexes were rendered anaemic by 2 injections of phenylhydrazine, 0.5 ml of a 0.5% solution on 2 successive days. This drug is chiefly active in anaemia induction by dramatically increasing the fragility of the existing erythrocytes, which begin to break up and are removed from circulation within a few days of the first injection. Table I gives the figures for numbers of erythrocytes and other circulating cells before and during recovery from anaemia. It will be seen that within 15 days of the first injection of phenylhydrazine, the blood is essentially devoid of mature erythrocytes. This closely parallels the findings of Grasso[2], in work on induced anaemia in the newt. On different days after the induction of anaemia animals have been bled by heart puncture and the blood cells incubated with nucleic acid precursors. This procedure was performed as previously described[3] except that bovine serum albumin (12 mg/ml)

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Table I. The numbers and types of cells present at different times after the induction of anaemia

Days after last phenylhydrazine injection	Number of cells present $\times 10^7$/ml blood Total expt. 1	expt. 2	Mean	Mature erythrocytes (%) expt. 1	expt. 2	Mean	Other circulating cells (%) expt. 1	expt. 2	Mean
5[a]	13.8	22.0	17.9	66	92	79	34	8	21
15	7.6	11.4	9.5	2	2	2	98	98	98
20	17.2	20.8	19.0	0	0	0	100	100	100
30	32.9	26.5	29.7	0	0	0	100	100	100
Control	70.1	70.4	70.2	99	99	99	1	1	1

Values were obtained by bleeding 2 animals for each time shown, i.e. 10 animals were used in all. [a] Many mature erythrocytes present on day 5 after the last phenylhydrazine injection are noticeably damaged and are later removed from circulation. Thus the drop in erythrocyte count from day 5 to day 15.

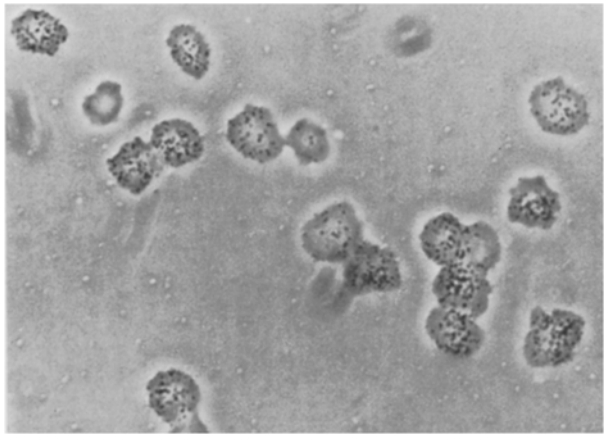

Fig. 1. Autoradiograph of blood cells from animal bled 15 days after last phenylhydrazine injection. Cells incubated with 200 μCi/ml of ^{3}H-thymidine for 6 h, exposed for 20 days at 4 °C. Phase-contrast.

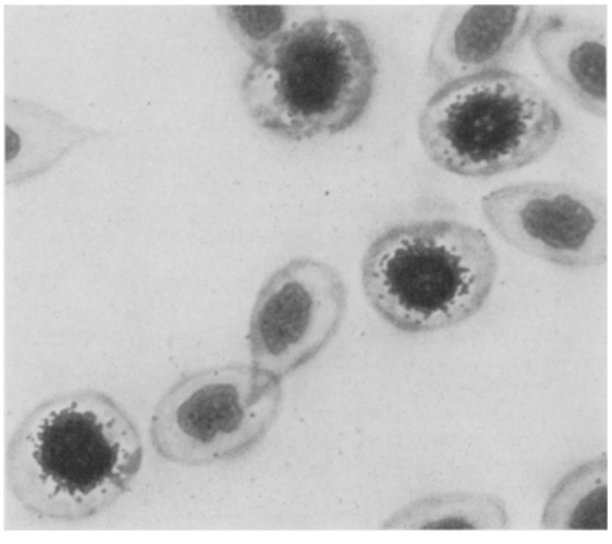

Fig. 2. Autoradiograph of blood cells from animal bled 30 days after last phenylhydrazine injection. Experimental conditions as indicated in legend to Figure 1 but also counterstained with Giemsa. The number of labelled cells in this photograph is rather higher than usual for day 30; however the figure demonstrates the characteristic appearance of the maturing erythrocytes typical of this stage of recovery from anaemia.

was sometimes added to solutions used for the washing of the blood cells to prevent lysis of white cells in the blood. In addition, blood smears were made on glass slides and subjected to staining with Wright's stain, Giemsa, and *o*-dianisidine[4]. The latter gives a brown colour specifically in the presence of haemoglobin, and thus is invaluable in determining when haemoglobin synthesis commences, and in which cells. Autoradiographs were also made of aliquots of cells from the in vitro cultures, as previously described[3], and the incorporation of tritiated thymidine used to determine the proportions of S phase cells in circulation. Table II tabulates our data on the proportions of cells, incorporating thymidine, and containing haemoglobin at different times after the induction of anaemia. Mitotic figures were occasionally seen, especially at 15 days after the last phenylhydrazine injection. Cells stained with *o*-dianisidine were also sometimes in mitosis, indicating that some cells in mitosis have already begun synthesizing haemoglobin.

We have also examined various organs for evidence of erythropoiesis by sectioning fixed material and by staining tissue imprints on glass slides. The spleen shows an approximately 2-fold increase in mass during anaemia but this is probably a response related to the extensive red cell destruction following phenylhydrazine treatment. There is no obvious indication of increased erythropoietic activity in the liver, bone marrow, kidney or spleen during anaemia. Interestingly, very few cells of any kind are present in the bone marrow of *Xenopus*, whether normal or anaemic, and those few are mainly leucocytes. The evidence from the in vitro thymidine incorporation studies would suggest that most blood cells arise by replication and division, in the circulation, of a small number of precursor cells (only 3.7×10^7/ml present at 5 days after phenylhydrazine injection). It is therefore possible that the failure to detect any change in erythropoietic activity in the organs studied may reflect an inadequate sensitivity of the histological techniques used to detect small changes which might be involved in producing the original precursor cells which are shed into the circulation.

The normal adult *Xenopus* gives a blood picture in which 99% of the cells are apparently mature erythrocytes and only 1% appear as non-haemoglobinized lymphocyte-like cells. Intermediates between the 2 cell types are rare or absent. This suggests that in normal erythropoiesis in

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Table II. The percentage of cells incorporating ^{3}H-thymidine and staining with *o*-dianisidine in phenylhydrazine injected toads

Days after last phenylhydrazine injection	Cells incorporating thymidine (%) [a] expt. 1	expt. 2	Mean	Cells stained with *o*-dianisidine (%) expt. 1	expt. 2	Mean
5	2.5	0	1.25	88.7	84.3	86.5
15	45.1	55.3	50.2	62.1	43.6	52.8
20	5.0	8.4	6.7	87.0	73.0	80.0
30	5.1	5.3	5.2	86.5	99.8	89.6
Control	0	0	0	99.0	99.0	99.0

Values were obtained by bleeding 2 animals for each time shown, i.e. 10 animals were used in all. [a] Experimental conditions as described in legend to Figure 1.

Xenopus most blood cell replenishment and erythrocyte maturation occur outside the circulation. But the process need only be slow since the amphibian erythrocyte has a life span of about 100 days[5] in circulation, or perhaps more. During the anaemia, many cells resembling lymphocytes make their appearance in the blood, at a concentration of 3.7×10^7 cells/ml on day 5 of anaemia, and the continued rapid production of blood cells which accomplishes recovery from anaemia takes place substantially by the rapid replication and/or differentiation of these cells and their derived cells. It therefore seems that essentially all the stages involved in erythropoiesis and erythrocyte differentiation can occur in the blood circulation in the anaemic amphibian. Granulocytes are also abundant and 15 days after the induction of anaemia they account for between 5 and 10% of all blood cells. Furthermore, haemoglobin synthesis begins in cells which still resemble small lymphocytes and certainly precedes later DNA replication, mitosis and division of these cells. Figures 1 and 2 are autoradiographs of blood smears from animals during early and later response to phenylhydrazine induced anaemia.

The findings in this communication will be discussed more fully in a future publication.

Résumé. On a détruit tous les érythrocytes de sujets adultes de *Xenopus laevis* par une double injection de phénylhydrazine. 15 jours après avoir provoqué l'anémie, on constate que le sang contient beaucoup de cellules ressemblant à des lymphocytes. L'examen de ces cellules laisse supposer que, chez ces animaux, l'érythropoièse se rétablit dans le circulation.

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[6] We are grateful to the Medical Research Council for financial support of some of this work.

Prolonged Survival of AKR Mice Following Allogeneic Bone Marrow Transplantation[1]

Spontaneous leukemia-lymphoma developing in inbred AKR mice resembles malignant lymphoma in man[2,3]. The incidence of spontaneous leukemia-lymphoma in AKR mice is only minimal until 6 months of age, but exceeds 90% before the animals reach one year of age[4]; 50% mortality is usually reached at about 9 months of age[5,6]. Treatments altering the natural history of AKR spontaneous leukemia-lymphoma may further our understanding of the disease in mice and may have relevance to its counterpart in man.

The experiments to be described were designed to assess the severity of graft-versus-host (GVH) disease in immunosuppressed young AKR mice transplanted with bone marrow and lymph node cells from allogeneic H-2 matched donors. Almost all deaths attributable to GVH disease occurred within 90 days following transplantation. Those AKR mice surviving 90 days (at which time they were 6 months of age), were observed daily through their lifespan. Immunosuppression followed by bone marrow and lymph node cell transplants was often associated with an increase in the mean survival time (MST) of those AKR mice not succumbing to GVH disease. Survival varied according to the donor strain.

Materials and methods. All mice were purchased from the Jackson Laboratory, Bar Harbor, Maine, and were 12 to 14 weeks old when used for these experiments. Female AKR (H-2^k) mice were given 400 R total body X-irradiation (TBR) and cyclophosphamide (CY) 185 mg/kg, followed by i.v. administration of 2×10^7 bone marrow and 10^7 lymph node cells from syngeneic, or H-2^k matched female donors. The H-2^k donor strains employed were B10·BR, CBA, C3H/He, C57BR/cd, C58, and RF.

[1] Supported by American Cancer Socitey Grant No. ET-55, a memorial to William Heller, Sr. and the Board of Trustees, Mount Sinai Medical Center.

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