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.

NESTA THOMAS and N. MACLEAN[6]

Department of Biology, Southampton University, Medical and Biological Sciences Building, Southampton S09 3TU (England), 29 March 1974.

[5] M. J. CLINE and T. A. WALDMAN, Am. J. Physiol. *203*, 401 (1962).

[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.

[2] M. OMINE and S. PERRY, Cancer Res. *33*, 2596 (1973).

[3] E. FREI, III, F. M. SCHABEL JR. and A. GOLDIN, Cancer Res. *34*, 184 (1974).

[4] D. METCALF, *The Thymus, Recent Results in Cancer Research* (Springer-Verlag, Inc., New York 1966), vol. 5.

[5] H. E. SKIPPER, F. M. SCHABEL JR., M. W. TRADER, W. R. LASTER JR., L. SIMPSON-HERREN and H. H. LLOYD, Cancer Chemother. Rep. 56, 273 (1972).

[6] E. S. RUSSEL, in *Biology of the Laboratory Mouse*, 2nd edn. (McGraw-Hill Inc., New York, 1966), p. 512.

Survival of AKR (H-2^k) mice following immunosuppression and transplantation of immunocompetent cells from allogeneic H-2^k donors

Group	No. AKR hosts[a]	Cell donors	No. Mice alive at 6 months of age[b]	MST[c] (days)	Range (days)	Significance between groups
1	58	AKR	57	273	185–523	3, 6, 7 ($p < 0.05$); 5 ($p < 0.01$)
2	60	C3H/He	10	287	182–408	
3	56	None	54	297	183–654	6 ($p < 0.05$); 5 ($p < 0.01$)
4	60	C58	18	316	198–538	
5	59	RF	49	350	205–757	
6	59	CBA	36	363	209–671	
7	58	B10·BR	22	377	185–683	
8	59	C57BR/cd	9	424	194–763	

[a] Host mice, ca. 3 months of age, received 400 R total body X-irradiation and 185 mg/kg cyclophosphamide followed by i.v. injections of 2×10^7 bone marrow cells and 10^7 lymph node cells. [b] Mice entered into the study and 'at risk' of developing spontaneous leukemia-lymphoma. [c] Mean survival time of animals surviving 90 days post-transplant.

Calculation of MST was based on those mice alive (surviving GVH disease) 90 days post-transplant. The AKR mice were then 6 months of age and 'at risk' of developing spontaneous leukemia-lymphoma. Mice that died were autopsied. Almost without exception, those mice that died after reaching 6 months of age, did so with autopsy findings characteristic of leukemia-lymphoma. Significance between groups was determined by analysis of variance.

Results and discussion. The overall results are summarized in the Table. The MST of 273 days for AKR control mice that received TBR, CY and syngeneic cells (group 1, Table) differed significantly ($P < 0.05$) from the MST of 297 days for AKR control mice treated with TBR, CY and no cells (group 3, Table). AKR mice that were recipients of cells from allogeneic donors had MST ranging from 287 to 424 days (groups 2, 4–8, Table).

AKR mice that received cells from C3H/He or C58 donors had MST similar to the control groups (cf. groups 2 and 4 with 1 and 3, Table). Those mice (groups 5–7, Table), that received cells from RF, CBA, or B10·BR donors, had significantly greater ($P < 0.05$ to $P < 0.01$) MST than those given syngeneic cells (group 1, Table). Furthermore, mice treated with cells from RF or CBA donors (groups 5 and 6, Table), had MST which were significantly greater ($P < 0.05$ to $P < 0.01$) than mice that were treated with TBR and CY only (group 3, Table). Each of the groups treated with cells from RF and C57BR/cd donors had one mouse that lived to be more than 2 years old. The longest MST of all experimental groups was found in the relatively small number of AKR mice than survived 6 months of age following transplantation of cells from C57BR/cd donors (group 8, Table). Substantial differences in MST between some groups proved not to be significant when small sample sizes were involved.

With regard to possible explanations for the observed increase in longevity within certain groups of AKR mice, it is evident that the immunosuppressive treatment per se did not have a significant effect on MST or the development of leukemia-lymphoma because the mortality pattern was similar to that known to occur in untreated AKR mice[5,6]. Similarly, the experience of receiving transplanted cells had no ameliorative effect of itself, as demonstrated by the failure of syngeneic cells to alter the development of the neoplasia.

There was no correlation between severity of early GVH disease and long-term survival. When one compares the effect of cells from C3H/He and C57BR/cd donors (groups 2 and 8, Table), the severity of GVH disease was equivalent (about 16% of the mice in both groups survived to 6 months of age), but a great (albeit not significant) difference in MST was found. Moreover, AKR mice that received cells from RF donors (group 5, Table) suffered only mild GVH disease (83% alive at 6 months of age), but the surviving mice fared better than mice transplanted with C3H/He donor cells. Thus, immunogenetic destruction of the thymus gland[7] due to GVH disease, does not appear to be a likely explanation for the prolonged survival observed in some groups of AKR mice.

The etiological agent associated with the development of AKR leukemia-lymphoma is the GROSS virus[8,9]; however, survival of experimental AKR mice did not appear to be related to the donor strain's resistance or susceptibility to Gross virus leukemogenesis. For example, C3H/He mice are relatively resistant to the Gross virus[8], yet AKR mice given cells from C3H/He donors (group 2, Table) died of spontaneous leukemia-lymphoma with no significant prolongation of MST. Conversely, C57BR/cd and B10·BR mice are highly susceptible to the Gross virus[8,9], but AKR mice that received cells from these two donor strains had the longest MST[10].

Zusammenfassung. Die Mehrzahl der AKR Mäuse, die nach Immunosuppression und Transplantation von Knochenmark und lymphoiden Zellen allogener H_2-identischer Spender die «Graft-versus-Host» Reaktion überlebten, zeigten signifikant verlängerte mittlere Überlebenszeiten gegenüber Kontrolltieren, welche mit Immunosuppression allein oder Immunosuppression plus syngenen Zellen behandelt wurden.

W. C. ROSE, M. M. BORTIN and E. C. SALTZSTEIN

The May and Sigmund Winter Research Laboratory, Mount Sinai Medical Center, Milwaukee (Wisconsin 53233, USA), and the Departments of Medicine and Surgery, The Medical College of Wisconsin, Milwaukee (Wisconsin, USA), 17 May 1974.

[7] D. E. UPHOFF, J. natn. Cancer Inst. *42*, 243 (1969).
[8] L. GROSS, Acta haematologia *23*, 259 (1960).
[9] F. LILLY and T. PINCUS, in *Advances in Cancer Research* (Academic Press, Inc., New York 1973), vol. 17.
[10] The authors thank A. A. LAPP, A. M. RHODES, J. J. MILLER, III, and E. R. REYNOLDS for expert technical assistance. We also express our thanks to Dr. M. TELLER for his comments on the data.