

flattened between its surface and the underside of the cover slip. This caused some of the protoplasts to assume an appearance similar to erythrocytes, with a dark outer edge and a light central area. It was in this central area that flickering was most readily visible.

The above technique is also useful for obtaining photographs of a large number of live organisms (particularly highly motile ones) at one time, since intact cells are not flattened by this procedure.

D. M. MILLER

Research Institute, Canada Department of Agriculture, London, Ontario

References

1. R. Blowers, E. M. Clarkson, M. Maizels, *J. Physiol.* **133**, 228 (1951).
2. O. E. Landman and S. Spiegelman, *Proc. Natl. Acad. Sci. U.S.* **41**, 698 (1955).

11 December 1962

Thymus: Its Role in Lymphoid

Recovery after Irradiation

Abstract. *Regeneration of spleen lymphoid centers after destruction of lymphoid tissues is dependent on the thymus.*

As a result of experiments with embryonic (1) and neonatal (2) thymus, a resurgence of interest has developed in the idea that the thymus represents the major primordium of the mammalian lymphoid immunological system (3). Experiments directed toward understanding the mechanics of thymus lymphopoiesis have shown that the thymus is an autonomous lymphoid organ capable of morphogenesis in vitro as well as upon transplantation (1, 4). Removal of the thymus from neonatal animals leads to depletion of lymphoid cells from various lymphoid organs as well as from the circulation (2).

That the thymus can play a role in adult life as well as in the embryonic and neonatal period has already been clearly demonstrated in studies of murine leukemias (5). A role for the thymus in normal lymphoid function has also been proposed (6, 7), but the nature of this role has not been well defined. The possibility that the thymus in the adult mouse influences lymphopoiesis in a manner analogous to that seen during development was examined in the experiments reported here.

To determine optimal experimental conditions, a series of experiments were

performed to establish the radiation sensitivity of thymus lymphoid differentiation. Thirty thymus rudiments were removed from 12-day-old mouse embryos. They were irradiated with 140 kv (peak), 5 ma x-rays with ½ mm Al filtration at a dose rate (in air) of 80 r/min. Doses ranged from 600 to 1500 r. The thymuses were then grown for 1 week in vitro under conditions known to promote differentiation of thymus lymphoid cells (4). Doses up to 900 r failed to interfere appreciably with subsequent lymphoid morphogenesis. In a series of exploratory runs 900 r was effective in destroying the lymphoid cells of the adult spleen. This dose was therefore chosen to assess the function of the adult thymus. Ten F₁(C₃H₂AKR) male mice were thymectomized at 3 to 4 weeks of age and irradiated with 900 r x-rays at 10 weeks of age. Ten nonthymectomized, irradiated litter-mates served as controls. Ten days after irradiation the animals were killed, and the spleens were prepared for histological examination. In nine out of ten control mice, early signs of recovery were seen in the accumulation of lymphoid cells in spleen lymphoid centers. In all ten thymectomized, irradiated mice, on the other hand, such lymphoid cell accumulations were lacking.

These experiments are particularly pertinent with regard to Miller's finding that recovery of immunological function after irradiation is impaired by thymectomy of adult animals (8); our results suggest the cellular basis for his observations. Neither in Miller's experiments nor in ours has it been determined whether the role of the thymus

is indirect—that is, through production of a lymphocytosis stimulating factor (7) or by providing a tissue environment conducive to differentiation of nonthymic cells (8)—or, on the other hand, direct, by contribution of cells which migrate to the spleen (3). Indeed, the alternatives are not mutually exclusive, for a few generative cells, migrating from the thymus to the spleen, might well serve as centers for induced differentiation of lymphoid cells. Experiments involving marked cells, extracts, and transplants are necessary before merits of various alternatives can be assessed critically (9).

ROBERT AUERBACH

Department of Zoology,

University of Wisconsin, Madison

References and Notes

1. R. Auerbach, *Develop. Biol.* **3**, 336 (1961); *Proc. Natl. Acad. Sci. U.S.* **49**, 1175 (1961); *Natl. Cancer Inst. Monograph* **11** (1963).
2. J. F. A. P. Miller, *Lancet* **2**, 748 (1961); *Ann. N.Y. Acad. Sci.* **99**, 340 (1962); *Proc. Roy. Soc. London B* **964**, 415 (1962); D. M. V. Parrott and J. East, *Nature* **195**, 347 (1962); C. Martinez, J. Kersey, B. W. Papermaster, R. A. Good, *Proc. Soc. Exptl. Biol. Med.* **109**, 193 (1962).
3. R. Auerbach, *Proc. Natl. Acad. Sci. U.S.* **49**, 1175 (1961); J. F. A. P. Miller, *Lancet* **2**, 748 (1961).
4. W. D. Ball and R. Auerbach, *Exptl. Cell Res.* **20**, 245 (1960); R. Auerbach, *Develop. Biol.* **3**, 336 (1961).
5. D. P. McEndy, M. C. Boon, J. Furth, *Cancer Res.* **4**, 377 (1944); L. W. Law and M. Potter, *J. Natl. Cancer Inst.* **20**, 489 (1958); H. S. Kaplan, *Cancer Res.* **21**, 981 (1961); J. F. A. P. Miller, *Brit. J. Cancer* **14**, 93 (1960).
6. F. M. Burnet, *Australasian Ann. Med.* **11**, 79 (1962); K. E. Fichtelius, *Acta Anat.* **19** (Suppl.), 1 (1953).
7. D. Metcalf, *Brit. J. Haematol.* **6**, 324 (1960).
8. J. F. A. P. Miller, *Nature* **195**, 1318 (1962).
9. Supported by grants from the U.S. Public Health Service (C-5281) and the National Science Foundation (NSF-G19384). I am grateful for the technical assistance of L. Kubai, E. M. Morin and A. Tallungen.

19 November 1962

Antigenicity of Polypeptides: Immunological Unresponsiveness to Copolymers of α -Amino Acids

Abstract. *Immunological tolerance toward three synthetic random copolymers of the α -amino acids, glutamic, lysine, alanine, and tyrosine, was produced by a single injection of the polymers into newborn rabbits. The tolerant state could be extended by an additional intravenous injection of antigen. Repeated injections of the polymer in adjuvant mixture could "break" the tolerant state.*

The antigenicity of random copolymers of L- α -amino acids has been investigated in rabbits (1-3), guinea pigs (4, 5), and man (6), with an aim of learning about the chemical basis for immunogenicity. Studies of the multi-chain polypeptide antigens (2) have also contributed to the existing knowledge on the molecular requirements for antigenicity. This study was undertaken

to determine whether random copolymers could induce a state of immunological unresponsiveness in rabbits similar to that induced by other nonliving antigens, mainly serum proteins (7).

The percentage composition in moles of the amino acids in the random copolymers used in this study is indicated by the subscript. The polymers glu₆₀ ala₄₀ (GA); glu₄₂ lys₂₈ ala₃₀ (GLA30),