

REACTIVATION IN VITRO OF IMMUNOCOMPETENCE IN IRRADIATED MOUSE SPLEEN*

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PLATE 9

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Total-body X-irradiation has been shown to suppress immune responses to a large variety of antigenic stimulations (1), followed, in the case of sublethal doses, by gradual spontaneous recovery. It has been demonstrated that such a spontaneous recovery may not be manifest if thymectomy precedes irradiation (2-4). Experiments using thymus grafts (4-6) or thymuses placed within diffusion chambers (7) indicated that the thymus might influence this recovery by the liberation of humoral factors. Although the idea of humoral factor activity has been repeatedly stated by various groups for radiation recovery as well as for the normal development in the neonate (8-11), its mechanism of action is still obscure. Several suggestions can be made to explain the process of thymus activity; e.g. the thymus may activate cells residing in the lymphoid-depleted (or immature) organs, or it may activate stem cells to migrate and develop in these tissues. However, humoral activity of the thymus may reflect only a part of its role in the immune system (cf. references 12 and 13). For example, circulation of lymphoid cells between lymphoid organs (14) provides an additional factor to be considered in studies on the mechanism of the thymus effect and for identification of the tissue origin of immunocompetent cells in a regenerating system.

The recent development of methods for induction of immune responses in vitro (15-17) and particularly the graft versus host response (17) enabled a direct approach to a study of reactivation of immunocompetence in isolated individual organs, and thus to trace the tissue origin of the competent cells and to follow the mechanism of the thymus effect.

A survey of lymphoid radiation recovery of mouse spleen (18) indicated that

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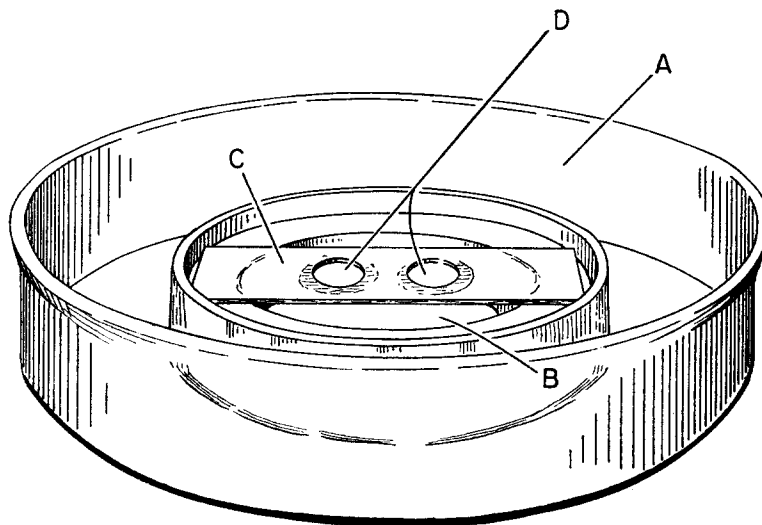
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lymphoid regeneration depends upon interaction of the irradiated spleen and bone marrow, and that this is enhanced by the thymus. The present study extended these experiments to analysis of immunological reactivity, attempting to test (a) what is the tissue origin of immunocompetent cells, (b) what is the role of the thymus in the process of regeneration of immune reactivity, and (c) whether immune reactivity and lymphoid development are separable systems.

Materials and Methods

Mice.—BALB/C/Au, C₃H/He/Au, C57Bl/6/Jax, and the F₁ hybrids (BALB/C × C57Bl) and (BALB/C × C₃H) were used in these experiments. Irradiation was performed on



TEXT-FIG. 1. Culture dish containing plastic mount used for graft versus host assay. A, Falcon dish; B, center depression in culture dish, used to contain medium; C, plastic mount for holding Millipore filters; and D, Millipore filter below hole in plastic; tissue is placed in each of the small wells bounded by filter below and plastic on sides.

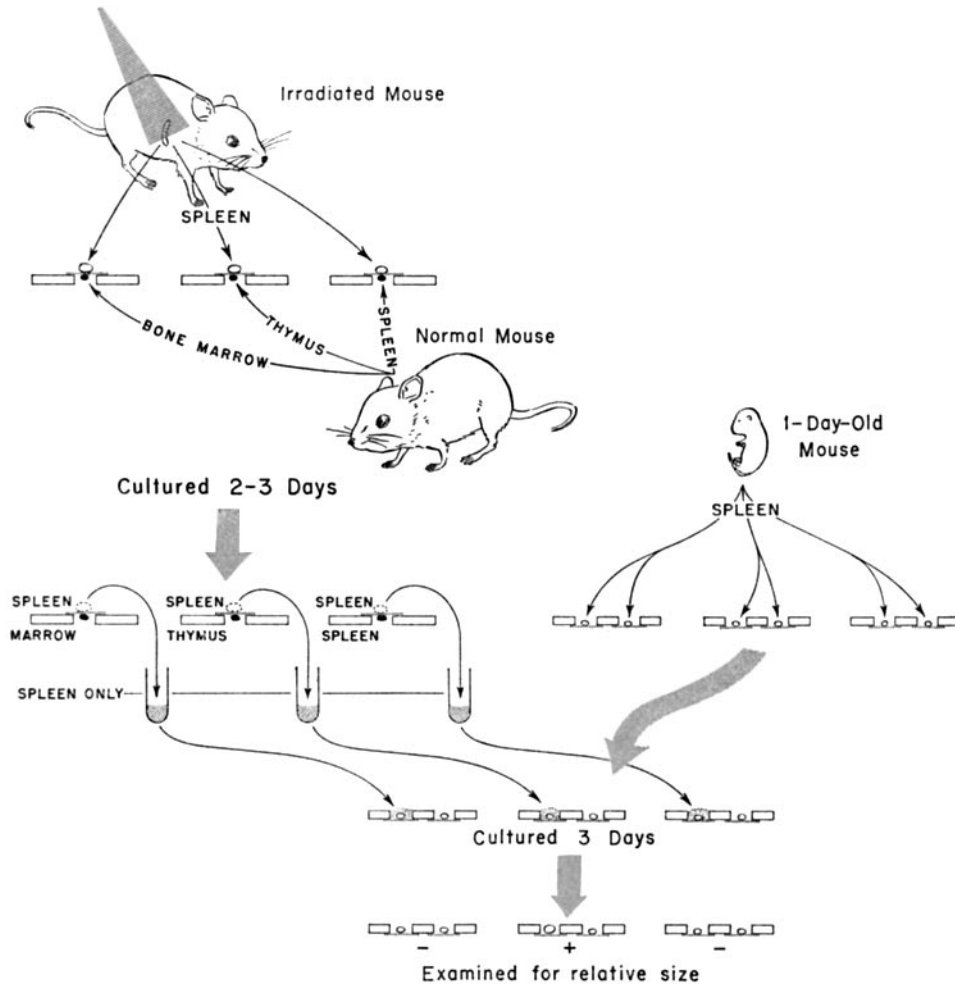
2- to 4-month-old male mice. Donors for thymus and bone marrow used in combination studies were 2-4 wk old.

Preparation of Tissue Explants and Culture Technique.—Methods were identical with those described in preceding reports (16-19). Transfilter combinations of tissues were prepared according to Grobstein's technique (19, 20, 21) using a chicken plasma clot to fasten tissue in the desired position.

Graft Versus Host Reaction.—The assay was performed in vitro as described previously (17). Splens from newborn mice were dissected into cross-sectioned slices, and pairs of slices were matched according to size, shape, and original topographical location in the spleen. Such pairs were then cultured in organ culture dishes (Falcon 3037¹) containing a plastic mount with two filter wells (Text-fig. 1). One of the explants was overlaid with the test cells, the

¹ Falcon 3037 dishes are the same dishes previously labeled 3010 or 3020. The absorbent paper normally found in these dishes has been omitted in these studies.

second serving as the control. The size of the explants was measured daily using an ocular micrometer in a dissecting microscope. In no case did the control fragment reach a size larger than the experimental fragment. Cultures in which the experimental fragment was larger than the controls on days 3-5 by more than 15% were scored as positive. Some of these



TEXT-FIG. 2. Schematic representation of experimental procedure used in transfilter studies. For description see text.

tissues were fixed at the end of this time and examined histologically to characterize the cellular changes, as a further criterion for the graft versus host response. Preparation of test cells was made from appropriate explants after 2-4 days of culture, by means of a gentle teasing with cataract knives. Equivalent cultures were pooled for this purpose, and cells (10^5 - 10^6) from two explants were added to one assay culture. When direct combinations were tested, all the tissues in the combination were used as source for the cell suspension; when transfilter combinations were made, the spleen was separated and tested alone (Text-fig. 2).

Immunization Procedure.—2-month-old C57Bl male mice were injected intraperitoneally with approximately 10^6 spleen cells from C₃H donors 5 days before irradiation.

X-Irradiation.—Mice were exposed to total-body irradiation of 550 R, using a General Electric Maxitron X-ray unit operated at 280–300 kvp, 20 ma, with 0.5 mm Cu and 1.0 mm Al added filtration, at a dose rate of 80 R/min as measured in air. Spleens were removed for culture 24 hr following irradiation. In one set of experiments (Table VI) performed at an earlier time, irradiation of 550 R was given at 5 ma, 140 kvp, with 0.5 mm Cu and 1.0 mm Al, at a dose rate of 20 R/min (cf. reference 18).

Histology.—Tissues were fixed in Zenker's solution, sectioned at 5–7 μ , and stained with hematoxylin and eosin.

EXPERIMENTAL

The first series of experiments attempted to determine whether irradiated spleen explants are competent to evoke a graft versus host reaction. Spleens

TABLE I
Splenomegaly Induction of Newborn F₁ (BALB/C × C57Bl) Explants by Cell Suspensions Obtained from Combinations of Irradiated Spleen (BALB/C) and Various Tissues of Syngeneic Origin

Tissue combination	No. of experiments	No. of spleens	No. of cultures	Incidence of splenomegaly
Irradiated spleen and bone marrow	1	4	10	0/8
Irradiated spleen and thymus	2	8	20	11/12
Irradiated spleen and liver	1	4	10	0/4
Irradiated spleen and kidney	1	4	10	0/4
Irradiated spleen only	2	8	20	0/12
Untreated control spleen	1	1	10	3/4

were removed from BALB/C mice 1 day after irradiation with 550 R, and fragments of these spleens were cultured for 2–4 days by means of the standard filter well method. Fragments of spleens from unirradiated donor BALB/C mice (control) were cultured in a similar fashion. When tested for the ability to evoke a graft versus host reaction, splenomegaly was found to be readily induced by the control suspension, whereas none of the irradiated spleens caused any overt enlargement of the host explant (Table I).

It was therefore decided to test whether splenomegaly can be induced by irradiated spleens following interaction with bone marrow, since such tissue combinations of irradiated spleen and bone marrow had been previously shown to become lymphoid (18). BALB/C-irradiated spleen fragments, combined with bone marrow, derived from syngeneic origin, were cultivated for 3 days. Subsequently, these cultures were tested against newborn spleen fragments. No splenomegaly could be detected in such cultures (Table I).

In the third experimental group, irradiated spleen fragments were combined with syngeneic thymuses for 2–3 days, and were subsequently tested for their

ability to induce splenomegaly. It was found that such thymus-spleen combinations led to enlargement of newborn F_1 spleens in 11 of 12 cases (Table I).

To test whether this result reflected any specific effect of the thymus, or whether this splenomegaly was caused by other factors, the experiment was repeated using a variety of tissues in combination with the irradiated spleen. It appeared, however, that neither liver nor kidney evoked this response (Table I).

Since reactivity resulted from combination of spleen with thymus, it was decided to test whether the observed reaction was truly immunological in nature or whether splenomegaly could be attributed, for example, to population and proliferation of the added cells within the newborn spleen environment, to synergistic stimulation of the thymus cells, or to some other nonimmunological mechanism resulting in growth. If splenomegaly was not a reflection of an im-

TABLE II

Analysis of the Immunological Nature of the Splenomegaly Reaction by Irradiated Spleen and Thymus Tissue Combinations: Comparison of Parental (BALB/C) and F_1 (BALB/C \times C57Bl) Tissue Combinations Tested against F_1 (BALB/C \times C57Bl) Newborn Spleen

Tissue combination	Strain origin		Incidence of splenomegaly
	Spleen	Thymus	
Irradiated spleen and thymus	F_1 (BALB/C \times C57Bl)	F_1 (BALB/C \times C57Bl)	0/4
Irradiated spleen only	F_1 (BALB/C \times C57Bl)	—	0/4
Irradiated spleen and thymus	BALB/C	BALB/C	3/4
Irradiated spleen	BALB/C	—	0/4

munological process then similar enlargement of the neonatal spleen would be expected in a syngeneic system. On the other hand, if splenomegaly was a reflection of the graft versus host response, it would be expected to occur only in allogeneic or semiallogeneic combinations.

The next experiment was therefore designed to test whether splenomegaly can be induced in vitro as a result of addition of cells from syngeneic irradiated spleen and thymus combination cultures. Spleens were obtained from irradiated F_1 (BALB/C \times C57Bl) mice and combined in culture with F_1 (BALB/C \times C57Bl) thymus. After 3 days of cultivation, suspensions from these cultures were prepared and added to F_1 (BALB/C \times C57Bl) newborn spleen cultures. Under these conditions, spleen enlargement was not detected (Table II). These results reinforced the conclusion that the splenomegaly observed in the previous experiments reflects a graft versus host reaction (cf. also reference 17).

One might argue, however, that singly isolated spleen fragments from irradiated donors are inactive in induction of splenomegaly due to atrophy which

may be partially overcome in the presence of thymus tissue. Thus, unresponsiveness might not reflect absence of immunocompetence but rather absence of lymphoid cells. To test whether an irradiated spleen can contain immunologically active cells under these experimental conditions, spleens were removed from preimmunized mice. The assumption was that since irradiation affects the initial stages of the immune response (cf. reference 1), preimmunized irradiated spleens should be reactive in vitro if the system permits adequate maintenance of responding cells.

Seven C57Bl mice were immunized against C₃H tissues. 5 days later, four mice were irradiated. Spleens were cultured 24 hr following exposure. Each

TABLE III
Spleno-megaly Induction by Spleen Cell Suspensions Obtained from C57Bl Mice Immunized against C₃H Antigens 5 Days before Irradiation

Spleen No.	Treatment	Spleno-megaly reaction			
		F ₁ (C ₃ H × C57Bl)		F ₁ (BALB/C × C57Bl)	
		+ Thymus	Control	+ Thymus	Control
1	Immunized, irradiated, and cultured	—	+	+	—
2	Immunized, irradiated, and cultured	—	+	+	—
3	Immunized, irradiated, and cultured	+	+	+	—
4	Immunized, irradiated, and cultured	+	—	+	—
5	Immunized and cultured control spleen		+		+
6	Immunized control spleen		+		+
7	Immunized control spleen		+		+

spleen was dissected into 10 fragments, 5 of which were singly isolated and 5 of which were combined with syngeneic thymus tissue. One spleen from an immunized, unirradiated donor was cultured as a control to test whether the culture system per se might reduce the competence of such immunized tissues. 3 days later, cultures were sacrificed and tested for the ability to induce spleno-megaly in F₁(C₃H × C57Bl) and F₁(BALB/C × C57Bl) cultures. The results (Table III) indicate that three of four irradiated C57Bl spleens induced spleno-megaly in F₁(C₃H × C57Bl) yet none of the four caused any detectable enlargement of F₁(BALB/C × C57Bl) newborn spleens. Following combination with thymus tissue, spleno-megaly occurred in both types of F₁ hybrids (2/4 and 4/4 respectively). The control unirradiated spleen led to spleno-megaly in both strains, and so did the control cell suspensions tested directly from the remaining two immunized spleens, which were not subjected to culture environment.

This selective effect of irradiation, suppressing the reaction to BALB/C and

not to C₃H antigens—towards which the irradiated mice had been immunized before exposure—lends further support to the conclusion that the splenomegaly reaction is a result of an immune response.

It was then decided to test whether the thymus effect can be explained by direct participation of thymus cells in causing the subsequent enlargement of

TABLE IV
Splenomegaly Induction in Newborn F₁ (BALB/C × C57Bl) Explants by Cell Suspensions of Irradiated Spleens Grown in Transfilter Combination Cultures with Normal Spleen, Irradiated Spleen, Normal Thymus, or Normal Bone Marrow

Tissue combination	Experiment No.	Total No. of spleens	Total No. of cultures	Incidence of splenomegaly
Irradiated spleen/thymus	1	4	20	4/8
	2	4	20	4/6
	3	4	20	3/8
	4	4	20	2/4
	5	4	20	2/4
	Total	20	100	15/30
Irradiated spleen/spleen	1	4	20	0/7
	2	4	20	0/6
	Total	8	40	0/13
Irradiated spleen/irradiated spleen	1	4	10	0/4
	2	4	10	0/4
	3	4	10	0/3
	4	4	20	0/4
	5	4	10	0/3
	Total	20	60	0/18
Irradiated spleen/bone marrow	1	4	20	0/10

neonatal spleen, or whether the thymus effect is indirect; i.e., by inducing competence in the irradiated spleen tissue.

Irradiated (BALB/C) spleen explants were combined with syngeneic thymuses across an intervening millipore filter barrier in standard fashion (19; Figs. 1 and 2). 2–3 days later the spleens were removed, and tested for their ability to induce splenomegaly in F₁(BALB/C × C57Bl) hybrid explants. Splenomegaly occurred in 15 of 30 cases (Fig. 3), whereas none of the controls, which were subjected to either irradiated spleen (13 cases), unirradiated adult spleen fragments (18 cases) caused any detectable enlargement (Table IV), (Fig. 4).

Since it has been shown that this splenomegaly reaction is an immunological

phenomenon, it was concluded that the thymus can induce reactivation of immunocompetence in the irradiated spleen explants.

It has been reported before (18) that such combinations of irradiated spleen and thymus did not lead to any morphologically detectable lymphoid regener-

TABLE V
Splenomegaly Induced in Newborn F₁ (BALB/C × C57Bl) Explants by Suspensions Obtained from Lethally Irradiated Spleen (BALB/C) Combined with Bone Marrow (BALB/C) and Thymus F₁ (BALB/C × C57Bl)

Tissue combination	No. of experiments	No. of spleens	No. of cultures	Incidence of splenomegaly
Spleen only	2	8	20	0/11
Spleen and bone marrow	2	8	20	0/11
Spleen and thymus	2	8	20	0/11
Spleen, thymus, and bone marrow	2	8	20	10/11

TABLE VI
Splenomegaly Induction in Newborn F₁ (BALB/C × C₃H) Explants by Cell Suspensions Obtained from Irradiated Spleen Combined with Bone Marrow and Thymus

Irradiated spleen	Strain origin of tissues combined with		No. of experiments	No. of spleens	No. of cultures	Incidence of splenomegaly
	Thymus	Bone marrow				
C ₃ H	—	—	4	12	16	0/15
C ₃ H	C ₃ H	—	1	2	5	0/3
C ₃ H	F ₁ (BALB/C × C ₃ H)	—	3	10	13	0/12
C ₃ H	—	C ₃ H	2	6	10	0/10
C ₃ H	—	F ₁ (BALB/C × C ₃ H)	2	6	8	0/7
C ₃ H	C ₃ H	C ₃ H	1	2	5	2/3
C ₃ H	F ₁ (BALB/C × C ₃ H)	C ₃ H	1	4	5	3/5
C ₃ H	F ₁ (BALB/C × C ₃ H)	F ₁ (BALB/C × C ₃ H)	2	6	8	0/7

ation, yet maintenance of some lymphoid elements during this period of cultivation was suggested.² One might wonder, then, whether the thymus affects those lymphoid cells that survived irradiation. The question was therefore raised whether under conditions in which such survival is limited the spleen is still capable of being reactivated by the thymus.

To test this, spleens were removed from lethally irradiated mice and com-

² Globerson, A., and R. Auerbach. Unpublished observations.

bined with either thymus, bone marrow, or both thymus and bone marrow; combinations were subsequently tested for their ability to induce splenomegaly. As shown in Table V, only the combination including both thymus and bone marrow was active, whereas the combination of spleen with thymus only, or with bone marrow only, were inactive.

Inasmuch as cells syngeneic to the neonatal spleen will not induce splenomegaly, it was possible to design a set of experiments to determine the origin of the immunocompetent cells present in such cultures (cf. also reference 24). Thus experiments were performed in which thymus or bone marrow was obtained from mice either syngeneic to the irradiated donor spleen or from the same F_1 hybrid strain as the newborn spleen. The results (Tables V and VI, suggest that the bone marrow contributed the reactive cells under these conditions.

DISCUSSION

The present experiments demonstrate that explants of spleens from irradiated mice regain competence to induce a graft versus host reaction upon interaction with thymus tissue. Furthermore, it has been shown that this thymus effect can be mediated across an intervening Millipore filter barrier. Similarly, inductive interactions of lymphoid tissues across membranes *in vitro* have been reported before for thymus development (12) and regeneration (23). That under such conditions the active factor is subcellular is indicated by the following: (a) such filters of 0.45 porosity are impermeable to lymphocytes under these conditions as demonstrated previously (cf. reference 12, 19-21); (b) in the present system an F_1 thymus, incapable of evoking splenomegaly in a syngeneic F_1 neonatal spleen, caused reactivity in combination with the irradiated parental spleen, excluding the possibility of direct participation of thymus cells in the reaction; and (c) furthermore, preliminary studies² have shown thymus activity in the system with thin filters of 0.3 or 0.1 μ porosity.

These results are in line with observations made *in vivo*, suggesting an indirect effect of thymus in radiation recovery (5-7) as well as in development during the postnatal period (9,10). Although it is impossible to tell at this stage whether the factor active *in vitro* is identical with any of the active extracts obtained from the thymus (cf. reference 11, 25-29) one could still draw some immediate conclusions about the mechanism of the thymus effect. Thus the thymus can affect the irradiated spleen via no essential intermediate organs, possibly by reactivating cells that survived irradiation. When such survival is limited (i.e. by applying different conditions of irradiation), bone marrow contributes the reactive cells, an observation which is in line with results obtained from *in vivo* studies of thymectomized radiation chimeras (5, 6).

Close examination of the irradiated spleen cultures at the stage when they become competent does not indicate any overt lymphoid regeneration; on the other hand, the combination of irradiated spleen with bone marrow has been

shown to be lymphoid (18), yet such cells do not elicit a detectable graft versus host reaction. It may therefore be suggested, that immunocompetence can be conferred with no histologically detectable regeneration of lymphoid structure of the tissue. Such a separation between morphological recovery and reactivation of immune responses has been indicated in several situations: (a) restoration of antibody-forming capacity by nucleic acid derivatives in irradiated rats was not accompanied by a simultaneous lymphoid regeneration (30); and (b) when thymuses were grafted within diffusion chambers into neonatally thymectomized mice or rats, immune reactivity was not always accompanied by lymphoid regeneration (9, 31). Whether reactivation of immune responses and induction of lymphoid regeneration are triggered by the same factor, or whether at least two factors are operating, is an open question at the present time.

SUMMARY

The restoration of immunological competence to irradiated adult mouse spleen was studied using an in vitro assay system. Competence of the spleen to evoke a graft versus host reaction in vitro was lost upon sublethal irradiation (550 R). Regeneration of immune competence occurred when such spleens were grown for 2-3 days in the presence of thymus tissue, but not when grown in the presence of a variety of other tissues. This thymic activity was demonstrated to occur across an intervening Millipore filter barrier. When lethal doses of irradiation were used, reactivation of immune competence did not occur unless both thymus and bone marrow were present. In this experimental situation the bone marrow appeared to supply the immunocompetent cells.

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BIBLIOGRAPHY

1. Taliaferro, W. H., L. G. Taliaferro, and B. N. Jaroslow. 1964. Radiation and Immune Mechanisms. Academic Press, Inc., New York. 152 pp.
2. Miller, J. F. A. P. 1962. Immunological significance of the thymus of the adult mouse. *Nature*. **195**:1318
3. Globerson, A., L. Fiore Donati, and M. Feldman. 1962. On the role of the thymus in recovery of immunological reactivity following x-irradiation. *Exptl. Cell Res.* **28**:455.
4. Globerson, A., and M. Feldman. 1964. Role of the thymus in restoration of immune reactivity and lymphoid regeneration in irradiated mice. *Transplantation*. **2**:212.
5. Miller, J. F. A. P., E. Leuchars, A. M. Cross, and P. Dukor. 1964. Immunologic role of the thymus in radiation chimeras. *Ann. N. Y. Acad. Sci.* **120**:205.
6. Feldman, M., and A. Globerson. 1964. The role of the thymus in restoring immunological reactivity and lymphoid cell differentiation in x-irradiated adult mice. *Ann. N. Y. Acad. Sci.* **120**:182.
7. Miller, J. F. A. P., D. Osoba, and P. Dukor. 1965. A humoral thymus mechanism responsible for immunological maturation. *Ann. N. Y. Acad. Sci.* **124**:95.

8. Levey, R. H., N. Trainin, and L. W. Law. 1963. Evidence for function of thymic tissue in diffusion chambers implanted in neonatally thymectomized mice: preliminary report. *J. Natl. Cancer Inst.* **31**:199.
9. Osoba, D., and J. F. A. P. Miller. 1963. Evidence for a humoral thymus factor responsible for the maturation of immunological faculty. *Nature.* **199**:653.
10. Levey, R. H., N. Trainin, and L. W. Law. 1963. Evidence for function of thymic tissue in diffusion chambers implanted in neonatally thymectomized mice. *J. Natl. Cancer Inst.* **31**:199.
11. Metcalf, D. 1966. The thymus. *In* Recent Results in Cancer Research. Springer Verlag New York, Inc., New York. **5**:1.
12. Auerbach, R. 1965. Experimental analysis of lymphoid differentiation in the mammalian thymus and spleen. *In* Organogenesis. R. De Haan, and H. Ursprung, editors. Holt, Rinehart, and Winston, New York. 539.
13. Parrott, D., and J. East. 1964. The immunological status of thymectomized animals—a survey. *Proc. Roy. Soc. Med.* **57**:147.
14. Harris, J. E., C. E. Ford, D. W. H. Barnes, and E. P. Evans. 1964. Cellular traffic of the thymus: experiments with chromosome markers: evidence from parabiosis for an afferent stream of cells. *Nature.* **201**:886.
15. Globerson, A., and R. Auerbach. 1965. Primary immune reactions in organ cultures. *Science.* **149**:991.
16. Globerson, A., and R. Auerbach. 1966. Primary antibody response in organ cultures. *J. Exptl. Med.* **124**:1001.
17. Auerbach, R., and A. Globerson. 1966. In vitro induction of the graft-versus-host reaction. *Exptl. Cell Res.* **42**:31.
18. Globerson, A. 1966. In vitro studies on radiation lymphoid recovery of mouse spleen. *J. Exptl. Med.* **123**:25.
19. Auerbach, R. 1961. Experimental analysis of the origin of cell types in the development of the mouse thymus. *Develop. Biol.* **3**:336.
20. Grobstein, C. 1956. Trans-filter induction of tubules in mouse metanephrogenic mesenchyme. *Exptl. Cell Res.* **10**:424.
21. Grobstein, C. 1957. Some transmission characteristics of the tubule-inducing influence on mouse metanephrogenic mesenchyme. *Exptl. Cell Res.* **13**:575.
22. Simonsen, M. 1962. Graft-versus-host reactions. Their natural history, and applicability as tools of research. *Progr. Allergy.* **6**:349.
23. Globerson, A., and R. Auerbach. 1965. In vitro studies on thymus and lung differentiation following urethan treatment. *In* Methodological Approach to Study of Leukemia. The Wistar Institute Press, Philadelphia. **4**:3.
24. Auerbach, R. 1966. Embryogenesis of immune systems. *In* Ciba Foundation Symposium on the Thymus. G. E. W. Wolstenholme and R. Porter, editors. J. A. Churchill, Ltd., London. 39.
25. Gregoire, C., and C. Duchateau. 1956. A study of lympho-epithelial symbiosis in thymus: Reactions of the lymphatic tissue to extracts and to implants of epithelial components of thymus. *Arch Biol. (Liege).* **67**:269.
26. Metcalf, D. 1959. Proceedings of the Third Canadian Cancer Conference. Academic Press, Inc., New York. 351.
27. De Somer, D., P. Denys, and R. Leyten. 1963. Activity of a noncellular calf thymus extract in normal and thymectomized mice. *Life Sci.* **1**:810.

28. Goldstein, A. L., F. D. Slater, and A. White. 1966. Preparation, assay, and partial purification of a thymic lymphocytopoietic factor (Thymosin). *Proc. Natl. Acad. Sci. U.S.* **56**:1010.
29. Hand, T., P. Caster, and T. D. Luckey. 1967. Isolation of a thymus hormone, LSH. *Biochem. Biophys. Res. Commun.* **26**:18.
30. Feldman, M., A. Globerson, and D. Nachtigal. 1962. The reactivation of the immune response in immunologically suppressed animals. *In* *Conceptual Advances in Immunology and Oncology*. University of Texas Press, Houston. 427.
31. Law, L. 1966. Studies of thymic function with emphasis on the role of the thymus in oncogenesis. *Cancer Res.* **26**:551.

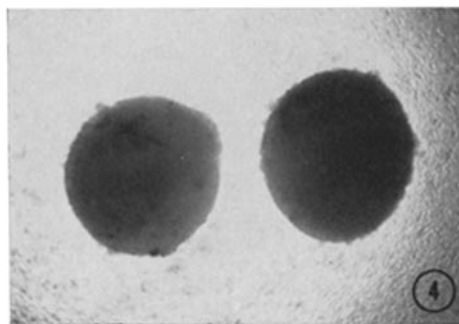
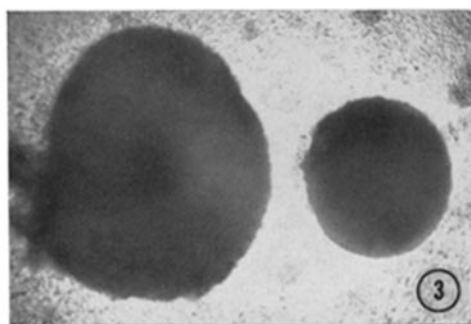
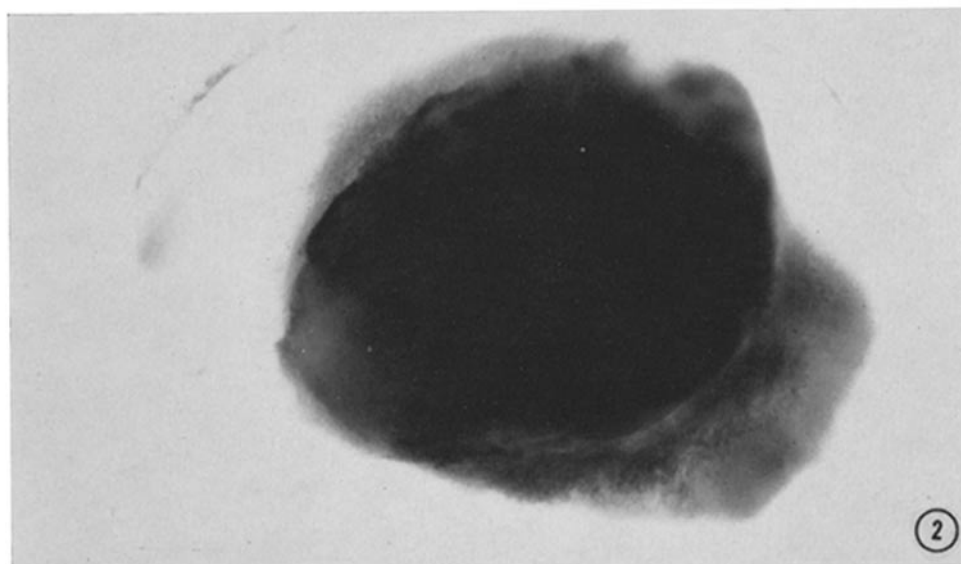
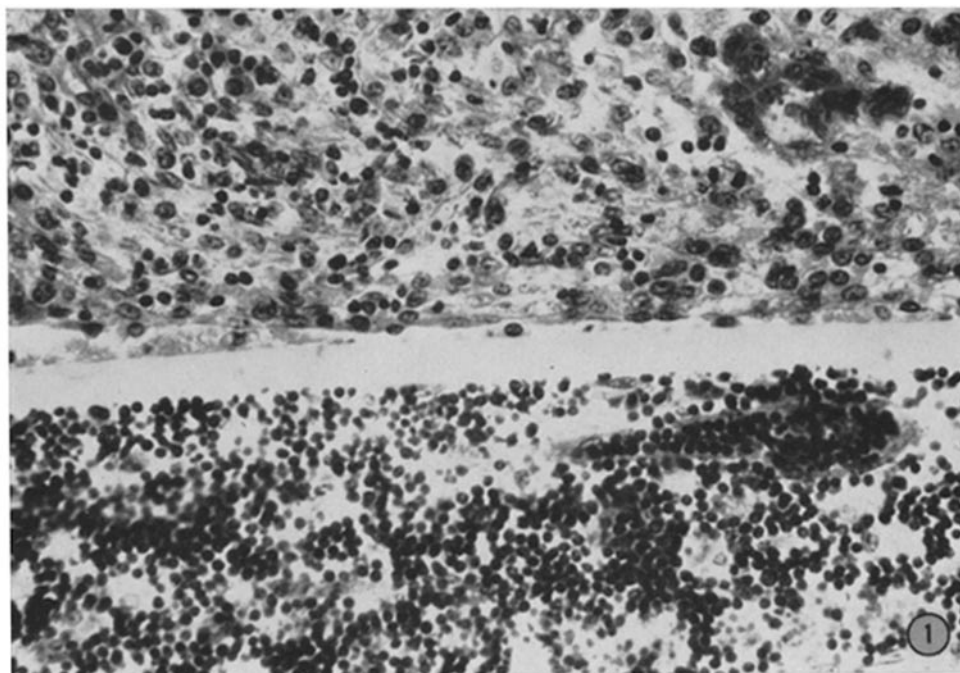
EXPLANATION OF PLATE 9

FIG. 1. Transfilter combination of irradiated spleen (top) and normal thymus (below). The Millipore filter of 25 μ thickness is seen separating the tissues. \times 260.

FIG. 2. Photograph of living culture. Spleen (darker) is on top of filter; thymus (lighter) is seen below the filter. \times 34.

FIG. 3. Host spleen explants placed side by side to facilitate comparison. Explants were matched in size at the start. 4 days later the explant receiving competent cells is considerably larger than the control. \times 37.

FIG. 4. Similar to Fig. 3. In this instance the test cells were not competent; the two explants remain comparable in size. \times 37.



(Globerson and Auerbach: Reactivation in vitro of immunocompetence)