# PRODUCTION OF RUNT DISEASE IN TOLERANT MICE BY THE INJECTION OF SYNGENEIC LYMPHOID CELLS*

By HENRY R. HILGARD,‡ M.D., CARLOS MARTINEZ,§ M.D., AND ROBERT A. GOOD,‖ M.D.

*(From the Department of Physiology and the Pediatric Research Laboratories, Variety Club Heart Hospital, University of Minnesota Medical School, Minneapolis)*

(Received for publication, July 29, 1965)

It is well established that the syndrome known as "runt disease", "homologous disease", or "secondary disease" can be produced by the introduction of immunologically competent lymphoid cells into allogeneic (homologous) individuals incapable of rejecting the donor cells. This syndrome has been produced in newborn mice (1), in adult mice previously made tolerant of the injected lymphoid cells (2), in adult mice pretreated with lethal doses of x-irradiation (3), and in $F_1$ hybrid mice injected with parental strain lymphoid cells (4). These diverse experimental preparations have three characteristics in common: (*a*) the injected donor cells are immunologically competent; (*b*) the recipient animal is unable to reject the donor cells; and (*c*) the donor cells are injected into a recipient which differs at strong histocompatibility loci from the donor.

The purpose of this paper is to describe a form of runt disease in mice which is produced by the injection of immunologically competent lymphoid cells into tolerant recipient mice of the *same strain* as the cell donors; the recipient mice, however, are lymphoid chimeras because of the prior induction of a tolerant state. In this form of runt disease, the injected syngeneic (isologous) lymphoid cells are presumably capable of immunologic attack only on the allogeneic lymphoid cells present in the recipient, and not against the tissues of the recipient itself. We will show further that tolerant recipient mice which are *not* lymphoid chimeras escape runting after injection with immunologically competent syngeneic lymphoid cells. These experimental findings indicate that an immunologic attack upon lymphoid and hematopoietic tissues, in the absence of attack against other tissues, is sufficient to cause runting.

We will also show that whereas the immunologic rejection of allogeneic

* Supported by the United States Public Health Service (HE-02085, AI-00798), the National Foundation, the American Cancer Society, and the Minnesota Heart Association.

‡ Research Fellow, Department of Pediatrics, University of Minnesota Medical School.

§ American Cancer Society Research Professor of Physiology.

‖ American Legion Memorial Heart Research Professor of Pediatrics and Microbiology.

lymphoid and hematopoietic cells present in chimeras results in runting, the rejection of similar cells which have not become established in host tissues does not result in runting. We therefore conclude that runting will occur only if the attacked cells are established within host tissues.

## *Materials and Methods*

*Mice.*—Highly inbred mice of the A and C3H/Bi strains and $F_1$ hybrid mice resulting from the cross between the A and C57BL/1 and the C3H and A strains have been used for these experiments. The A, C3H, and C57BL/1 strains have been maintained in our colony by strict brother-sister mating since 1956, and are directly descended from the inbred colony of the late Dr. J. J. Bittner.

*Irradiation.*—Mice were given total body x-irradiation in a compartmented lucite container with plywood surround. The factors were: 220 kv, half value layer 0.89 mm copper, 15 ma, distance 60 cm, 1.0 mm aluminum plus 0.25 mm copper filtration.

*Skin Grafting.*—Grafting of allogeneic skin was performed by the method routinely employed in this laboratory (5). Grafts of abdominal skin approximately 2 cm square were rotated 180 degrees and sutured onto the back of recipient mice with 5–0 surgical silk. No bandages or dressings of any kind were used. For at least 4 weeks after grafting, mice were housed individually in plastic cages with free access to Purina laboratory chow and tap water. Grafts were inspected twice weekly and were considered fully accepted if the grafts had maintained at least their original size and if the entire graft was growing hair in a direction opposite that of the hair on the surrounding host skin.

*Viable Spleen Cell Suspensions.*—Spleen cell suspensions were prepared by slicing spleens into 5 or 6 pieces and then expressing the splenic pulp gently from the capsule in a loose-fitting Potter-Elvehjem tissue homogenizer. All suspensions were prepared in lactate-Ringer's solution and were injected within 1 hour after preparation.

*Disrupted Spleen Cell Suspensions.*—Homogenates of spleen cells were prepared in a tight-fitting tissue homogenizer containing lactate-Ringer's solution and then subjected to four cycles of freezing and thawing as previously described (6). The spleen cell suspensions were checked under the microscope for the presence of intact cells following staining with trypan blue. In no instance could intact cells be detected after this treatment.

*Induction of Tolerance with Viable Spleen Cells.*—

*A strain mice:* Two-month-old mice of the A strain were made tolerant of (A × C57BL/1)$F_1$ hybrid skin grafts by submitting them to 600 roentgen in air of total body x-irradiation followed immediately by an intravenous infusion of 200 million (A × C57BL/1)$F_1$ hybrid spleen cells into a lateral vein of the tail. Two weeks later these mice received $F_1$ hybrid skin grafts. They were used for further experimentation 4 weeks after grafting if the grafts had been fully accepted.

*C3H strain mice:* Newborn mice of the C3H strain were made tolerant of A skin grafts by injecting them intravenously *via* the orbital branch of the facial vein within 24 hours after birth with 3 to 5 million viable spleen cells obtained from adult A strain donors. These mice were grafted with A strain skin at 5 weeks of age and were used for further experimentation 6 weeks after grafting if the grafts had been fully accepted.

*Induction of Tolerance with Disrupted Spleen Cell Suspensions.*—C3H mice were injected intraperitoneally with disrupted spleen material obtained from adult A strain mice according to the following schedule: during the 1st week of life, mice received 0.1 of a spleen equivalent of the disrupted spleen material divided into two injections, one given within 24 hours after birth and the other 3 days later. During the 2nd, 3rd, and 4th weeks mice received a total of 0.2, 0.4, and 1.0 of a spleen equivalent respectively, given by twice weekly injections 3 days

apart. Mice were grafted with A skin at 5 weeks of age and were used for further experimentation 6 to 12 weeks after grafting if the grafts were fully accepted.

*Induction of Runt Disease in Tolerant mice.—*

*A strain mice tolerant of (A × C57BL/1)$F_1$ hybrid skin grafts:* One group of tolerant mice received an intraperitoneal injection of 250 million spleen cells from normal adult A strain donors. A second group was injected intraperitoneally with 250 million spleen cells from A strain donors which had been immunized 1 month previously to C57BL/1 tissue by a single intraperitoneal injection of 50 million C57BL/1 spleen cells. A third group was injected with 250 million (A × C57BL/1)$F_1$ hybrid spleen cells.

*C3H mice tolerant of A strain skin grafts:* To facilitate a graft *versus* host reaction in this strain combination, all tolerant C3H mice were given sublethal x-irradiation of 400 r in air immediately prior to the injection of donor spleen cells. Three groups of C3H mice made tolerant of A by the injection of viable A strain spleen cells at birth received either (*a*) 400 r and an injection of 150 million C3H spleen cells intravenously, or (*b*) 400 r and an injection of 150 million (C3H × A)$F_1$ hybrid spleen cells intravenously, or (*c*) 400 r only. A group of C3H mice made tolerant of A by the injection of disrupted spleen material received 400 r and an intravenous injection of 150 million C3H spleen cells. Cell injections were given *via* one of the lateral veins of the tail within 1 hour after irradiation.

*Evaluation of Runt Disease.*—Mice were evaluated on the basis of daily weight changes and clinical appearance. At death, mice were examined in the gross and tissues prepared for microscopic examination.

## RESULTS

*Induction of Runting in A Strain Mice Tolerant of (A × C57BL/1)$F_1$ Hybrid Skin Grafts by the Injection of Spleen Cells from A Strain Donors.*—A strain mice which were tolerant of (A × C57BL/1)$F_1$ hybrid skin grafts by giving them lethal irradiation and an intravenous injection of 200 million hybrid spleen cells were used in this experiment. At the time of this experiment these mice were 4 months old, 8 weeks postirradiation and injection, and were bearing a fully accepted (A × C57BL/1)$F_1$ hybrid skin graft placed 6 weeks previously. Each mouse in a group of 11 mice was injected intraperitoneally with 250 million spleen cells from normal A strain donors. The mice in this group developed significant weight loss beginning on the 8th day after injection, reaching a mean weight loss of 15 per cent of their preinjection body weight on the 14th day after injection (Fig. 1). Ten of the 11 injected mice exhibited lethargy, a hunched appearance, and ruffled fur during the period of severe weight loss. Three succumbed to the disease process on days 15, 15, and 19 after injection (Table I). The remaining 8 mice eventually made a full recovery, and within 1 month after injection had nearly regained their preinjection body weight. All surviving mice had rejected their $F_1$ hybrid skin grafts within 21 days after injection.

To determine if the disease process observed in the previous experiment was in fact the result of an immunologic attack by the injected A strain lymphoid cells against the hybrid lymphoid cells of the tolerant recipient, another group of 10 tolerant A strain mice were injected with spleen cells from A strain

donors immunized 4 weeks previously to C57BL/1 tissue. The mice in this group showed acceleration of the runting process. By the 6th day after injection they showed a mean body weight loss of 24 per cent of their preinjection body weight, and most had developed clinical evidence of disease by the 5th post-

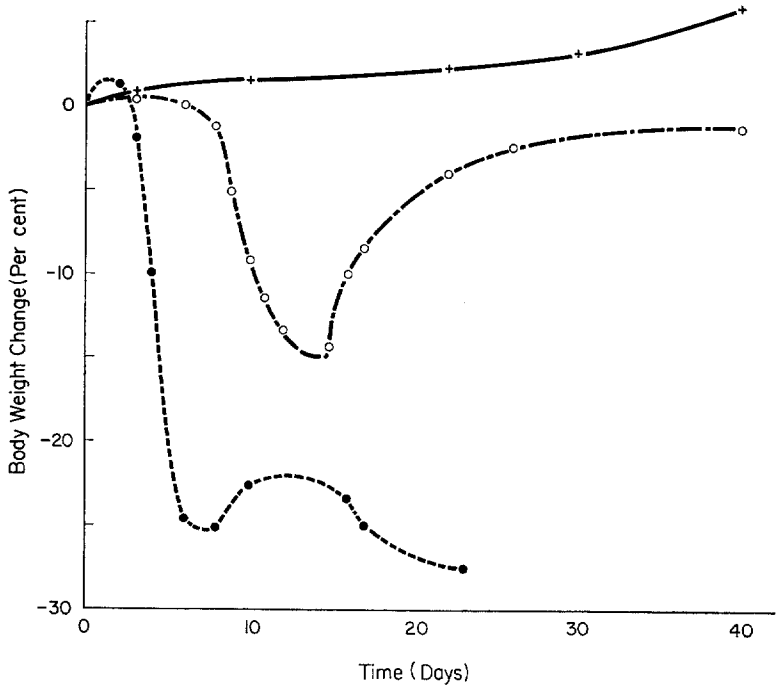


FIG. 1. Body weight changes in 4-month-old A strain mice tolerant of (A × C57BL/1)$F_1$ hybrid skin grafts after injection with spleen cells of various origins. (Tolerance was induced at 2 months of age by giving 600 r of x-irradiation and an intravenous injection of (A × C57BL/1)$F_1$ hybrid spleen cells.) – – – • – – –, injected with spleen cells from A immune to C57BL/1 donors; — – – ∘ – – —, injected with spleen cells from normal A strain donors; and —— + ——, injected with spleen cells from (A × C57BL/1)$F_1$ hybrid donors. All were injected intraperitoneally on day 0 with 250 million spleen cells.

injection day. Eight of 10 mice died, and by the 21st postinjection day surviving mice had rejected their hybrid skin grafts. It was apparent from the acceleration of the runting process achieved by the injection of immunized cells that runting was the result of an immune attack by the injected A strain lymphoid cells against the (A × C57BL/1)$F_1$ hybrid lymphoid tissue present in the recipients. A group of 9 tolerant mice were injected with spleen cells from (A × C57BL/1)$F_1$ hybrid donors and served as cell injection controls. These mice showed no weight loss and their hybrid skin grafts remained intact.

*Failure of A Strain Mice Injected with Large Quantities of (A × C57BL/1)$F_1$ Hybrid Spleen Cells to Develop Runting.*—Since the runting process observed in the previous experiments resulted from an immune attack by A strain lymphoid cells against (A × C57BL/1)$F_1$ hybrid lymphoid cells, we wished to determine if a similar runting process could be produced when normal A strain mice were in the process of attacking and rejecting a massive cellular allograft of (A × C57BL/1)$F_1$ hybrid lymphoid cells. Nine 2-month-old A strain mice were each given intravenous injections of a total of 850 million viable (A × C57BL/1)$F_1$

TABLE I

*Effect of Injection of Spleen Cells of Various Origins into A Tolerant to (A × C57BL/1) Recipients**

| No. of mice in group | Donor of injected spleen cells | Recipient | No. deaths / No. mice | Day of death |
|---|---|---|---|---|
| 11 | Normal A | A tolerant to (A × C57BL/1) | 3/11 | 15, 15, 19 |
| 10 | A immune to C57BL/1 | A tolerant to (A × C57BL/1) | 8/10 | 13, 19, 20, 21, 21, 23, 25, 28 |
| 9 | (A × C57BL/1) | A tolerant to (A × C57BL/1) | 0/9 | None |

* The recipient A strain mice were made tolerant of (A × C57BL/1)$F_1$ hybrid skin grafts by giving them 600 r of x-irradiation and an intravenous injection of 200 million hybrid spleen cells at 2 months of age. They were 4 months of age when used for this experiment and were bearing fully accepted (A × C57BL/1)$F_1$ hybrid skin grafts. On day 0 of this experiment all mice received an intraperitoneal injection of 250 million spleen cells from adult donors of the strain indicated.

hybrid spleen cells (approximately equivalent to 6 spleens). Each mouse received four injections of at least 200 million cells per injection *via* the lateral tail veins within a 48 hour period. The spleen cells were given intravenously in order to afford the greatest opportunity for the cells to set up a chimeric state in the recipient lymphoid tissue similar to that which exists in mice made tolerant by injections of viable allogeneic lymphoid cells. These recipient mice, however, showed no weight loss or other evidence of clinical disease, and in fact gained weight as well as non-injected control mice (Fig. 2). They rejected hybrid skin grafts placed on the 21st day after injection within 10 days after grafting, showing that they had not become tolerant to the hybrid tissue. This experiment, then, demonstrates that the process of rejection of a massive quantity of allogeneic lymphoid cells is insufficient to cause clinical disease. We conclude that whereas the rejection of non-established hybrid lymphoid cells

causes no runting, the rejection of hybrid lymphoid cells which have become established in a chimeric animal results in a runting syndrome.

*Effect of Injection of C3H Spleen Cells into C3H Mice Tolerant of A Strain Skin Grafts.*—The foregoing experiments showed that runt disease can result from an immune attack by injected lymphoid cells on allogeneic lymphoid tissue in a chimeric recipient. We therefore investigated the possibility that tolerant mice which were *not* lymphoid chimeras would escape runting when injected with syngeneic lymphoid cells. Because we have been unable to produce tolerance in A strain mice of (A × C57BL/1)$F_1$ hybrid skin by the injection of disrupted cell material, we used C3H mice tolerant of A strain skin grafts for

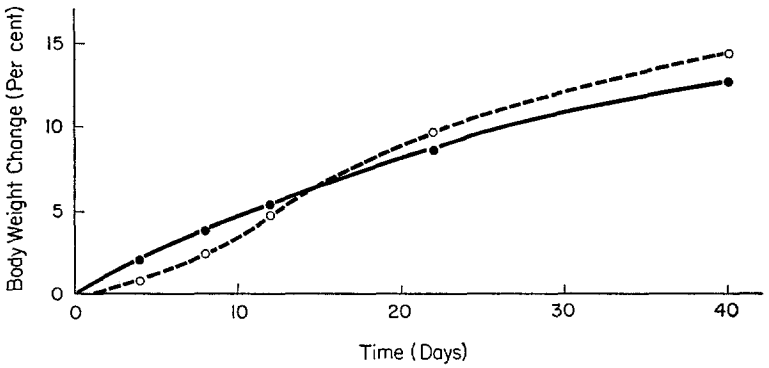


FIG. 2. Body weight changes in 2-month-old A strain mice injected with 850 million (A × C57BL/1)$F_1$ hybrid spleen cells given intravenously in 4 injections within a 48 hour period, the last injection given on day 0. – – – ○ – – –, A strain mice injected with 850 million (A × C57BL/1)$F_1$ hybrid spleen cells; and —— • ——, non-injected A strain mice.

this experiment. C3H mice were made tolerant of A either by injecting them at birth with viable A strain lymphoid cells or by giving them repeated injections of non-viable disrupted cell material from A strain donors. These C3H mice had been tolerant of A strain skin grafts for at least 6 weeks when they were used for this experiment. All tolerant mice were given 400 r of x-irradiation prior to injection of C3H spleen cells in order to facilitate an immune attack by the injected C3H cells against the A strain lymphoid tissue of the recipients. Irradiation of recipient mice has been shown to facilitate the graft *versus* host reactivity of C3H cells against A tissues (7), and it facilitates the production of runt disease in other strain combinations as well (8–10). A group of C3H mice tolerant by the injection of viable A strain cells at birth received 400 r of x-irradiation followed immediately by an intravenous injection of 150 million spleen cells from normal adult C3H donors. These mice showed significant weight loss after irradiation and injection (Fig. 3). On the 5th day after irradiation and injection they exhibited a mean body weight loss of 10 per cent of their prein-

jection body weight. However, they showed only slight evidence of clinical disease, and regained their initial body weight by the 20th postinjection day. The A strain grafts these mice were bearing at the time of injection were all rejected by the 28th postinjection day. Control groups of C3H mice tolerant by the injection of viable A strain cells at birth received either 400 r only, or

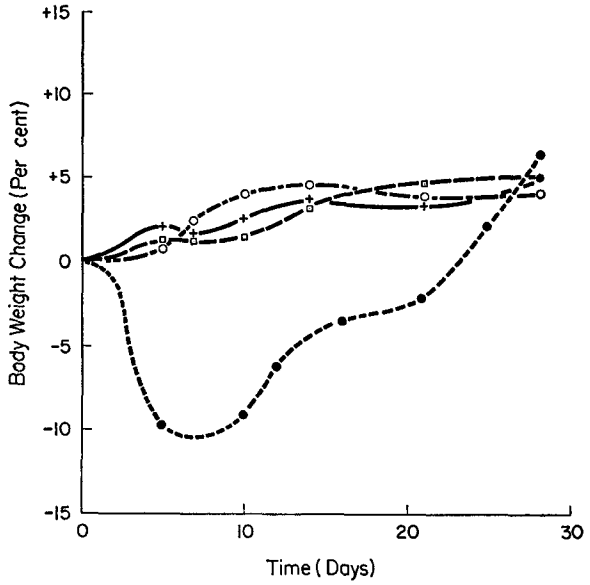


FIG. 3. Body weight changes in C3H mice tolerant of A strain skin grafts after irradiation and injection of spleen cells. – – – • – – –, C3H tolerant to A (tolerance resulting from injection of viable A strain lymphoid cells at birth) receiving 400 r plus 150 million spleen cells from C3H donors; —— + ——, C3H tolerant to A (tolerance resulting from repeated injections of non-viable A strain cellular material) receiving 400 r plus 150 million spleen cells from C3H donors; – — ○ – —, C3H tolerant to A (tolerance resulting from injection of viable A strain lymphoid cells at birth) receiving 400 r only; and — — □ — —, C3H tolerant to A (tolerance resulting from injection of viable A strain lymphoid cells at birth) receiving 400 r plus 150 million spleen cells from (C3H × A)$F_1$ hybrid donors. All spleen cell injections were given within 1 hour after x-irradiation intraperitoneally on day 0.

400 r plus an intravenous injection of 150 million (A × C3H)$F_1$ hybrid spleen cells. Mice in these two groups showed no periods of weight loss, no other clinical signs, and no evidence of rejection of their A skin grafts (Table II).

A group of 6 C3H mice which had been injected with disrupted spleen material to induce tolerance to A received 400 r of x-irradiation and an intravenous injection of 150 million C3H spleen cells. These mice, which presumably were not lymphoid chimeras, showed no weight loss or clinical disease, and rejected their A strain skin grafts within 28 days after irradiation and injection. It

appears, then, that mice which do not harbor viable allogeneic lymphoid cells escape this form of runting.

TABLE II

*Effect of Irradiation and Injection of Spleen Cells on C3H Tolerant to A Mice**

| No. of mice in group | Donor of injected spleen cells | Method used to induce tolerance in the C3H tolerant to A recipients | No. of tolerant mice exhibiting weight loss / Mice in group |
|---|---|---|---|
| 6 | C3H | Viable A strain spleen cells injected at birth | 5/6 |
| 6 | C3H | Non-viable cell material from A strain spleens‡ | 0/6 |
| 9 | None | Viable A strain spleen cells injected at birth | 0/9 |
| 9 | (C3H × A)$F_1$ | Viable A strain spleen cells injected at birth | 0/9 |

* All mice in all groups received 400 r of x-irradiation. Spleen cells were injected intravenously in a dose of 150 million within 1 hour after irradiation. No deaths occurred in any experimental group.

‡ Non-viable cellular material was given repeatedly, twice a week, intraperitoneally, for the first 4 weeks of life.

## DISCUSSION

These experiments demonstrate that runt disease can be produced by the injection of lymphoid cells into syngeneic recipient mice which are lymphoid cell chimeras. We have shown that this form of runting is the result of an immunologic attack by the injected lymphoid cells against the allogeneic lymphoid cells in the recipient, since if the donor of the injected lymphoid cells has been previously immunized against the allogeneic cell population of the recipient, the onset of the runting process is much more rapid and the end result more frequently fatal. It should be emphasized that when lymphoid cells are injected into syngeneic tolerant recipients the *only* tissues of the recipient which can be attacked by the injected cells are the descendants of the allogeneic lymphoid cells used originally to induce the tolerance. In other forms of runt disease, the injected cells which induce runting are capable of attacking any or all of the tissues of the recipient, since the recipient is an animal of a different strain from the donor. Spleen cells were used in the present experiments for the induction of tolerance, so it is reasonable to assume that only cells of the lymphoid and hematopoietic series have colonized the recipients. It is concluded that the immunologic rejection of lymphoid and hematopoietic tissue is sufficient to

cause the runting syndrome. This conclusion is consistent with the finding that the most characteristic and striking pathologic changes during runting are found in the lymphoid tissues (11). Furthermore, our observation that tolerant animals, if they are *not* lymphoid chimeras, escape runting after the injection of syngeneic lymphoid cells, also implicates the destruction of lymphoid and hematopoietic tissues as a critical factor in the pathogenesis of runt disease.

Failure of body growth, which is the hallmark of the runting syndrome, also occurs in other experimental and clinical situations in which depletion of lymphoid tissue is a prominent finding. Mice subjected to neonatal thymectomy (12, 13) or given lethal irradiation and small amounts of syngeneic bone marrow (14) often develop growth failure and fatal wasting associated with lymphoid depletion. Similarly, ataxia telangiectasia (15) and agammaglobulinemia of the Swiss type (16, 17) in man both involve deficient body growth and hypoplastic lymphoid tissues. It is therefore tempting to speculate that these diseases and classical runt disease have a common pathogenesis related to hypoplastic lymphoid tissue. Recent experiments, however, have shown that neonatally thymectomized mice reared in a germfree environment do not develop growth failure or wasting whereas mice which are inoculated with allogeneic lymphoid cells in a germfree environment develop fatal runting (18, 19). It therefore appears that sepsis may be more important in the pathogenesis of postthymectomy wasting than in classical runt disease.

We have also shown that there is a special relationship in the tolerant animal of the allogeneic lymphoid cells to their hosts. The rejection of a massive cellular allograft of (A × C57BL/1)$F_1$ hybrid spleen cells by normal A strain mice produces no evidence of runting. Why then should the rejection of (A × C57BL/1)$F_1$ hybrid cells in a tolerant A strain mouse result in runting? Our working hypothesis is that immunologic damage to lymphoid tissue which has become *established* in a chimera results in severe runting, whereas the rejection of *non-established* lymphoid tissue is not a life-threatening process. Immunologic damage to established lymphoid tissue could cause the runting syndrome either by predisposing to infection or causing the liberation of toxic substance(s) directly within the lymphoid tissues. Although Howard and Woodruff (20) and Blaese *et al.* (21) have shown that mice during runting have a severe immunologic deficit, the finding mentioned earlier that runting will occur in a germfree environment suggests that infection is not the cause of death in runt disease. On the other hand, it has recently been shown by Ramseier and Streilein (22) that lymphoid cells from unsensitized genetically dissimilar mice when mixed and inoculated into the skin of irradiated hamsters provoke cutaneous hypersensitivity reactions. Such hypersensitivity reactions, if they occurred in the lymphoid tissues of runting animals, could conceivably cause the liberation of toxic substances which in turn might be sufficiently destructive to result in the growth failure characteristic of runting.

SUMMARY

When chimeric A strain mice tolerant of (A × C57BL/1)$F_1$ hybrid skin grafts are injected with spleen cells from normal A donors the recipients develop weight loss, clinical evidence of runting, and death in some animals. Similar recipients injected with spleen cells from A strain donors immunized against C57BL/1 tissue show a more rapid onset of the runting process and increased mortality. Runting in these experiments therefore results from an immune attack by the injected A strain lymphoid cells against the (A × C57BL/1)$F_1$ hybrid tissue harbored by the chimeric recipients. Since the hybrid tissues of the chimeric recipients were derived from spleen cell populations we conclude that the immunologic rejection of lymphoid and hematopoietic tissue is sufficient to cause the runting syndrome.

C3H mice tolerant of A strain skin grafts because of the prior injection of viable or disrupted A strain spleen material were given 400 r of x-irradiation and an injection of C3H spleen cells. Only the chimeric C3H mice harboring viable A strain cells developed weight loss and clinical evidence of disease, showing again that runting occurs only when an attack can be made against viable lymphoid and hematopoietic tissue.

Normal A strain mice injected intravenously with 850 million (A × C57BL/1)$F_1$ hybrid spleen cells reject hybrid skin grafts and do not develop runting, whereas the rejection of similar hybrid tissue present in chimeric A strain mice results in runting. It is concluded that runting will occur only when the immunologic attack is directed against lymphoid and hematopoietic tissue which has become established within host tissues. The possibility that runting may result from hypersensitivity reactions occurring in the lymphoid tissues is discussed.

BIBLIOGRAPHY

1. Billingham, R. E., Studies on the reaction of injected homologous lymphoid tissue cells against the host, *Ann. New York Acad. Sc.*, 1958, **73**, 782.
2. Martinez, C., Smith, J. M., and Good, R. A., Runt disease in adult tolerant mice induced by intravenous injection of immunologically competent cells, *Proc. Soc. Exp. Biol. and Med.*, 1961, **106**, 572.
3. Trentin, J. J., Induced tolerance and "homologous disease" in X-irradiated mice protected with homologous bone marrow, *Proc. Soc. Exp. Biol. and Med.*, 1957, **96**, 139.
4. Fiscus, W. G., Morris, B. T., Jr., Session, J., and Trentin, J. J., Specificity, host-age effect, and pathology of homologous disease induced in unirradiated $F_1$ hybrid mice by transplantation of parental lymphoid tissue, *Ann. New York Acad. Sc.*, 1962, **99**, 355.
5. Martinez, C., Smith, J. M., Aust, J. B., and Good, R. A., Acquired tolerance to skin homografts in mice of different strains, *Proc. Soc. Exp. Biol. and Med.*, 1958, **97**, 736.

6. Martinez, C., Smith, J. M., Blaese, M., and Good, R. A., Production of immunological tolerance in mice after repeated injections of disrupted spleen cells, *J. Exp. Med.*, 1963, **118,** 743.
7. Martinez, C., Smith, J. M., and Good, R. A., Mutual tolerance of donor and host lympho-reticular cells in lasting tolerance, *Transplant. Bull.*, 1962, **30,** 513.
8. Schwartz, E. E., Upton, A. C., and Congdon, C. C., A fatal reaction caused by implantation of adult parental spleen tissue in irradiated $F_1$ mice, *Proc. Soc. Exp. Biol. and Med.*, 1957, **96,** 797.
9. Cole, L. J., and Ellis, M. E., Delayed deaths in sublethally X-rayed $F_1$ hybrid mice injected with parental strain spleen cells, *Science*, 1958, **128,** 32.
10. Kaplan, H. S., and Rosston, B. H., Studies on a wasting disease induced in $F_1$ hybrid mice injected with parental strain lymphoid cells, *Stanford Med. Bull.*, 1959, **17,** 77.
11. Billingham, R. E., Defendi, V., Silvers, W. K., and Steinmuller, D., Quantitative studies on the induction of tolerance of skin homografts and on runt disease in neonatal rats, *J. Nat. Cancer Inst.*, 1962, **28,** 365.
12. Good, R. A., Dalmasso, A. P., Martinez, C., Archer, O. K., Pierce, J. C., and Papermaster, B. W., The role of the thymus in development of immunologic capacity in rabbits and mice, *J. Exp. Med.*, 1962, **116,** 773.
13. Miller, J. F. A. P., Effect of neonatal thymectomy on the immunological responsiveness of the mouse, *Proc. Roy. Soc., London, (Series B)*, 1962, **156,** 415.
14. Barnes, D. W. H., Loutit, J. F., and Micklem, H. S., "Secondary disease" of radiation chimeras: a syndrome due to lymphoid aplasia, *Ann. New York Acad. Sc.*, 1962, **99,** 374.
15. Peterson, R. D. A., Kelly, W. D., and Good, R. A., Ataxia-telangiectasia. Its association with a defective thymus, immunological deficiency disease and malignancy, *Lancet*, 1964, **1,** 1189.
16. Tobler, R., and Cottier, H., Familiäre Lymphopenie mit Agammaglobulinämie und schwerer Moniliasis, *Helv. Paediat. Acta*, 1958, **13,** 313.
17. Good, R. A., Martinez, C., and Gabrielsen, A. E., Clinical considerations of the thymus in immunobiology, *in* The Thymus in Immunobiology (R. A. Good and A. E. Gabrielsen, editors), New York, Hoeber-Harper, 1964, 3.
18. McIntire, K. R., Sell, S., and Miller, J. F. A. P., Pathogenesis of the post-neonatal thymectomy wasting syndrome, *Nature*, 1964, **204,** 151.
19. Wilson, R., Sjodin, K., and Bealmear, M., The absence of wasting in thymectomized germ-free (axenic) mice, *Proc. Soc. Exp. Biol. and Med.*, 1964, **117,** 237.
20. Howard, J. G., and Woodruff, M. F. A., Effect of graft *versus* host reaction on the immunological responsiveness of the mouse, *Proc. Roy. Soc., London, (Series B)*, 1961, **154,** 532.
21. Blaese, R. M., Martinez, C., and Good, R. A., Immunologic incompetence of immunologically runted animals, *J. Exp. Med.*, 1964, **119,** 211.
22. Ramseier, H., and Streilein, J. W., Homograft sensitivity reactions in irradiated hamsters, *Lancet*, 1965, **1,** 622.