erative even after their transmission to the next generation. Or the treated plants may use only a portion of the hormone and sucrose for fruit formation and store the rest in their seeds. If this were so, these seeds might germinate with an initial extra reserve of hormone and sucrose and, therefore, would not require any further chemical treatment for fruit formation. Which of these explanations is more acceptable will depend upon the performance of the progeny in subsequent generations. They may retain the ability to produce fruits generation after generation, or they may show progressive reduction in their fruiting ability in proportion to the utilization of hormone and sucrose initially supplied to the parental plants for fruit formation.

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29 January 1969

Graft versus Host Inhibition: Fetal Liver and Thymus Cells to Minimize Secondary Disease

Abstract. *Long-lived radiation chimeras were produced in mice differing at the major histocompatibility locus. Survival occurred in lethally irradiated recipients inoculated with allogeneic fetal liver and allogeneic fetal thymus cells in combination. The survival rate was equal or superior to that of mice with transplanted syngeneic fetal, neonatal, or adult hematopoietic cells.*

Clinical application of transplantation of allogeneic bone marrow has been deterred, chiefly because of complications after transplant from infection due to immune inadequacy (*1*) and from graft-versus-host (GVH) disease (*2*). The two problems are inversely related in that attempts to improve the immune status worsen the GVH reaction and efforts to minimize GVH disease tend to increase immune inadequacy.

Shortly after GVH disease was established as a cause of delayed deaths in radiation chimeras transplanted with allogeneic cells, Uphoff reported (*3*) inhibition of secondary disease in the mouse by the use of transplanted fetal liver cells. We have shown that suspensions of fetal liver cells are immunologically incompetent against transplantation antigens (*4*). This finding, and the results reported here, suggest that the delayed deaths which occur in lethally irradiated mice subsequently treated with allogeneic fetal liver cells are probably not due to a graft-versus-host reaction. Others have made the same suggestion based on different data (*5*).

In the case of mice, radiation chimeras often have impaired immune capability (*6*). The immune inadequacy of radiation chimeras with transplanted fetal liver cells may be exaggerated when compared with that of mice with transplanted adult hematopoietic cells. The fetal lymphoid cells, or lymphoid precursors, may not yet have come under the influence of the donor's thymus when the cells were removed for transplantation. In addition, the thymus glands of the host mice are injured by the x-irradiation. Thus, in the radiation chimeras with transplanted fetal liver cells, the precursors of antigen-reactive cells (*7*) may not have received adequate thymic "immunological instruction" before transplantation and may not benefit by thymic influence in the host mice after transplantation.

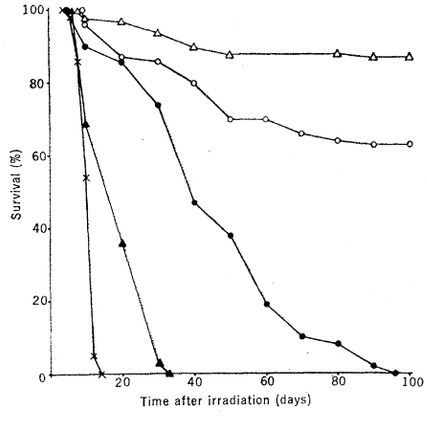

Fig. 1. Survival of CBA/J mice that received 950 r of total body x-irradiation plus 2×10^7 syngeneic or allogeneic (A/J) adult spleen or bone marrow cells. △, Syngeneic adult spleen (52); ○, syngeneic adult bone marrow (51); ▲, allogeneic adult spleen (59); ●, allogeneic adult bone marrow (50); X, radiation controls (150). Numbers in parentheses indicate number of mice in each group.

We used donor and recipient mouse strains which differed at the H-2 locus, the major histocompatibility locus in mice. Host mice were lethally irradiated. Inoculation of these mice with allogeneic fetal liver cells and with fetal thymus cells, in combination, resulted in long-lived chimeras.

Inbred CBA/J (*H-2*^{k}) and A/J (*H-2*^{a}) mice were used. Pregnancies were timed at the Jackson Laboratory, Bar Harbor, Maine. The hosts were CBA mice, 3 to 4 months of age. Liver hematopoietic cells were obtained from fetuses at day 13 and day 18 and from 5-day-old mice. Thymus cells were obtained from fetuses at day 18 and from 5-day-old mice. Donors of adult spleen and bone marrow were 3 to 4 months of age. Cells were collected, processed, counted, and tested for viability (*8*). X-irradiation conditions were: 280 kv at 20 ma; rate in air was approximately 55 r/min; air distance was 82 cm; and 0.7 mm Cu half-value layer. The mice were placed in a perforated, partitioned Plexiglas container on a rotating platform. A single total-body exposure (TBR) of 950 r was given. Then 10^7 or 2×10^7 viable cells were administered intravenously within 4 hours after exposure to radiation. When both liver and thymus cells were given, the suspensions were prepared from different mice, the injections were made separately, and 10^7 cells of each type were administered. Two untreated mice from each irradiated group served as controls. To test chimerism, A-strain skin grafts were used. Chi-square analysis was used to test differences between survival rates.

The results of survival of radiation chimeras that received syngeneic and allogeneic adult spleen and bone marrow are shown in Fig. 1. Whereas 87 percent of mice inoculated with syngeneic adult spleen cells survived 100 days, none of the mice that received allogeneic adult spleen cells survived beyond day 33. Mice that received allogeneic bone marrow cells survived longer than those given allogeneic adult spleen cells; however, all were dead within 96 days. Over the 100-day test period, syngeneic spleen cells provided better survival than syngeneic bone marrow cells ($P < .001$).

Survival curves for mice that received syngeneic or allogeneic fetal liver cells

are presented in Fig. 2. Survival curves for 60 CBA mice that received syngeneic or allogeneic liver cells from 5-day-old mice are not included in the figure. Their survival curves do not differ significantly from mice that received syngeneic or allogeneic liver cells from fetuses at day 18. The survival curves for recipients of syngeneic and allogeneic liver cells from 13-day fetuses are parallel between day 10 and day 100. Fetal liver cells obtained at day 18 provided higher survival rates than 13-day cells in syngeneic ($P < .001$) recipients, but there was no effect in allogeneic ($P > .15$) recipients. Both 13-day and 18-day allogeneic fetal liver cells provided better long-term survival than either allogeneic adult spleen cells or bone marrow (Fig. 1). In addition, 27 CBA mice were given 10^7 syngeneic fetal or neonatal thymus cells alone. The thymus cells conferred no protection, and the mice died at the same rate as the radiation controls.

Survival of mice treated with liver cells in combination with thymus cells are shown in Fig. 3. Of the mice treated with syngeneic 18-day fetal liver and thymus cells, 86 percent survived 100 days. Recipients of syngeneic liver and thymus cells from 5-day-old mice had a better survival rate than any other group; 97 percent were alive at 100 days. However, this was not statistically different from syngeneic fetal liver and thymus cells (86 percent), syngeneic 18-day fetal liver cells alone (90 percent, Fig. 2), or syngeneic adult spleen cells (87 percent, Fig. 1).

The most significant finding was that allogeneic fetal liver cells in combination with allogeneic fetal thymus cells conferred excellent protection and long-term survival of 92 percent (Fig. 3). The survival rate of these radiation chimeras transplanted with allogeneic fetal liver and fetal thymus cells was similar to that of mice inoculated with syngeneic liver and thymus cells from 5-day-old mice. The survival rate was higher than that of recipients of syngeneic adult bone marrow ($P < .005$). Liver cells from 18-day fetuses was the next best allogeneic cell type tested with 57 percent survival at 100 days. The combination of fetal liver and thymus cells afforded significantly higher survival ($P < .002$). Of the mice treated with allogeneic liver and thymus cells from 5-day old donors, only 13 percent lived 100 days.

Thirteen long-lived CBA mice which had been treated with A-strain fetal liver cells or fetal liver and fetal thymus cells were tested for chimerism with A-strain skin grafts. None of the skin grafts were rejected in the 90- to 100-day period of observation. This is strong evidence that these mice were true chimeras.

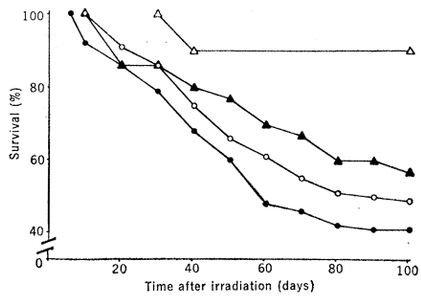

Fig. 2. Survival of CBA/J mice that received 950 r of total body x-irradiation plus 2×10^7 syngeneic or allogeneic (A/J) liver cells from 13-day fetuses or 10^7 syngeneic or allogeneic (A/J) liver cells from 18-day fetuses. △, Syngeneic 18-day fetal liver (30); ○, syngeneic 13-day fetal liver (142); ▲, allogeneic 18-day fetal liver (30); ●, allogeneic 13-day fetal liver (160).

The data on survival of mice treated with syngeneic and allogeneic adult hematopoietic cells (Fig. 1) supported the concept of an inverse relation between GVH disease and immune inadequacy. The spleen is predominantly a lymphoid organ. Fewer than 25 percent of bone marrow cells in the mouse are lymphoid (*9*), and most of these are immunologically incompetent (*10*). Suspensions of adult spleen cells, in these experiments, gave good long-term protection in syngeneic recipients but caused acute GVH disease in allogeneic recipients. Bone marrow, with fewer immunologically competent cells, did not protect syngeneic recipients as well, but it provided better long-term protection to allogeneic hosts.

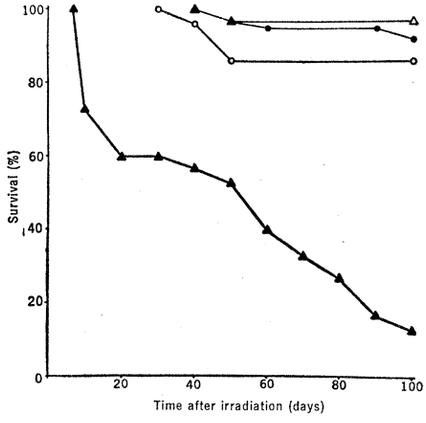

Fig. 3. Survival of CBA/J mice that received 950 r of total body x-irradiation plus 10^7 fetal or neonatal liver cells and 10^7 fetal or neonatal thymus cells from syngeneic or allogeneic (A/J) donors. △, Syngeneic liver and thymus cells from 5-day-old mice (31); ○, syngeneic liver and thymus cells from 18-day fetuses (28); ▲, allogeneic liver and thymus cells from 5-day-old mice (30); ●, allogeneic liver and thymus cells from 18-day fetuses (37).

The similarity of the survival of syngeneic and allogeneic radiation chimeras with transplanted liver cells from 13-day fetuses (Fig. 2) is further evidence that fetal liver is immunologically incompetent against transplantation antigens (*4*). Syngeneic cells do not cause death that is a result of a GVH reaction. With a similar survival curve, it is, therefore, most likely that the cause of death in the allogeneic chimeras was due to immune inadequacy rather than to GVH disease. The higher survival rate of mice treated with syngeneic 18-day fetal liver cells (Fig. 2), compared to 13-day cells, is what might be anticipated. Present understanding of the ontogenesis of the mammalian immune apparatus (*11*) suggests that cells from 18-day fetal liver would have a greater degree of immune capability than those from 13-day fetal liver.

Survival of radiation chimeras protected with 13- or 18-day fetal liver cells (Fig. 2) is greater if the cells are syngeneic rather than allogeneic. These differences may reflect a more favorable environment for cell growth in the syngeneic recipients; stem-cell spleen colony formation is known to be repressed in allogeneic recipients (*12*).

The 92 percent survival at 100 days of radiation chimeras transplanted with allogeneic fetal liver and thymus cells was substantially superior, compared to the survival of mice treated with any of the other allogeneic cells. This is a demonstration of the additional protection furnished by the fetal cell combination, particularly when compared with the 13 percent survival at 100 days of chimeras that received allogeneic liver and thymus cells from 5-day-old donors. The experimental conditions were such that the only difference between these two groups was the 6-day difference in the age of the cell donors. Using a similar experimental model, Congdon, Goodman, and Ferrebee (*13*) showed that allogeneic fetal thymus in combination with allogeneic adult bone marrow produced secondary disease. Our results suggest that both the thymus cell and the hematopoietic stem-cell source must be

fetal in order to prevent secondary disease.

Over the long period, the 92 percent survival of radiation chimeras inoculated with allogeneic fetal liver and thymus cells is presumptive evidence that these mice have an adequate immune system. In addition, GVH disease was at the minimum in view of the relative absence of secondary disease and the healthy and vigorous appearance of the mice. Our results suggest that a state of immunological tolerance exists in these allogeneic radiation chimeras, despite the histocompatibility differences between the strains of mice tested. We believe that the postulated inverse relation between immune inadequacy and GVH disease has been abrogated by the use of fetal liver cells and fetal thymus cells in combination.

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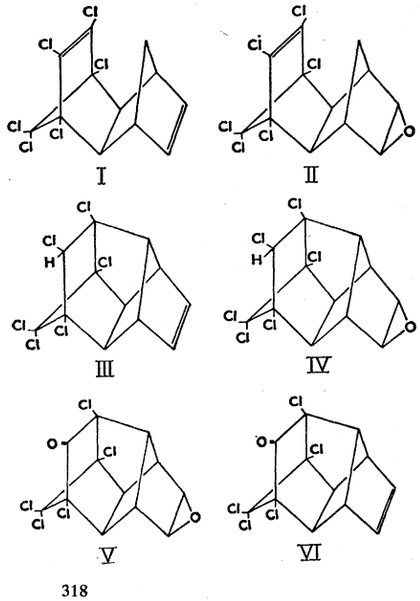
Insect Metabolism of Photoaldrin and Photodieldrin

Abstract. *Photoaldrin and photodieldrin, sunlight products of aldrin and dieldrin, are rapidly metabolized to a more toxic material by flies and mosquito larvae. It is suggested that this conversion is the cause for the enhanced toxicities of the photoproducts.*

Solar irradiation of the cyclodiene insecticides aldrin (I) and dieldrin (II), either as dry films (*1*, *2*) or in aqueous solution (*3*), yields the photoisomers photoaldrin (III) and photodieldrin (IV). The latter is two to ten times more toxic than dieldrin is to several vertebrates (*2*, *4*), while both photoisomers are more toxic to insects (*1*, *2*). Photoaldrin is as much as 11 times more toxic to mosquito larvae than aldrin (*5*). In order to determine the possible reason for their increased toxicity, we have investigated the metabolism of compounds III and IV in insects.



Each compound (0.285 mg/ml) was applied topically in acetone (1 μl) to 50 female multiresistant *Musca domestica* L. (4 days old) (*6*). After 2 hours, the flies were immobilized with carbon dioxide and rinsed with acetone. The flies were then homogenized in 10 ml of water and the homogenate was extracted with 50 ml of a mixture of hexane and isopropanol (3:2); it was then extracted with 30 ml of hexane. The extract was dried over anhydrous sodium sulfate and analyzed on a gas chromatograph (MicroTek MT-220), equipped with a Ni^{63} electron-capture detector and a glass column (122 by 0.159 cm) containing 0.15 percent DC-710 on Corning-0201 glass beads DMCS, 60 to 80 mesh. The chromatograph was operated at column, detector, and injection port temperatures of 135°, 230°, and 215°C, respectively, and at a flow rate of 60 ml/min. A peak with a retention time equal to that of Klein's metabolite (V) [a metabolite of dieldrin in male mice (*7*)] was obtained from flies treated with photoaldrin or photodieldrin. In addition to compound V, flies treated with photoaldrin also contained photodieldrin. Tissue extracts of flies to which aldrin or dieldrin had been applied did not contain compound V. Identical retention times for our metabolic material and compound V were also observed on the very same instrument and column (DC-200) reported on recently (*7*). Although this is the only evidence available at present for the formation of compound V in insects, the finding is logical on the basis of the structures of the applied starting materials, compounds I and II.

The metabolite, after topical application to susceptible flies, caused 52 percent mortality within 4 hours, compared to 28 percent for equimolar amounts of photoaldrin and no mortality for dieldrin. The increased and more rapid toxicity of compound V has been observed earlier (*7*).

In another experiment, 500 mosquito larvae [*Aedes aegypti* (L.)] were placed in 500 ml of water containing 0.014 part per million of photoaldrin or photodieldrin for periods of 1, 2, and 4 hours; the mortality was then determined, and the larvae were prepared for chromatographic analysis. The results (Table 1) indicate that compound V is formed very rapidly. After photoaldrin treatment, only compound V was found in the larval tissue after 4 hours. At this time 90 percent of the larvae were dead. Treatment with photodieldrin resulted in 78 percent mortality after 4 hours, by which time 90 percent of the absorbed photodieldrin had been converted to

Table 1. Concentration (ng/larva) of materials and percent mortality (M) after varying periods of exposure to 0.014 part per million of photoaldrin (III) and photodieldrin (IV).

Exposure time (hr)	Compound (ng)		M (%)	Compound (ng)		M (%)
	III	V		IV	V	
1	1.6	0.2	5	0.99	0	0
2	1.5	2.6	22	1.2	2.3	10
4	0	4.5	90	0.5	4	78