ceptus was a teratoma. (ii) Female PR-2054 partially aborted a fetus at 5 months. An enlarged head caused a fetal dystocia which was manually relieved. The head was nearly twice normal size. There was hypogenesis of both ear auricles (anotia). The enlarged head was most likely due to internal hydrocephaly associated with a defective foramen magnum similar to the Arnold-Chiari malformation in man. The interparietal portions of the occipital bone were present, but the portion of the occipital bone forming the posterior half of the foramen magnum was absent. The neural arches of the first and second cervical vertebrae were also missing. The left and right anterior limbs were absent (amelia). The posterior extremities were macerated by the chewing of the mother and therefore could not be assessed. (iii) Female 374 had a grossly normal fetus that was taken by Caesarean section. This monkey was mated during each of two menstrual cycles. She was treated with thalidomide after the second mating but was not treated after the first mating. On the basis of weight, size, and ossification of the skeleton, the fetus was judged to be 4 months old. Therefore, conception must have occurred at the time of the first mating, when the female was not treated. (iv) Female 815 had a fetus taken by Caesarean section. It was grossly malformed and had a facial capillary hemangioma over the bridge of the nose and on the cheeks. The right anterior extremity consisted of a stub of soft tissue connected to a single digit. The finger contained a distal and intermediate bony phalanx. The left anterior limb was absent (amelia). Only a tiny nub of soft tissue was present to represent an extremity. The left posterior extremity had a stub of soft tissue intervening between the trunk and a foot. Small plaques of bone, thought to be a femur, tibia, and fibula, were in the stub of tissue representing the thigh and leg. A tarsal region was apparent but it was not possible to ascertain whether the cartilage primordia of the tarsal (ankle) bones were present. Five metatarsal bones were observed. The second and third were half the length of the others. This resulted in a retraction of their associated (second and third) digits, the toes appeared to be shortened in relation to the others. Each of the five digits had three bony phalanges. The first digit was not the hallux (big toe) because it was not in a position of apposition to the other digits and contained three bony phalanges instead of the usual two. The right posterior extremity was similar to the left except that only the left second metatarsal bone was shorter than the others. This resulted in a retraction of the associated digit causing the second toe to appear shortened in relation to the others. The only grossly abnormal finding of the viscera was dilated ureters.

The malformations described in these two monkeys were anatomically identical to the deformities reported in children (*2*) whose mothers had taken thalidomide during pregnancy. The anomalies in the monkeys reported here are of such distinctive character that it would be highly unlikely that they could have occurred, even in these limited cases, merely by chance. The birthrate of anomalous offspring among monkeys (*Macaca rhesus*), according to Lapin and Yakovleva (*9*), constitutes 0.48 percent. They reported that defective cardiac development is the most common congenital malformation in the monkey. Their findings would further strengthen our position that thalidomide produced the skeletal defects in our monkeys. The skeletal system in both man and the monkey was the primary target for malformations induced by thalidomide.

Note added in proof: Two additional, typically malformed fetuses were recovered from the thalidomide-treated monkeys (2062 and 2063). After 3 months of pregnancy, female 1162 aborted a teratoma. The drug treatment period for monkeys 2063 and 1162 was from day 34 to 40.

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Transplantation Tolerance Induced in Adult Mice by Protein Overloading of Donors

Abstract. *Treatment of donor animals with unrelated antigens can regularly inhibit reactivity of spleen cells against the host, with subsequent induction of specific immune tolerance in the recipient animals if the barrier to histocompatibility is weak. Mice made tolerant in this way accept skin grafts from donor strain animals. In the case of strong differences (in H_2 locus) or heterologous (rat-mouse) combinations, the inhibition of the reactivity of the transferred lymphoid cells against the host is partial, and the skin-graft survival time is significantly prolonged.*

As a result of the treatment with protein antigen, adult animals become immunologically unresponsive to the antigen. Moreover, the treated animals neither produce antibodies nor develop delayed type hypersensitivity to a different antigen if the latter is given a few days after the beginning of the treatment with the first antigen (*1*). In such treated animals, rejection of the homografts is significantly delayed (*2*), and when spleen cell donors are similarly treated, the rate of mortality due to the reaction of these cells against the recipients is reduced by 50 percent (*3*). These observations were recently confirmed by Miller, Martinez, and Good (*4*) who showed that administration of five strong bacterial antigens to the donors of spleen cells reduces the reactivity of these cells against the host.

The finding that the severity of the reaction of the grafted cells against the host (graft-host reaction) could be reduced is particularly meaningful because of the possibility of inducing permanent tolerance in adult animals to foreign tissues. If an irradiated animal transfused with foreign lymphoid cells does not succumb to the graft-host reaction, it may then become tolerant to the tissues of the donor animals, if an appropriate amount of cells has been injected (*5*).

To test this possibility, a three-step procedure was followed: (i) treatment of the donors with large doses of an antigen; (ii) transfer of the spleen or bone marrow cells of the treated animals into irradiated recipients; (iii) graft of the recipients with skin of the donor strains. Three combinations were used: Balb/c spleen cells injected into DBA/2 mice (weak histocompatibility barrier), C57Bl spleen cells injected into C3H

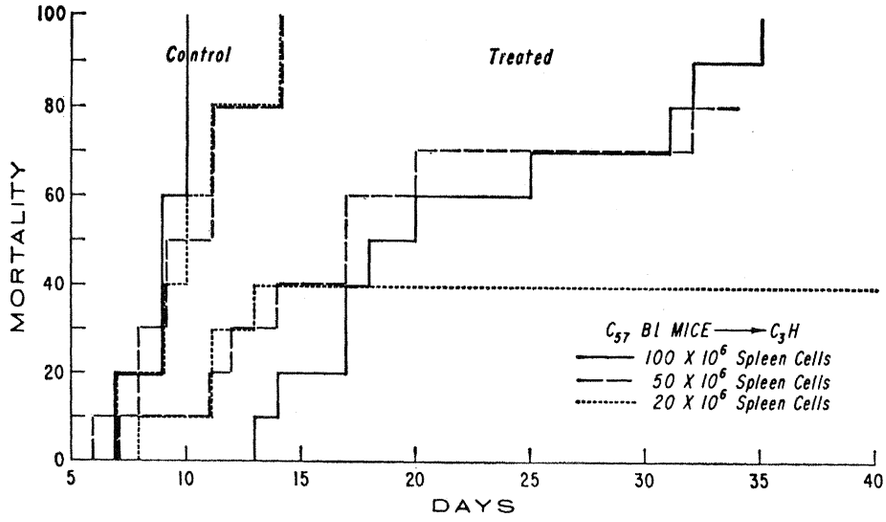

Fig. 1. Mortality rate of irradiated (500 r) recipient C_3H mice given various amounts of spleen cells from control and Hcy-treated C_{57} B1 mice.

mice (strong H_2 locus histocompatibility), and spleen or bone marrow cells of inbred Fisher rats injected into Balb/c mice. In all cases the animals were adults 2 to 4 months old. The donor mice were treated either with rabbit γ-globulin (RGG), the dose being 100 mg per day, intravenously (i.v.) and intraperitoneally (i.p.) for 10 days, or with *Limulus polyphemus* hemocyanin (Hcy), the dose being 100 mg per day i.v. and i.p. for 7 days. The rats were treated with Hcy (240 mg per day i.v. for 10 days). The recipient mice were irradiated at 500 r (200 kv for 4.1 minutes) 24 hours before the cell transfer. The spleens of the donors were removed, sliced, and gently squeezed through a square of nylon stocking (*6*), suspended in Hanks' solution, and centrifuged for 10 minutes at 1400 rev/min. Tests with trypan blue (0.5 percent) regularly showed that approximatively 90 percent of the cells were viable. Varying amounts of these cells were slowly injected intravenously. The bone marrow cells were obtained by aspiration of the femur and were prepared in the same manner as the spleen cells. Grafts of free skin were applied to the survivors of the graft-host reaction, 25 to 35 days after the cell transfer, since complete tolerance in adult animals is established slowly (*7*).

The results of the experiment where there was a weak histocompatibility barrier (Balb/c and DBA/2 mice share the same H_2d locus) are shown in Table 1. Whereas the DBA/2 mice which received spleen cells from control Balb/c donors presented some external signs of a graft-host reaction (loss of weight, diarrhea) and four out of 25 died, the mice which received similar or even larger amounts of spleen cells from RGG-treated donors apparently did not have a graft-host reaction, and none died. Tolerance was almost regularly induced in the group of treated animals: only in the group of mice injected with the smaller number of cells (100×10^6) did one of the nine reject the graft; one accepted the graft but kept it in a deteriorating condition. In the control group only four of the 21 survivors became fully tolerant. The others either rejected the graft or kept the graft in a deteriorating condition (as evidenced by shrinking and sparse hair regrowth). In the combination (Balb/c ⟶ DBA/2 mice) the induction of tolerance in irradiated adult animals has been observed in a relatively high proportion of the surviving recipients (*5*, *8*). It is nevertheless significant that in the experiments reported here the graft-host reaction was completely inhibited, and a long-lasting and perhaps permanent tolerance was regularly achieved among the recipients given cells from treated donors. This tolerance is specific: two of the control and three of the treated tolerant mice were grafted with the skin of C3H mice and all of them rejected this graft. On these mice the Balb/c skin graft remained in a healthy state.

The results of the experiment in which there was a strong difference in histocompatibility at the H_2 locus are shown in Fig. 1. All the irradiated C3H recipients injected with spleen cells from control C57B1 donors died in 14 days with external signs of severe graft-host reaction (loss of weight, diarrhea, emaciation). In another group of controls which received 10×10^6 spleen cells, only one of five mice died on the 9th day. The mortality rate in the group

Table 1. Results of spleen cell transfer from control and RGG-treated Balb/c mice to irradiated (500 r) DBA/2 mice.

No. cells ($\times 10^6$)	No. mice	Time of death (days)	No. survivors	Grafts		
				Rejected (days)	Deteriorating condition on day 120	Healthy to 120th day
			Control			
200 (1 spleen)	15	32, 36, 44	12	42, 58, 61, 65, 69	5	2
100	10	12	9	27, 35, 48	4	2
			RGG-treated			
500	5	0	5	0	0	5
250 (1 spleen)*	6	0	6	0	0	6
100	9	0	9	32	1	7

* In the treated animals the spleen was enlarged.

Table 2. Results of spleen and bone marrow cell transfer from control and Hcy-treated Fisher rats into irradiated (500 r) Balb/c mice.

No. cells ($\times 10^6$)	No. mice	Time of death (days)	No. survivors	Skin graft survival (days)
		Control spleen		
20	5	5, 6, 11, 11, 14	0	
50	5	8, 9, 13, 14	1	20
		Control bone marrow		
50	5	34, 36, 37, 37, 41	0	
		Hcy-treated spleen		
20	5	9, 11, 11, 14, 18	0	
50	5	15	4	17, 23, 23, 26
		Hcy-treated bone marrow		
50	5	9, 14, 62*	2	40, 52

* The skin graft was healthy at the time of death.

of C3H mice injected with the same number of spleen cells from Hcy-treated C57Bl mice was considerably delayed, and most of the group given 20×10^6 cells survived indefinitely. Two of the mice injected with 50×10^6 cells survived to the 34th day; one of them died from anesthesia on that day during the graft procedure, and the other died on the 60th day still bearing a healthy skin graft (26 days old). The six survivors of the group given 20×10^6 treated spleen cells and the four survivors of the control group given 10×10^6 cells were grafted with C57B1 skin. The control mice rejected the graft with a vigorous reaction of the second set type in 4 to 5 days. The six mice of the group of treated mice also rejected the graft, but this followed a delayed pattern (in 10, 14, 15, 16, 19, and 20 days). Thus, although no tolerance to the donor tissues has been induced in the recipients in this strong histocompatibility combination, the severity of the graft-host reaction has been considerably reduced, and some prolongation of the survival time of the skin grafts has been observed.

In Table 2 are shown the results of the third experiment, that on the combination of Fisher rats and Balb/c mice. The control mice injected with either spleen or bone marrow cells were with one exception dead between the 5th and the 41st day. The rat skin grafted on the survivor was sloughed and rejected in 20 days. Most of the mice injected with cells from Hcy-treated rats survived indefinitely. In the four survivors of the mice given 50×10^6 spleen cells the rat-skin grafts were sloughed and rejected between the 17th and the 26th day. Of the three grafted mice of the group given bone marrow cells, one died 30 days later with its graft still in a healthy state, while the two others rejected the rat-skin grafts on the 40th and 52nd day after some regrowth of hair on the graft. Thus, 93 percent of the control mice died after the transfer of rat cells, and the one survivor did not become tolerant. Among the mice transfused with cells from Hcy-treated rats only 60 percent died. Most of the survivors rejected their grafts, but three of them showed a significant prolongation of the period of graft survival.

In irradiated mice some survival (3 to 10 percent) has been observed from graft-host reaction up to the 100th day, if the mice had been treated with rat marrow or spleen cells (*9*). On the average, one half of these survivors did tolerate rat tissue grafts for a prolonged period of time (*10*). These results of prior work are rather similar to our findings for the group of control animals. The observation that 40 percent of the mice of the treated group survived the graft-host reaction indefinitely shows that the spleen and marrow cells of the Hcy-treated rats are significantly less reactive than the cells of normal animals.

The aforementioned results further support the idea that a permanent tolerance to foreign tissues could be induced in irradiated adult animals by transfusion of a rather limited number of lymphoid cells. The main obstacle to such a procedure is the activity of these cells against the host. Consequently, if by any means the lymphoid cells of the donor could be rendered less active against the recipient, a sufficiently large number of such cells could be safely injected in order to induce in adult irradiated animals a specific tolerance to the donor tissues. Treating the donors with an unrelated antigen may prove to be one means of attaining the aforementioned goal. Our results show that even in the stronger histocompatibility combinations, an important reduction of the severity of the graft-host reaction can be produced. In these experiments only two commonly available antigens (RGG and Hcy) were used. Other antigenic materials, of bacterial or viral origin for example, might be more effective in this regard. Another possibility is that when the graft-host reaction lasts for a long period, as in our experiments, other forms of medication may facilitate the recovery of recipient animals from the graft-host disease without being prejudicial to the establishment of the specific immune tolerance to the donor's tissues.

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Vitamin K Compounds in Bacteria That Are Obligate Anaerobes

Abstract. *A naphthoquinone-dependent strain of* Bacteroides melaninogenicus *has been used in a microbiological assay to survey bacteria for compounds of the vitamin K group. Organisms known to contain vitamin K, as well as several bacteria that are obligate anaerobes, produced substances which satisfied the naphthoquinone requirement of the assay organism. Vitamin K was chemically isolated from strains of* Bacteroides melaninogenicus, Bacteroides fragilis, *and* Veillonella alcalescens.

Compounds of the vitamin K group are widely distributed in plants, animals, and microorganisms. It has been suggested that these compounds function in phosphorylation accompanying electron transport during oxidative metabolism (*1*). This hypothesis is supported in part by what is known of the distribution of vitamin K compounds in various bacteria; in particular, the naphthoquinones are absent from cytochrome-free organisms and chemoorganotrophic anaerobes (*2*, *3*, *4*). The anaerobe, *Chromatium*, has been found to contain naphthoquinones of the vitamin K group, but this organism represents a special case, being a photosynthetic, cytochrome-containing sulfur bacterium (*5*). However, a naphthoquinone was recently isolated from the lactic acid bacterium, *Streptococcus faecalis* (*6*). In addition, certain strains of *Bacteroides melaninogenicus*, a strict anaerobe, require naphthoquinones for growth, which suggests that these compounds may be important in anaerobic chemoorganotrophic metabolism (*7*, *8*). We now report data indicating that compounds of the vitamin K group are present in a variety of obligately anaerobic chemoorganotrophic bacteria.