posed. The trance-like state also revealed a dominant delta frequency of 0.5-2 cycles per second. A "beta" wave with an amplitude of 5-10 μv was seen superimposed on the slow wave ("theta"). The alpha-like wave appeared to be essentially absent under conditions of hypothermia, hyperthermia, and supine (trance-like state) position. *Optic lobes.* Electroencephalographs from the optic lobes demonstrated a predominant slow wave of 6-8 cycles per second with an amplitude of 5-10 μv. This frequency and amplitude was markedly depressed under hypothermia. The electrical activity appeared to double in amplitude under hyperthermia. While the animal was in a trance-like state, a well-defined slow wave of 5-6 cycles per second was seen as the most significant change recorded from this area of the brain. *Cerebellum.* Electrical activity as recorded from the cerebellum was most pronounced under hypothermia and in the supine trance-like state; in both states a well-defined "theta" wave of 4-6 cycles per second with an amplitude of 10-15 μv was seen.

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Transfusion Experiments and Survival Studies in Parabiosis Intoxication.* (30554)

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When F_1 hybrid mice are placed in parabiotic union with mice of one of their parental strains, a lethal wasting syndrome, known as parabiosis intoxication frequently develops in the hybrid partner. This syndrome is characterized by anemia, weight loss, hunched posture, ruffled fur, and early death(1).

There has been some debate concerning the etiology of parabiosis intoxication. Some investigators maintain that a graft-*vs*-host reaction, elicited by passage of parental strain lymphoid cells into the hybrid partner is primarily responsible(2-4). Evidence for this is the clinical similarity of parabiosis intoxication and homologous disease, the fact that both conditions usually occur when the strains involved differ at H-2 histocompatibility locus, and the presence of lymphoid depletion in both(2,5). Hall and Hall expressed the opinion that the anemia, which is due primarily to shunting of blood to the parental strain partner(1), is a sufficient cause of the intoxication syndrome(6). Another line of evidence suggesting that intoxication is not etiologically related to homologous disease is the finding of Eichwald *et al* that intoxicated hybrid parabionts will recover if they are separated from their parental strain partners(7). All attempts to reverse runt disease or homologous disease have been unsuccessful so far when reversal was attempted more than 24 hours after the initiation of the process (8). Additional evidence that anemia is the principal cause of clinical intoxication and death was reported by Hilgard *et al*(1). When parental strain mice, preimmunized against one of their F_1 hybrid strains, were placed in parabiotic union with these hybrid mice, the anemia in the hybrid partners was more severe than in a control group in which the parental strain partners had not been preimmunized. Clinical intoxication also ap-

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peared earlier and mean survival time was much reduced.

It occurred to us that if anemia were the major or sole cause of parabiosis intoxication then syngeneic blood transfusions into the hybrid partner might be expected to ameliorate or prevent the syndrome. Furthermore, hybrid mice separated from parental strain partners after intoxication had developed should recover, particularly if transfused with syngeneic blood immediately after separation. In the experiments to be reported we found that transfusion of the hybrid mouse in parabiosis was followed by a rapid shunting of blood to the parental strain partner, with little change in the clinical picture. If parabiosis was discontinued 6 or 10 days after union, syngeneic blood transfusions into the hybrid partner immediately following separation were ineffective in preventing death, which occurred after progressive wasting. The evidence suggested that parabiosis intoxication, in the particular strain combination employed, involved the effects of both anemia and the graft-*vs*-host reaction. Also transfusion to normal hematocrit levels was of little value in prevention or treatment of the graft-*vs*-host reaction.

Materials and methods. Inbred mice of the A strain and F_1 hybrids resulting from the cross between A and C_{57} Bl/1 were employed in all experiments. Four experimental groups were prepared as follows: (1) A strain mice joined with $(AxC_{57}$ Bl/1$)F_1$ hybrids, (2) A strain mice joined with $(AxC_{57}$ Bl/1$)F_1$ hybrids followed by separation 10 days later, (3) A strain mice joined with $(AxC_{57}$ Bl/1$)$ F_1 hybrids followed by separation 6 days later, and (4) $(AxC_{57}$ Bl/1$)F_1$ hybrids injected with A strain lymphoid cells (homologous disease controls). Each group was divided into 2 subgroups. In a control subgroup, no transfusions were given. In a second subgroup, one to 3 syngeneic blood transfusions, each of 1.0 ml, were given to the hybrid mouse when anemia was present (Groups 1 and 4) or immediately after separation from parental strain partners (Groups 2 and 3). No more than 3 transfusions could be given because of the induration of the tail resulting from subcutaneous extravasation of blood during the intravenous injection. In all instances parabiotic partners were of the same sex, between 1½ and 2 months of age, and of approximately equal body weight. Parabiosis was of the celomic type, performed according to the technique reported by Hilgard *et al*(9). Parabiotic partners were separated under ether anesthesia. After separation and debridement, the muscle layers were closed with catgut and the skin margins were united with 11 mm wound clips. Homologous disease was produced in Group 4 by the intravenous infusion of 150 to 175 million A strain spleen cells into the $(AxC_{57}$ Bl/1$)F_1$ hybrids. Suspensions of cells were prepared by a method previously described(10). Hematocrit determinations were made by standard methods(11), using blood obtained from the tail veins; these were done serially every 3 or 4 days. The mean final hematocrit value refers to the mean of the last routine hematocrit determinations before death of the animals. Where the meaning is clear from the context, subgroups are designated as groups.

Results are presented in Fig. 1 and 2 and Table I. *Parent-hybrid pairs.* Following parabiosis with parental strain mice, the mean hematocrit values in the hybrid partners showed a progressive decrease from the day 0 value of 47% to 37% on the 5th day to 21% on the 12th day (Fig. 1a). The parental strain partners showed a progressive increase from 49% on day 0 to 72% on the 12th day. When the hybrid partners were transfused on the 4th day, the hematocrit rose from 35% on the 4th to 41% on the 5th; by the 7th day it had fallen to 32% which is very near the value for the nontransfused control hybrids. On day 5, the parental strain partners of transfused hybrids had a mean hematocrit value of 68%, compared to 56% for the parental strain partners of the nontransfused hybrids. This increase was statistically significant ($p<0.001$). It thus seems likely that the transfused blood was rapidly shunted to the parental strain partner. A second transfusion into the hybrid on day 7 was ineffective also; the mean hematocrit value of the 8 hybrids in which transfusion was successful was 3.3% lower the following day and was only 3% above that of the con-

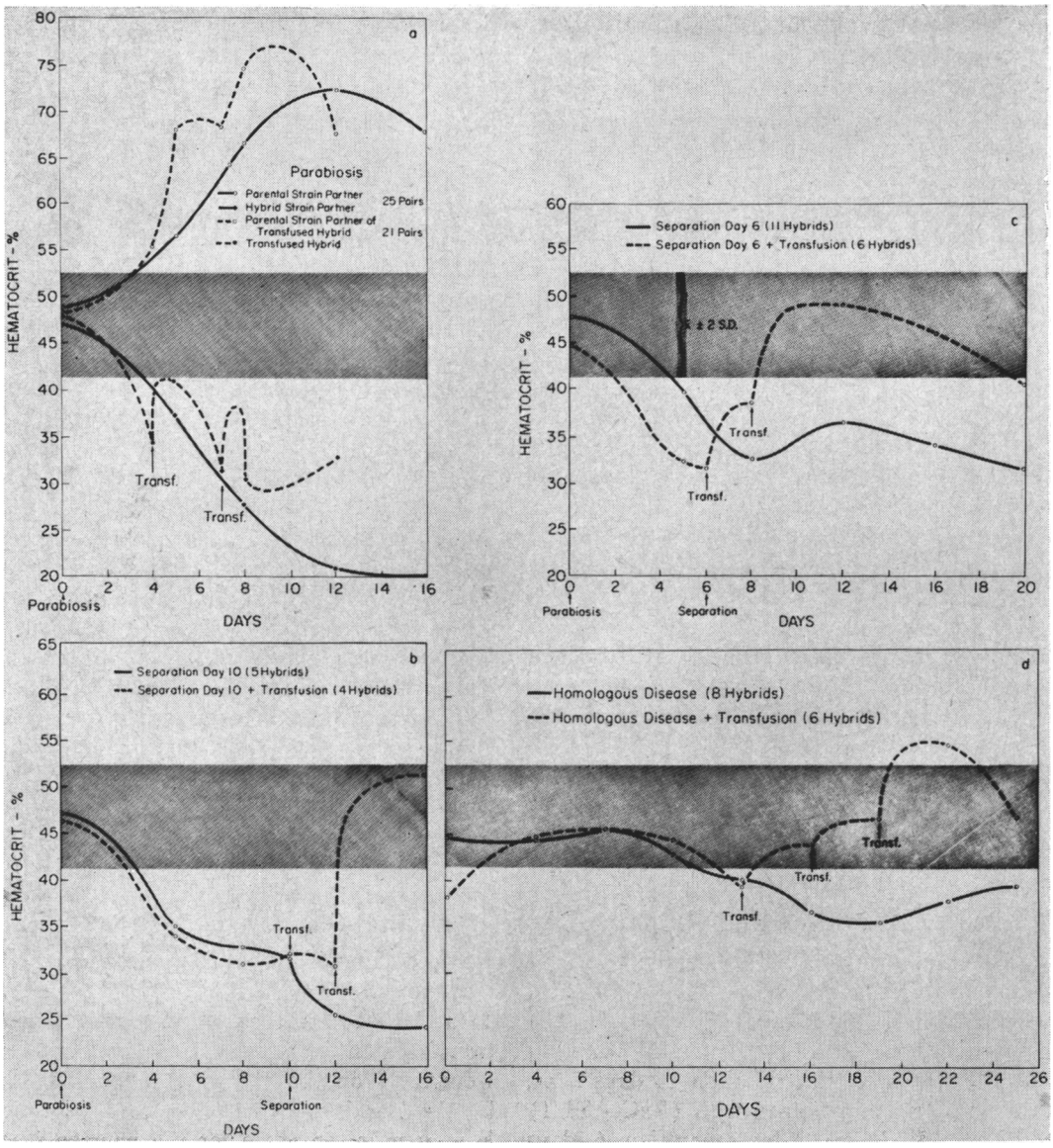

FIG. 1 a. Mean hematocrit values for both partners in A↔(A×C_{57}Bl/1)F_1 parabiosis pairs. Determinations were done serially on the same animals within each group. Values plotted in this and subsequent graphs are for animals which succumbed. Shaded area in each graph is mean ± 2 S.D. based on random determinations in 219 normal (A×C_{57}Bl/1)F_1 mice. b. Mean hematocrit values in (A×C_{57}Bl/1)F_1 hybrids separated 10 days after parabiosis with A strain partners (Group 2). c. Mean hematocrit values in (A×C_{57}Bl/1)F_1 hybrids separated 6 days after parabiosis with A strain partners (Group 3). d. Mean hematocrit values in (A×C_{57}Bl/1)F_1 hybrids suffering from homologous disease (Group 4).

trol group. The parental strain partners had a statistically significant ($p<0.02$) increase in hematocrit over the control parental strain partners. On day 12 the transfused hybrids had a hematocrit value of 33%, considerably higher than the control value of 21% but nevertheless below the normal range of 47.0 ± 5.6 for this hybrid strain.†

Whereas in the nontransfused hybrid-parental strain pairs the hybrid partner died

† Mean ± 2 S.D. based on random determinations in 219 (AxC_{57}Bl/1) F_1 mice.

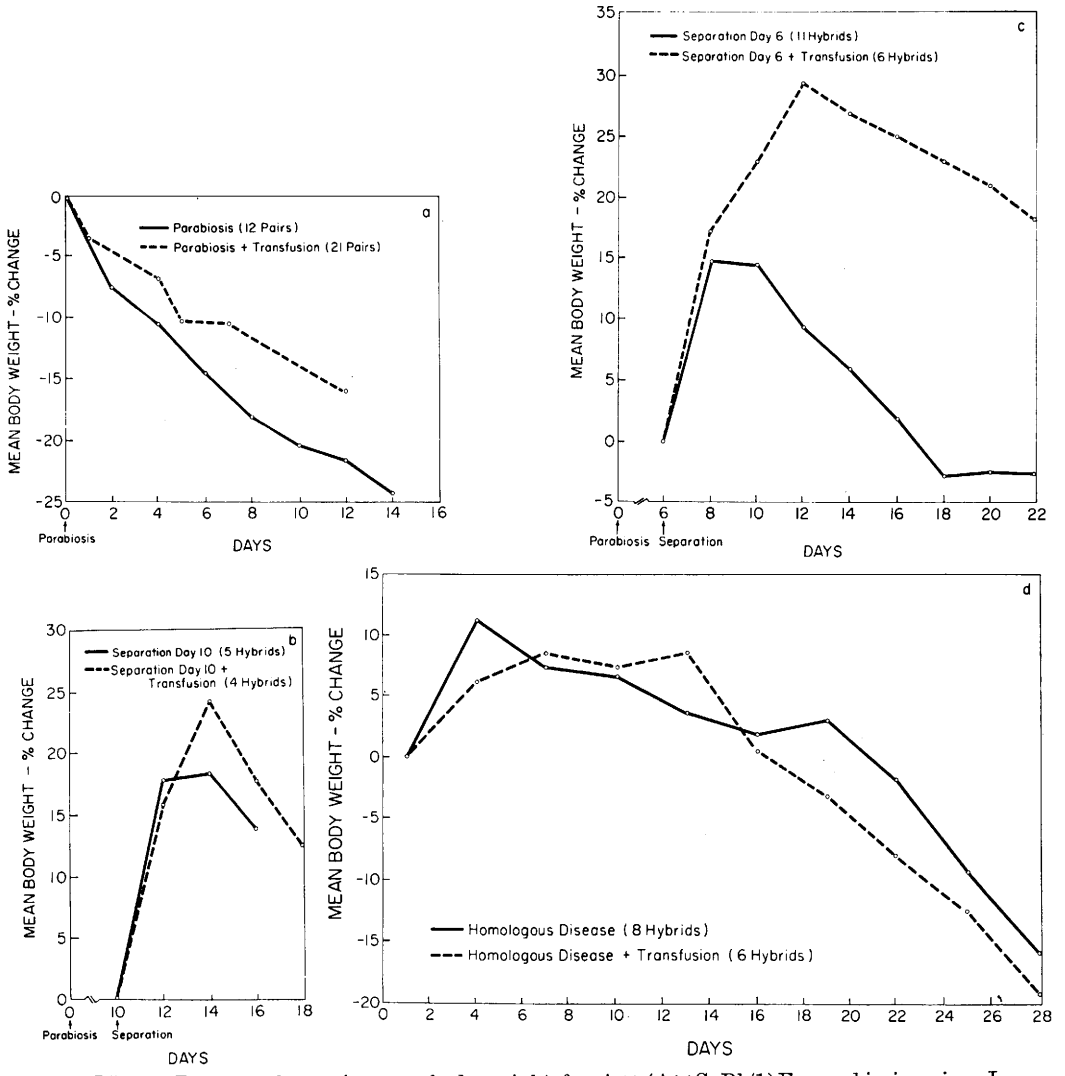

FIG. 2a. Per cent change in mean body weight for A↔(A×C_{57}Bl/1)F_1 parabiosis pairs. In this and subsequent groups, values are included only for animals which died. b. Mean weight curves for (A×C_{57}Bl/1)F_1 hybrids separated from A strain partners 10 days after parabiosis. c. Mean weight curves for (A×C_{57}Bl/1)F_1 hybrids separated from A strain partners 6 days after parabiosis. d. Mean weight curves for (A×C_{57}Bl/1)F_1 hybrid mice suffering from homologous disease.

first in all cases, in the transfused group in 5/21 pairs the parental strain partner was the first to succumb. This was probably the result of the increased polycythemia since autopsy examination showed numerous infarcts in the lungs.

Following parabiosis there was a progressive decrease in mean body weight of the pairs (Fig. 2a). A similar decrease occurred in the transfused group, although of slightly lesser degree. Signs of parabiosis intoxication, *i.e.*, hunched posture, ruffled fur, and lethargy, were evident in most hybrid parabionts of both groups by the 7th day.

Survival time was not appreciably different for the two groups: 11.9 ± 1.2‡ days for 12 nontransfused pairs and 11.2 ± 0.9‡ days for 21 transfused pairs.

In summary, transfusion of the hybrid partner in parabiosis with a parental strain partner was unsuccessful in raising the he-

‡ ± S.E. of mean.

TABLE I. Survival Data of Hybrid Mice in Parabiosis and Suffering from Homologous Disease, With and Without Syngeneic Blood Transfusion.

Group	Mortality at 2 mo		Mean survival (days ± S.E.)		Final hematocrit (%)*	
	No transfusion	Transfusion	No transfusion	Transfusion	No transfusion	Transfusion
1. Parabiosis	12/12	21/21	11.9 ± 1.2	11.2 ± .9‡	23.1	28.6
2. Parabiosis (10 days) → separation	8/8	10/10	19.5 ± 4.1	19.2 ± 2.0	30.4	51.5
3. Parabiosis (6 days) → separation	11/12	6/8	22.1 ± 4.0†	25.9 ± 7.1†	34.1	44.2
4. Homologous disease	8/10	6/10	26.5 ± 2.1†	26.7 ± 1.6†	38.6	52.7

* Mean value of last routine hematocrit determinations.
† For animals which succumbed.
‡ In 12 pairs, the hybrid partner succumbed first (mean survival 13.0 ± 1.6 days), in 5 pairs the parental strain partner (mean survival 8.8 ± 1.91 days) and in the remainder both partners were found dead.

matocrit value of the hybrid mouse to a normal range because of rapid shunting of blood to the parental strain partner, and the clinical picture was not improved.

Parabiosis with separation at 10 days. Following separation the nontransfused group showed an accentuated hematocrit drop, which was probably due to hemorrhage during the separation procedure (Fig. 1b). The transfused group received blood on both day 10 and 12 resulting in a hematocrit value of 51% on day 16, compared to 24% for the control group. This is within the normal range indicated above. Weight curves for the 2 groups of mice were strikingly similar, however. Following separation from parental strain partners, the hybrid mice in both transfused and nontransfused groups showed a rapid increase in body weight for 4 days, followed by a rapid decrease ending in death of all animals (Fig. 2b). Mean survival time for the 8 nontransfused mice was 19.5 ± 4.1 days and for the 10 transfused mice 19.2 ± 2.0 days. Thus blood transfusions to normal hematocrit levels were ineffective in altering the clinical course and ultimate fate of hybrid mice separated from parental strain partners 10 days after parabiosis.

Parabiosis with separation at 6 days. Following separation, the nontransfused mice showed a slight increase in hematocrit from day 8 to day 12, followed by a slowly progressive decrease thereafter: mean hematocrit values for 11 mice were 33% on day 8, 36% on day 12, and 31% on day 20 (Fig. 1c). The basis of the only minimal erythropoietic response to the anemia was found to be immunologic, and is evaluated in detail in a subsequent paper on the graft-*vs*-host reaction in parabiosis intoxication. The transfused mice received blood on day 6 and day 8, resulting in mean hematocrit values of 49% on day 12, 46% on day 16, and 40% on day 20.

Weight curves after separation were somewhat different for the two groups (Fig. 2c). The nontransfused mice showed a 15% increase in body weight in the two days following separation, followed by a progressive decrease ending in death in 11/12 animals. The transfused mice showed a 30% increase in body weight over a 6-day period following separation. Thereafter a progressive decrease ended in death for 6/8 animals.

Mean survival time of the nontransfused mice which succumbed was 22.1 ± 4.0 days, and of the transfused mice, 25.9 ± 7.0 days. These values do not differ significantly. They are significantly greater than those for hybrid mice remaining in parabiosis with parental strain partners, in the case of the nontransfused ($p<0.02$) and transfused ($p<0.05$) groups respectively.

In summary, separation at 6 days with or without transfusion resulted in a significant prolongation of survival over the mice remaining in parabiosis; separation plus transfusion was followed by a greater weight gain and

a 17% reduction in mortality, (from 11/12 to 6/8) as compared to the nontransfused controls.

Homologous disease. In the nontransfused group, a decrease in the hematocrit was first evident on day 10, and a minimum value of 35% was attained on day 19 (Fig. 1d). This is a milder degree of anemia than occurred in the hybrid mice in parabiosis with parental strain partners. A second group of hybrid mice suffering from homologous disease was transfused on days 13, 16 and 19. This resulted in mean hematocrit values of 44%, 48%, 55% and 46% on days 16, 19, 22 and 25 respectively. These values are within or just above the normal range. Mean weight curves for the nontransfused and transfused groups of mice were very similar: an initial normal increase for about one week followed by a progressive decrease ending in death (Fig. 2d). The mortality for the nontransfused group was 8/10 and for the transfused group, 6/10. Mean survival times for the animals which succumbed were 26.5 $\pm$ 2.1 days and 26.7 $\pm$ 1.6 days, respectively. These values do not differ significantly. Thus the only difference in the transfused group was a 20% reduction in mortality rate.

Histologic examination was carried out on the hybrid mice in both subgroups of all 4 groups. Similar pathologic changes were present in all hybrids: lymphoid depletion, amyloidosis, liver lesions and bowel wall abscesses. These are characteristic of a graft-*vs*-host reaction(3).

Discussion. Attempts to prevent anemia in the hybrid partners remaining in parabiosis with parental strain mice were unsuccessful because of rapid shunting of the transfused blood to the parental strain partners. The absence of any beneficial effect is therefore not surprising. Previous investigations in this laboratory(9) on the anemia-polycythemia picture in parent-F_1 hybrid parabiosis combinations showed that the hybrid-to-parent shunt proceeded in 2 phases: an initial phase perhaps dependent on a vascular pressure gradient from F_1 hybrid to parent, and a later phase in which this pressure gradient was sustained by an immunological reaction of the parental strain partner against the hybrid. In our experiments, the first transfusion was given during the initial phase and the second at a time when an immune reaction was already manifest(9). The decreased effectiveness of the second transfusion, compared to the first, suggests that the immune reaction had produced an increase in the pressure gradient.

If the anemia due to shunting of blood to the parental strain partner were the sole cause of parabiosis intoxication, then separation from the parental strain partner should be followed by a rapid and complete recovery of the hybrid mouse, particularly if it is transfused immediately after separation. The recovery, however, lasted only a few days following which the animals lost weight progressively and died. The clinical course was similar to that of the homologous disease control group. Histologic examination of the hybrid parabionts confirmed the clinical diagnosis of a graft-*vs*-host reaction.

These studies provide evidence that anemia is an etiologic factor also in parabiosis intoxication. The significant increase in survival time of the hybrid mice separated 6 days after parabiosis, compared to the nonseparated hybrids suggests that some factor(s), presumably mainly the anemia, are responsible for the shorter survival time of the nonseparated hybrid parabionts. By means of separation, the continuous bleeding of the hybrid into the parental strain partner is terminated. In addition, the hybrids separated at 6 days gained weight and appeared normal for an additional 4 days, a time interval in which clinical intoxication was already pronounced and in which some deaths already had occurred among the nonseparated parabionts. Furthermore, during the same time interval there was only minimal morbidity and no deaths among the homologous disease controls.

These experiments also show the negligible value of transfusion in treatment of the graft-*vs*-host reaction. In the hybrid parabionts separated at 6 days, and in the homologous disease group, transfusion was associated with a reduction of mortality of 17% and 20% respectively. In both groups there were a few survivors among the nontransfused con-

trols; presumably the number or reactivity of parental lymphoid cells was insufficient to kill the hybrid and a state of tolerance to the hybrid ensued. It is possible, therefore, that transfusion, by some mechanism, was able to swing the balance toward tolerance and recovery in several additional doubtful cases. In the strain combination used, anemia due to the graft-*vs*-host reaction was mild, in the range of 30-40%. In other strain combinations, and in other species, in which a more severe immunologic anemia might occur, transfusions would be more useful in eliminating this as a cause of death, but again it seems likely that most animals would inevitably succumb, for the basic cause of death in homologous disease is still an enigma.

Summary. (1) Transfusion of the hybrid mouse in parabiosis with a parental strain partner was followed by shunting of blood to the latter. The hybrid mouse was not benefited. (2) When the hybrid mouse was separated from the parental strain partner 10 days after union, the hybrid mouse showed a temporary weight gain, followed by progressive wasting and death. Transfusion of the hybrid mouse to a normal hematocrit level did not alter this course of events. (3) When separation and transfusion of the hybrid were carried out 6 days after parabiosis, there was a greater gain in weight than in nontransfused controls, and a reduction in mortality from 11/12 to 6/8. In both transfused and nontransfused groups survival time was significantly prolonged, compared to hybrid mice remaining in parabiosis. (4) Transfusion of hybrid mice suffering from homologous disease resulted in a reduction of mortality from 8/10 to 6/10 but otherwise the course of the disease was not altered. (5) Parabiosis intoxication, in the strain combination employed, appears to involve the effects of both anemia and the graft-*vs*-host reaction. (6) Syngeneic blood transfusion is of little value in treatment of the graft-*vs*-host reaction in the strain combination employed.

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Influence of Phenoxybenzamine on Vascular Responses to Vasoactive Agents.* (30555)

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Phenoxybenzamine (Dibenzyline) has been reported to be effective in increasing survival and altering the typical responses observed in endotoxin, traumatic, and hemorrhagic shock(8,9,10,11). One explanation of the protection afforded by phenoxybenzamine in endotoxin shock is that it suppresses the action of pressor agents such as epinephrine and norepinephrine, thus increasing blood flow to

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