Cross Circulation in Normal and Intoxicated Parabionts.* (27726)

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There are strong indications that parabiosis intoxication, a syndrome peculiar to animals in the parabiotic state characterized by anemia of one animal and polycythemia of the other, has its origin in an immunological incompatibility between the parabionts(1). Similarity between parabiosis intoxication and a graft-*versus*-host reaction, such as runt disease, has been pointed out(2,3). The actual mechanism for the characteristic peripheral blood findings in the parabionts is still a matter of debate. Currently, there are 2 hypotheses as to the etiology of the hyperemia-anemia symptoms, both of which presume unequal exchange of blood between parabionts. One hypothesis holds that unequal exchange occurs because of mechanical trapping of the anemic animal's erythrocytes in the hyperemic partner. It is believed that once transferred to the hyperemic rat the anemic animal's erythrocytes become so "sticky" and agglutinable due to antibody coating that they can not readily pass back through the small vascular connections between parabionts so that flow is predominantly in one direction (4). The other states that shock is the predisposing factor which causes decrease in peripheral vascular tone in one animal; a pressure gradient is thus established between partners and unequal exchange occurs(5,6). The experimental model of the hyperemia-anemia of spontaneous parabiosis intoxication which results from section of the spinal cord of one member of a normal pair has been described and offered as substantiation for the latter hypothesis(5).

The exchange of erythrocytes and plasma in intoxicated parabionts was investigated in the present experiment to quantitate the rate of cross circulation and to determine whether or not unequal exchange actually occurs. Cross-circulation rates were determined in a large number of normal pairs as controls for intoxicated animals; these data also served to amplify the observations on cross-circulation rates previously made by 2 groups of investigators(7,8).

Materials and methods. Sprague-Dawley female rats from Holtzman Co. were used in the study of normal parabionts. To increase the incidence of intoxication, rats of Sprague-Dawley origin from Holtzman were paired with Sprague-Dawleys from Charles River Laboratories or with rats of Wistar origin from Carworth Farms. The symbols used for these various combinations are as follows:

H-H	Holtzman	(Sprague-Dawley)	-Holtzman pairs.
H-CR	"	"	"	-Charles River (Sprague-Dawley) pairs.
H-CF	"	"	"	-Carworth Farms (Wistar) pairs.

Animals were paired at 30-35 days of age according to the method of Bunster and Meyer (9). Pairs were characterized as intoxicated by the erythematous or pale appearance of ears, feet and tail subsequently corroborated by hemoglobin or microhematocrit determinations. Pairs in which anemic partners differed from hyperemic approximately by a factor of two in the parameters given above were considered as being in a state of intoxication.

Rates of cross circulation of erythrocytes and plasma were determined simultaneously with $NaCr^{51}O_4$ tagged erythrocytes and with radio-iodinated human serum albumin (RISA, Abbott), respectively. Chromium^{51} tagged erythrocytes were prepared *in vitro* and after saline washings were finally suspended in isotonic saline to which RISA had been added. One ml of tagged elements containing approximately 6 μc Cr^{51} and 1-2 μc I^{131} was injected at zero time into the tail vein of one member of a pair. Blood was removed at intervals from both partners by cardiac puncture, heparinized and centrifuged; exactly 0.1 ml plasma and 0.1 ml packed red cells from each sample were placed in a deep well chamber and activity determined on a scaler (Nuclear-Chicago Model 186). No allowance for

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decay was necessary since counting was delayed until all samples from a single pair were collected.

Results were expressed as percentage of total activity in each member of the pair. When one animal of a pair was injected, it was assumed first that 100% of the activity was located in the injected animal and secondly, that for a time, there would be greater flow toward the uninjected rat than in the opposite direction. The half-time transfer (T ½), *i.e.*, one-half the time theoretically needed for the tagged elements to come to equilibrium between partners, was calculated for each pair by plotting the difference in percentage of total activity between injected and uninjected partners logarithmically against time; T ½ is the point on this straight line curve where it intersects the 50% level.

There were several technical difficulties with the experimental method. When plasma and cells were separated, 0.3% of Cr^{51} activity remained in the plasma and approximately 11% of I^{131} activity remained with the erythrocytes. Since the standard variation in counting is approximately equal to the square root of the number of counts, 0.3% error in 5000 counts (the level usually obtained per minute with the quantity of isotope used in this experiment) is not significant. An 11% error is significant in 5000 counts; therefore, erythrocyte counts were considered to have an 11% error. The error, however, was considered to be comparable in all animals and no adjustment was made in counting. This assumption was verified for pairs in which large hematocrit differences occurred by injecting a group of 11 intoxicated pairs with tagged erythrocytes only. Another complication was loss of tag from the vascular system. The loss of Cr^{51} by decay or excretion was less than 0.5%/hour, but the loss of I^{131}, probably because of activity of the thyroid, was between 1% and 2%/hour.

Results. Normal pairs. Fig. 1 shows the half-time transfer of erythrocytes and plasma in 33 H-H female control pairs less than one month after surgical union. The half-time transfer for RBCs averaged 58.4 ± 4.7 minutes and for plasma 72.7 ± 6.8 minutes. Since there was considerable variation, this difference in circulation rate between cells and plasma was found not to be significant ($P > .05$), but plasma transfer was the same as or greater than that for RBCs in all but one of 33 pairs.

TABLE I. Half-Time Transfer in Normal H-H Parabionts.

Time after pairing (mo)	No. pairs	Half-time transfer* RBC	Plasma
<1	33	58.4 ± 4.7	72.7 ± 6.8
10	16	77.4 ± 10.6	103.1 ± 10.9
15	8	76.3 ± 13.2	—

* Time in min. ± stand. error.

Cross circulation was reinvestigated in some of these same pairs at 10 and 15 months after union. Results are presented in Table I. Although average RBC half-time transfer times were increased, no statistically significant differences were noted because of the greater variability in circulation times among older pairs. Plasma transfer time was considerably greater than erythrocyte transfer time at 10 months, but, once again, there was no statistically significant change. Plasma half-time transfer at 10 months was significantly elevated over that at less than one month ($P < 0.02$).

Intoxicated pairs. The half-time transfer in H-CR pairs is shown in Fig. 2. Before the onset of signs of intoxication, 5-14 days after pairing, 19 H-CR pairs with an average hematocrit of 39.3 ± 0.9% showed an erythrocyte T ½ of 52.3 ± 9.2 minutes. This was similar to H-H control pairs (58.4 min). The remaining curves in Fig. 2 represent 2 groups of intoxicated H-CR pairs, 11 injected with tagged erythrocytes only and 9 pairs doubly tagged. Tagged cells and RISA were injected into the anemic partners. In the doubly tagged pairs, in which hemoglobin averaged 6.3 ± 0.8 g% for anemic and 18.6 ± 0.6 g% for hyperemic partners, erythrocyte half-time transfer averaged 18.9 ± 4.3 hours and half-time transfer for plasma averaged 13.1 ± 3.3 hours. In the group of 11 pairs injected with tagged erythrocytes only, as a control for double tagging, hematocrits averaged 26.5 ± 1.8% for the anemic members and 50.7 ± 0.7% for the hyperemic. RBC T ½ was 15.4 ± 3.3 hours which was not statistically differ-

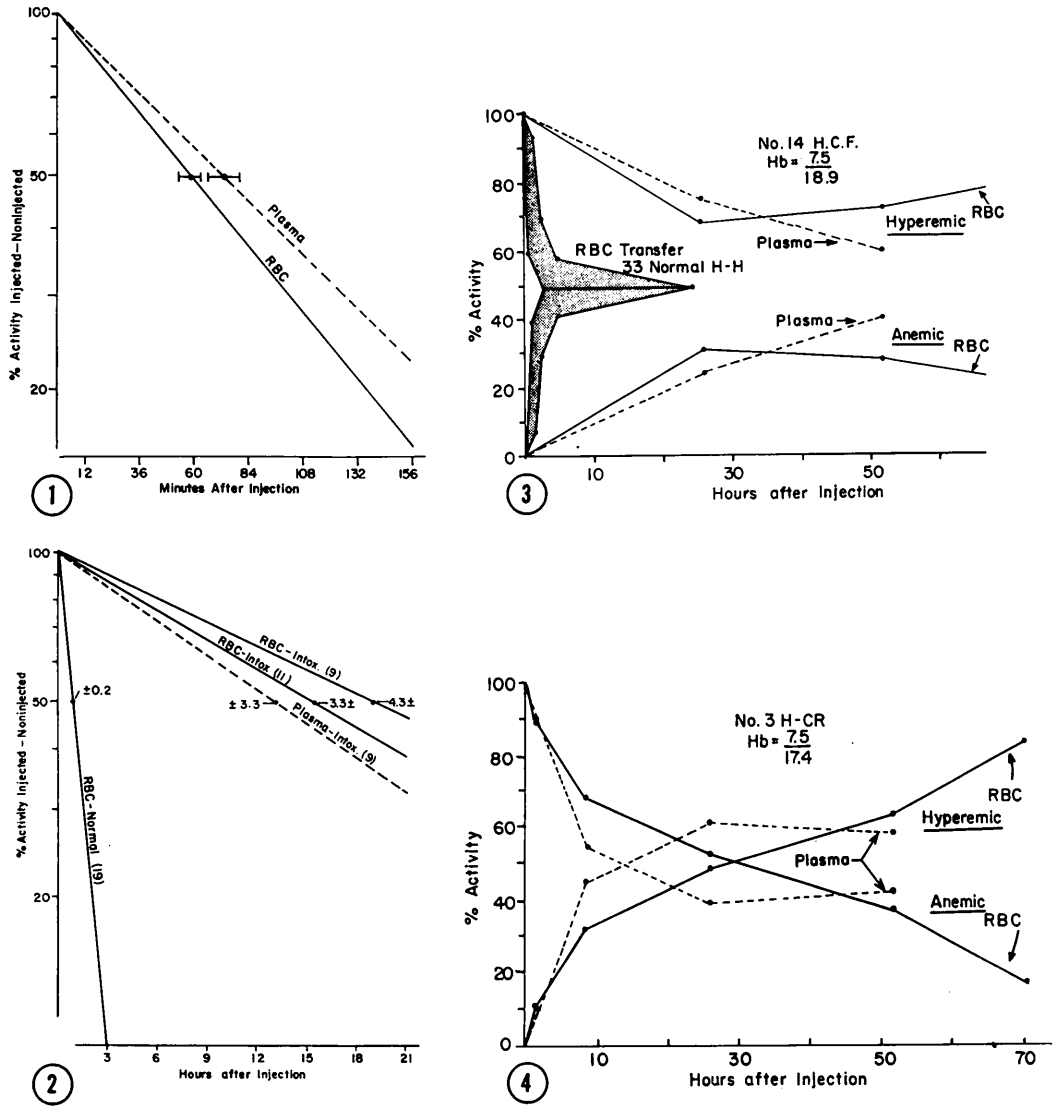

FIG. 1. Half-time transfer of erythrocytes and plasma in 33 normal H-H pairs less than 30 days after union. The ordinate is on a logarithmic scale. Horizontal lines on the curves denote stand. error.

FIG. 2. Half-time transfer ± stand. error in normal and 2 groups of intoxicated H-CR pairs. The anemic partner was injected in intoxicated pairs. The ordinate is on a logarithmic scale.

FIG. 3. Erythrocyte and plasma transfer in a typical intoxicated H-CF pair. Tagged elements were injected into the hyperemic animal. Erythrocyte transfer curves for 33 normal H-H pairs included for comparison.

FIG. 4. Erythrocyte and plasma transfer in a typical intoxicated H-CR pair. Tagged elements were injected into the anemic partner.

ent from RBC T ½ of intoxicated pairs injected with doubly tagged suspension.

This experiment was repeated with 6 H-CF (Wistar) and 4 H-H intoxicated pairs. It was found that cross-circulation times in H-CF pairs prior to onset of intoxication were similar to H-H control values and after onset of intoxication resembled those found in intoxicated H-CR pairs. Circulation times were investigated successfully in 4 intoxicated H-H parabionts. The average T ½ for RBCs was 5.8 ± 2.6 hours, for plasma 5.8 ± 1.7 hours.

These 4 intoxicated H-H parabionts showed circulation times which were increased significantly ($P<.001$) over those in normal H-H pairs but were significantly shorter ($P<.001$) than those in intoxicated H-CR and H-CF pairs.

Several H-CR and H-CF pairs were tested at 19-21 days after union when crusting at the line of junction was evident and dehiscence of the junction had begun. There was no transfer of tagged elements in these pairs.

Fig. 3 and 4 are graphic records of 2 typical intoxicated pairs injected with tagged elements. Fig. 3 illustrates injection of tagged elements into the hyperemic partner of a typical intoxicated H-CF pair; the hyperemic partner was injected in 5 such pairs. The areas into which all the curves for 33 normal H-H pairs fall have been included for comparison. The intoxicated pair showed transfer of 30% of the activity into the anemic animal 26 hours after injection, but then the tagged elements showed a tendency to return to the hyperemic animal. Equilibrium was not reached even at 70 hours after injection. There was an indication that plasma was moving toward equilibrium, but the clearance of RISA from plasma proceeding at a rate between 1% and 2% per hour precluded accurate determination beyond 50 hours.

Fig. 4 illustrates transfer in a typical H-CR intoxicated pair in which the anemic partner was injected with tagged cells and plasma. This graph shows that equilibrium was reached in the plasma sometime between 10 and 15 hours after injection and that erythrocyte equilibrium took place at approximately 30 hours after injection; the faster transfer of plasma as contrasted with the pattern in normal pairs was the usual finding in intoxicated pairs. The flow of tagged elements continued beyond equilibrium so that at 70 hours after injection more than 80% of total activity was found in the non-injected partner, the hyeremic. Once again, because of clearance of RISA from plasma, activity could not be ascertained accurately beyond 50 hours although there was a tendency for plasma to move toward equilibrium. In 2 intoxicated pairs with faster cross-circulation times than those shown in Fig. 4, plasma activity actually was at equilibrium at 50 hours whereas 70% of tagged erythrocytes had moved into the hyperemic partners.

Discussion. These results indicate that slowing of cross circulation must now be added as another symptom of parabiosis intoxication. This profound slowing of circulation was found at the height of the hyperemia-anemia, and animals which were farthest apart, genetically speaking, exhibited it most strikingly; H-CR and H-CF pairs showed cross-circulation rates slowed as much as 15-20 times over normal, whereas in the few H-H intoxicated pairs studied the circulation had only slowed by a factor of 5 or 6. Between the 14th and 21st days after union of H-CR and H-CF animals, pairs either die as a result of severe hyperemia-anemia or the union between the animals breaks down and transfer between parabionts ceases. This cessation of transmission has been described for mouse parabionts in which each partner has an allele at the H-2 locus not carried by the other, but it occurs earlier than found with rats in the present experiment(11). Despite the slowing and subseqeunt cessation of circulation between the 2 animals, the present study demonstrated by injection of the hyperemic partner that some exchange occurs in intoxication but most of the tagged elements remain in the injected animal. Injection of the anemic partner of a pair demonstrated dramatically that unequal exchange does indeed take place between animals in a state of intoxication.

In contradistinction to the pattern seen in normal pairs, the demonstration that plasma transfer was faster than erythrocyte transfer in intoxication suggests that the flow of erythrocytes may be specifically impeded. This might lend support to the hypothesis of intoxication based upon agglutination of erythrocytes as proposed by Chute and Sommers (4). We can neither support nor contradict the hypothesis of decreased vascular tone as the causative factor in intoxication as held by Hall and Hall(5). However, our results suggest that their use of the spinal cord sectioned animal as a model of intoxication is not realistic because it does not take into account the slowing of cross circulation.

In the present experiment the half-time transfer of erythrocytes in a large number of normal Holtzman (Sprague-Dawley) parabionts investigated at less than one month after union confirms the observations of Huff, *et al.*(7) who used Fe^{59} tagged cells in Wistar pairs united at one month of age. On the other hand, Fleming, Caldwell and Jacobs (8), who utilized P^{32} tagged cells, reported cross-circulation rates 2-3 times faster than those found previously by Huff *et al.* Fleming *et al.* attributed their divergent results to the age of their animals and the duration of parabiosis since their rats were paired at 21 days of age and were studied at 21 and 34 weeks after union. Our animals, which were the same strain as those used by Fleming *et al.*, were approximately the same age at pairing and one group (10 months) was investigated at a similar time after union. We are unable to explain the discrepancy between the experiments but P^{32} tagging has largely been replaced by Cr^{51} labeling because of the loss of P^{32} label during the first hour(10).

The rate of circulation of plasma in normal pairs in the present experiment is somewhat different from previous results of Huff *et al.* (7) who, using Na^{24} as a tag, reported plasma transfer 2-3 times longer than we found and 2-3 times longer than erythrocyte exchange. Diffusion of Na^{24} from the vascular system, which would prevent accurate appraisal of plasma movement, probably explains the dissimilar results in the 2 experiments; use of RISA obviated this difficulty. Our normal pairs, less than one month after union, exhibited an average plasma circulation rate which was statistically similar to that for erythrocytes, but individual rates were as long or longer than those for erythrocytes in all but one of the pairs. This apparent disparity in transfer times between cells and plasma may represent the difference between swifter central flow of erythrocytes and slower peripheral flow of plasma.

Cross circulation in normal pairs reinvestigated 10 and 15 months after union showed a tendency for greater variation because of occasional pairs with long half-time transfer times; erythrocyte cross circulation had slowed on the average and plasma transfer was significantly increased in rate. This finding was in contrast to the report of Fleming *et al.*(8) who suggested on the basis of a small number of pairs restudied 13 weeks after initial injection that cross-circulation time decreases with the duration of parabiosis.

Summary. Erythrocyte and plasma transfer times determined simultaneously with Cr^{51} tagged cells and radio-iodinated human serum albumin (RISA) in normal parabionts at less than 30 days after union were statistically similar to each other, but plasma half-time transfer was as long or longer than that for erythrocytes in all but one of 33 pairs. Normal pairs reinvestigated 10 and 15 months after surgical union showed some slowing of cross circulation. Pairs in a state of intoxication, as determined by clinical appearance corroborated by differentials in peripheral blood values, showed a great slowing of cross-circulation time. In addition to this slowing of cross-circulation time the intoxicated pairs exhibited plasma transfer faster than that for erythrocytes which was opposite to the pattern for normal parabionts. When injected into the anemic animal, the majority of tagged erythrocytes crossed to the hyperemic rat demonstrating unequal exchange; when injected into the hyperemic animal, the erythrocytes tended to remain in that animal although some mixing did occur.

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Acute Inhibition of Thyroid Function in the Rat Produced by Rabbit Antibody to Beef Thyrotrophic Hormone.*† (27727)

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Rabbit antibody produced in response to injection of bovine thyrotrophic hormone (TSH) effectively neutralizes the thyroid stimulating activity of injected rat pituitary extract(1). Since the anti-beef TSH antibody interferes with the effects of exogenous rat TSH, it seemed likely that the antibody could also inactivate circulating endogenous hormone in this animal. An analogous experiment, the production of acute diabetes mellitus in the rat has already been accomplished by Wright(2) using an anti-beef insulin antibody produced in the guinea pig. In the following experiment thyroid function has been measured continuously in the rat by the *in vivo* counting of neck radioactivity following injection of radioactive iodine; the effects of injection of antibody were then tested during the course of a thyroid I^{131} release curve. An abstract of this work has been published(1).

Methods. Antibody to bovine TSH was produced in rabbits by the method of Werner *et al.*(3). Adult male New Zealand rabbits were injected in multiple intracutaneous sites with an emulsion of Thytropar (Armour Laboratories) in Freund's adjuvant. Three series of injections of 3 units each were given at 10-day intervals. The initial injection was with the complete adjuvant; later injections used incomplete adjuvant. Plasma was harvested 10 days after the last injection and at intervals after booster injections of one unit and was stored in the frozen state. Thyroid I^{131} release curves were determined by serial neck counts of groups of adult male rats injected with 40 μc of radioiodine 24 hours prior to the initial neck count(4).

Results. (Fig. 1). Following a control period in which initial I^{131} release rates were determined one group of 6 rats was injected intraperitoneally once a day for 3 days with plasma from TSH-immunized rabbits. Each animal was given a total of 8 ml. Another group of 9 animals was given 8 ml of normal rabbit plasma. In the antibody treated groups there was prompt cessation of I^{131} release persisting for at least 6 days. Although there is a suggestion from the curve that return towards normal function had begun about 6 days after injection, it is possible that inhibition was present even on the seventh day. To determine when the antibody induced thyroid inhibition became significant statistically, comparisons by the t test were made for successive sets of neck counts following injection: at 16 hours post injection, P value was $<.1$; at 35 hours, the mean neck counts were significantly different ($P<.01$) and remained so for each succeeding set of determinations ($P<.01$ to $<.001$). Even though significant differences in mean neck counts were not apparent until 35 hours after antibody injection, it is possible to estimate roughly the time of onset of antibody effect by extrapolation of the post-injection curve back to the pre-injection curve. The point of intersection falls 90 minutes after injection.

Discussion. Acute inhibition of thyroid I^{131} release in the rat follows injection of rab-

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