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Protection Against Tourniquet Shock Afforded by Parabiosis.* (21991)

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When rats are placed in parabiosis one of them may thereby be protected against otherwise fatal consequences. It has been shown for example, that survival of one is prolonged after total nephrectomy(1), a lethal dosage of x-rays(2,3) or bilateral adrenalectomy(4), when the co-twin is normal. Similarly the usually severe diabetes following either pancreatectomy(5) or toxic doses of alloxan(6) is either minimized or abolished by the presence of a normal partner. Recently we have reported that when one of a normal pair of parabiotic rats is subjected to transection of the spinal cord just caudad to C7, the operated rat develops erythremia, with increased hemoglobin, erythrocyte count and hematocrit, whereas the intact co-twin concurrently develops a rapidly fatal anemia(7). This study was interpreted to indicate that the capillary hypotension induced on one side of the capillary anastomosis by spinal transection led to a decrease in the amount of blood exchanged from operated to intact, while the amount traversing the vascular bed from intact to operated remained normal. These changes, typical of spontaneous parabiosis intoxication (8), suggest the latter to be due to a shock-like state in one of the partners.

The present study was undertaken to determine whether the disparity in blood transfer could also be induced by subjecting one of a pair to tourniquet shock, or whether the usually lethal effects of this intervention would be modified by the presence of a normal twin.

Materials and methods. The animals used in these studies were females of the Holtzman strain. Thirteen single animals weighing 100-139 g served as controls, and 10 pairs of parabionts, in union for 2 weeks and together weighing 200-280 g, constituted the parabiotic group. Parabiosis was performed essentially by the method of Bunster and Meyer(9). Tourniquets were applied to both hind limbs of the singles and to both hind limbs of one of the partners of each pair. The method was similar to that described by Rosenthal(10) and Stopack(11). A #8 elastic band was looped 5 times around a hollow tube of 16 mm diameter, each hind limb was then drawn through the tube and the elastic ligature slipped off so as to constrict the limb as close to the body as possible. The ligatures were removed 5 hours later. Rectal temperature, erythrocyte count, hematocrit and hemoglobin determinations were taken on all animals prior to ligation, and at 12, 36, and 48 hours after its removal, where survival permitted. Purina Laboratory Chow and tap water were available to the rats at all times.

Results. Of the 13 single rats to which the tourniquets were applied, 5 died within 12 hours of removal of the ligatures and all but one had succumbed by 48 hours thereafter.

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TABLE I. Effect of Tourniquet Shock on Single and Parabiotic Rats.

A—Single rats

Time	No. rats	Rectal temp., °C	Erythrocytes, millions cu/mm	Hemoglobin, g/100 cc	Hematocrit
Before tourniquet	13	38.0 ± 1.4*	7.50 ± .30	14.9 ± .3	42 ± 1.2
12 hr after removal	8	29.4 ± 1.2	10.43 ± .36	20.1 ± .5	62 ± 1.0
36 " " "	2	28.8 ± .8	11.15 ± .83	21.5 ± .9	65 ± 3.1
48 " " "	1	38.4	9.80	15.3	51

B—Parabiotic pairs

Time	No. pairs	Rectal temp., °C Left†	Rectal temp., °C Right	Erythrocytes, million cu/mm Left	Erythrocytes, million cu/mm Right	Hemoglobin, g/100 cc Left	Hemoglobin, g/100 cc Right	Hematocrit Left	Hematocrit Right
Before tourniquet	10	38.2 ± .3	38.2 ± .3	7.57 ± .26	8.15 ± .39	14.6 ± .3	15.0 ± .6	42 ± 1.2	44 ± 1.7
12 hr after‡	10	37.5 ± .2	37.7 ± .2	9.12 ± .39	8.05 ± .50	16.4 ± .2	14.6 ± .2	50 ± 1.1	43 ± 1.6
36 " "	10	38.0 ± .2	37.9 ± .2	7.09 ± .32	6.69 ± .59	13.0 ± .3	12.3 ± .9	37 ± 1.4	36 ± 2.3
48 " "	10	38.2 ± .1	38.2 ± .2	7.02 ± .42	6.66 ± .46	12.8 ± .4	12.6 ± .8	37 ± 1.6	38 ± 2.4

* ± S.E. of the mean. † Animal with tourniquet applied. ‡ After removal.

Rectal temperatures declined rapidly, and there occurred a marked hemoconcentration as evidenced by increase in the red-cell count, hemoglobin and hematocrit which was progressive until death. The results are given in Table IA.

The behavior of parabiotic rats was in marked contrast. Following removal of the tourniquets from the limbs of the one animal, there occurred a transient hemoconcentration at 12 hours, reflected in the hemogram, but no alteration in rectal temperature. However, at the 12-hour interval hemoconcentration was not nearly as marked in these as in single animals similarly treated, and whereas it was progressive in the latter it promptly reverted towards normal in the parabionts. Further, although the edema of the limbs was as marked in parabionts as in singles following ligature removal, and indeed in one the limbs amputated 4 days thereafter, none of them succumbed. The hemograms of the untreated partners indicated an anemic tendency. This was detectable at the 36-hour interval, was still present at 48 hours, and recovered slowly thereafter. Inasmuch as each of the pairs had tended to stabilize within 48 hours, no further blood determinations were made, although survival was followed for 2 weeks. The results are summarized in Table IB.

Discussion. Since the chief connection between the twins is vascular, and since the recovery from transient hemoconcentration in the injured rat was accompanied by hemodilution in the untreated partner, it seems reasonable to attribute the protective effect of parabiosis to the availability of the normal partner's blood to the injured twin. The magnitude of this interchange was sufficient to induce changes in the hemogram of the untreated animal in most cases although not to the same degree as has been true of untreated rats, attached to twins subjected to spinal transection, which invariably succumb. It would seem likely that the erythrocyte and fluid reserves available to the untreated twin are often sufficient to enable it to maintain an almost normal hemogram in the face of demands by the damaged twin. Only when the reserves are strained, as when a normal animal is attached to a twin with a spinal transection(7), to a dying or dead partner (12), or perhaps when one is attached to the hyperemic member of a pair with spontaneous parabiosis intoxication(12), is the resulting demand upon the vascular fluids of the normal partner sufficiently severe as to induce a fatal anemia. This is believed to be due to whole blood transfusion of one rat by its partner, with the latter undergoing hemodilution in an effort to sustain the declining blood volume as discussed elsewhere(13).

Summary. 1. Elastic ligatures applied to both hind limbs of young female rats for 5 hours produced shock and death in 12 of 13 single rats; a marked and progressive decrease

in body temperature and an increase in hemoconcentration invariably occurred in those which succumbed. 2. Tourniquets applied similarly to the limbs of one partner failed to produce a single death in 10 pairs of parabionts. A transient hemoconcentration was observed in the injured partner at 12 hours although rectal temperatures remained stable. Anemia occurred in the untreated member of many of the pairs, and is believed to reflect transfusion of the shocked rats, with compensatory hemodilution in an effort to sustain the blood volume.

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Inhibition of Lipid Synthesis by Alpha-Phenyl-N-Butyrate and Related Compounds. (21992)

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Cottet *et al.* have reported that feeding alpha-phenylbutyrate (phenylethylacetate) can lower serum cholesterol levels in the normal rat(1,2) and in patients with various forms of hypercholesterolemia(3). Since the drug did not prevent hypercholesterolemia in cholesterol-fed rats(1), it presumably does not act by inhibiting intestinal absorption; but the mechanism of action remains to be established. The present studies show that *in vitro* the drug strikingly inhibits synthesis of both cholesterol and fatty acids from sodium acetate-1-C^{14}.

Methods. Male Sprague-Dawley rats maintained on Purina rat chow to the time of sacrifice were decapitated and livers rapidly removed and chilled. 350-450 mg liver slices made with the Stadie-Riggs microtome were incubated with sodium acetate-1-C^{14} at 37°C in Krebs-Ringer phosphate buffer, pH 7.4, from which Ca++ and Mg++ were omitted. $C^{14}O_2$ was collected in a KOH center well and converted to $BaC^{14}O_3$ for assay of radioactivity. At the end of incubation the slices were drained of buffer and homogenized for 3 minutes in 50 ml 1:1 acetone-ethanol in a Waring blendor.* After centrifugation the supernatant was taken to dryness on a steam bath under nitrogen, and the dried residue saponified by autoclaving 4 hours at 15 pounds with 5 ml 3 N KOH. Then 5 ml absolute ethanol were added and the non-saponifiable lipid extracted with three 30-ml aliquots of n-heptane. This was back-washed with 1 N KOH and water. Aliquots of the concentrated heptane extract were dried on steel planchets and counted directly to determine ***non-saponifiable lipid radioactivity***. In several experiments

* We are indebted to Dr. Joseph H. Bragdon for this simple procedure which he has found to give essentially complete extraction of liver lipids.