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Stem cell in peripheral blood: a study by parabiosis of irradiated and non-irradiated animals. I. New approach to parabiosis as a method for hematologic study*

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Abstract

1. For the purpose to obtain parabiotic rats having well maintained humoral circulation, the author observed parabionts having coerio-anastomosis and vascular anastomosis. 2. In the parabiotic rats having coerio-auastomosis when one of the parabionts was prevented from taking food and water by mouth sealing, the animals died within 5 to 6 days just as the control animals subjected to complete starvation, indicating that in coerio-anastomosis no appreciable humoral exchange was established between the two parabionts. 3. In vascular parabiosis having cross anastomosis of the aortas with polyethylene tubules, the animals died about 24 hours after the operation because of blocking thrombosis formed in the polyethylene tubules. 4. In the vascular parabiosis having cross anastomosis of the aortas by the homologous thoracic aorta animals did not survive through the operation but those having parallel anastomosis of the aortas survived after the operation for 3 weeks at largest and they seem to serve as useful tool for the parabiosis experiment.

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STEM CELL IN PERIPHERAL BLOOD: A STUDY BY PARABIOSIS OF IRRADIATED AND NON-IRRADIATED ANIMALS

I. NEW APPROACH TO PARABIOSIS AS A METHOD FOR HEMATOLOGIC STUDY

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Parabiosis, the surgical union of two living animals, has been originally developed as a valuable tool for the research of the transmission of humoral factors aud is widely used in a variety of research fields, e. g. hematology, immunology, endocrinology, etc. In the hematologic field, this technique has been applied for the research of the transmission of cellular elements, e. g. the transmission of "Stem Cells" from non-irradiated to lethally irradiated animals (1).

At preesent, we have three methods of parabiosis; skin anastomosis with the union of skin only (BENT, 1863) (2, 3) skin and muscle anastomosis meaning the union of both muscle and skin, and coerio-anastomosis with the union of skin, muscle and abdominal cavities, SAUERBRUCH and HEYDE, 1908 (4) Among these the coerio-anastomosis is considered to be the best in respect to the humoral communication between two animals and is now most widely used. However, it is quite uncertain to what extent the two animals may be kept under the well communicated humoral exchange condition by these methods.

For example, the body fluid exchange test as estimated by observing the distribution of various substances such as organic dyes, bacteria, nucleated red cells, red cells labelled with Fe^{59}, P^{32}, Cr^{51}, etc, introduced into one of the animals often gave poor results (7, 8, 15, 16) a marked difference was found in the cellular constituents of the peripheral blood between non-irradiated and lethally irradiated parabionts jointed by coerio anastomosis; i. e. red cell and white cell counts of the normal partner were usually 2 times greater than those the irradiated one, though the average rate of cross exchange of blood was estimated as 0.64 per cent of the blood volume per minute (9). All these data indicate some incompleteness of humoral and cellular communication between two parabionts

even in the coerio-anastomosis. So, the author aimed to observe how long the mouth-sealed rat would survive through the supply of nourishment from the normal parabiont in coerio-anastomosis.

I) *Observations on the Mouth-Sealed Rat with Unsealed Partner in Coerio-Anastomosis :*

Fifteen adult male rats of Wister strain of about 200 g body weight, about 3 months old, were used. Ten of these animals were used for coerio-anastomosis, making 5 pairs of parabionts. The other five animals served as controls having their mouths sealed. As the technique for the parabiosis, the method devised by BUNSTER add MEYER 1933 (10) was used. Each animal had his littermate as parabiont.

Ten days after the union, the mouth of one animal was sealed completely by suturing both lips to keep the animal from taking food and water. In the five starving controls their mouth was sutured. The intact partners were kept on standard diet (Oriental Kobo) and water, but all the mouth-sealed parabionts died from starvation within 5—6 days after the mouth suture, and one day later the normal par tner died. All the control animals also died 5 to 6 days after the mouth suture as in the case of the mouth sealed parabiont. The results indicated that there developed no appreciable humoral communication between the two animals joined by the coerioanastomosis.

Then, the author tried to join two animals by vascular parabiosis with arterial anastomosis so as to obtain the parabiotic animals having a complete exchange circulatory system.

II) *The Rats Joined with Vascular Parabiosis by Using Polyethylene Tubules :*

Forty adult male rats of Wister of about 3 months old, 200—300 g body weight, were used. With these animals 20 pairs of litter mate parabiotic animals having aortic anastomosis were obtained (Fig. 1).

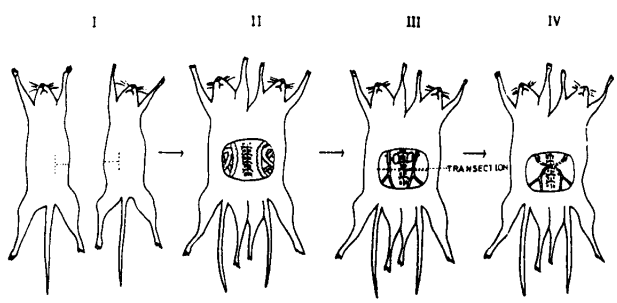

Fig. 1 Schematic drawing of the procedure of the vascular parabiosis by polyethylene tubes

To connect the aorta, polyethylene tubules of 1.5 mm in diameter were used. Prior to the operation, the animals were anesthesized with sodium Nembutal (60 mg/ml Abbott) by injecting a diluted solution (1 : 10 v/v) intramusculary, 0.5 cc per 100 g body weight. The supposed partners received lateral abdominal insisions, about 2.5 cm in length, left or right, respectively, and then cross sections in the middle of the first incisions reaching to the left and right lumbar muscle were made to form "T" sections. (Fig. 1—I). Two animals were joined by suturing the skin of the dorsal sides of the wounds and then the lumbar muscle (Fig. 1—II).

The abdominal cavities were opened and the abdominal aorta was exposed dissociating the retroperitoneum from the retroperitoneal tissues. Then the abdominal aorta was clamped with bulldog clamps at two points just above the bifurcation of the iliac artery and just under the renal artery and transected in the middle (Fig. 1—III). Then the heparinized tubules of 2 cm in length were inserted first into the distal end of aortal in both animals and ligated with 3—0 silk suture and the tubules were filled with blood by slightly loosening the clamps. Then each tubule conitaining blood was connected to the proximal end of the aorta of the partners. The two upper bulldog clamps were removed at the same time. The conjugated vessels were covered carefully with the peritoneum and the muscles and skin were sutured.

All the animals died within 24 hours and autopsy revealed the thrombotic obstruction of the polyethylene tubules.

III) *The Rats Joined with Vascular Parabiosis by Transplanting Homologous Aorta :*

In this experiment 100 male Wister rats of about 3 months old weighing 250—300 g were used. Parabiotic partners were of the same litter and chosen from those of nearly the same body weight, less than 10 g in difference. The animals were divided into two groups ; one group consisting of 40 animals was parabiosed by cross anastomosis of the aorta by the routine method (Fig. 2A). and another group of 60 animals was parabiosed in the opposite direction by pararell anastomosis of the aorta (Fig. 2B).

The thoracic aorta from young rats of 30—40 days old served as grafting materials. Rats were sacrificed by decapitation, opening the thoracic and abdominal cavities, the lung, heart, liver and diaphragm were removed in order to expose the aorta. Then the abdominal aorta was cut off at the middle point. A stainless needle core inserted from the cut end into thoracic and arc aorta was slightly raised and each intercostal arteries was ligated and cut off from the aorta in the order from the lower to the upper ones. Then the aorta was taken out and preserved in cold saline

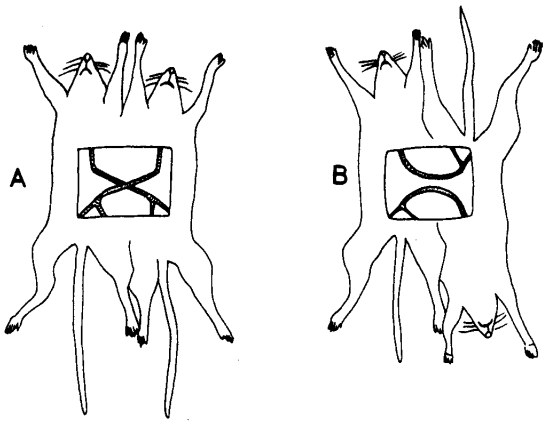

Fig. 2 Diagrammatic drawing of two ways of vascular parabiosis
A: Cross anastomosis of aorta
B: Parallel anastomosis of aorta

and used within 30 minutes. Before use, the aorta was cut off at both ends, just under the subclavical branch and just above the coeliacal branch, and the thoraice aorta was used as the graft.

A) *The Rats Joined with Cross Anastomosis of Aortas :* Twenty parabiotic couples were formed by this method. Prior to the operation the animals were anesthesized by the method as described in the foregoing anastomosis and they underwent the operation in nearly the same way as those in the vascular anastomosis with polyethylene tubules with some modification. After dissociating the retroperitoneum, the left kidney was removed after ligating the left renal artery. Then the aortas were clamped at two points just as in the rats that had the aortic anastomosis with polyethylene tubules and transected at the middle point of the abdominal aortas. The abdominal aortas were filled with heparin solution (100 units) and the needle core having fresh aorta to be grafted was inserted into the distal end of aorta of the host so as to have

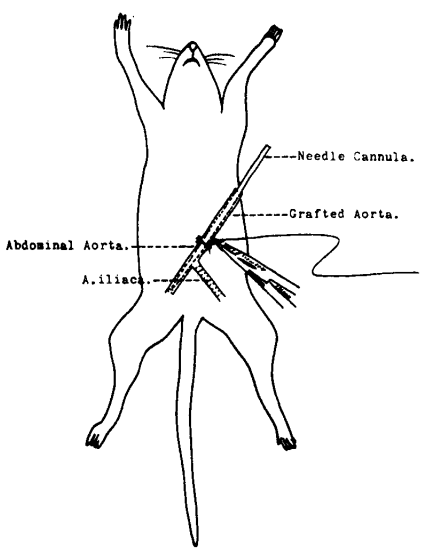

Fig. 3 Mode of anastomosis of the grafting aorta with the distal end of the host aorta by using needle core

the ends of two aorta come in close contact, then loosening the clamps (Fig. 3). The junction of two aortas was sutured at two points by 8—0 nontraumatic monofilament nylon suture under the binocular stereomicroscope and the cemented with Alon Alpha (SANKYO Co), avoiding the adventitial tissue to turn into the inner part of the aorta.

Finally, the needle cores were removed and the grafted aortas clamped. In the similar way into the proximal end of the aorta was inserted the needle core through the cut end of the left renal artery. The end of the needle core was further inserted into the end of the aorta just grafted to the partner animal (Fig. 4) and the suture of the aorta was made just as

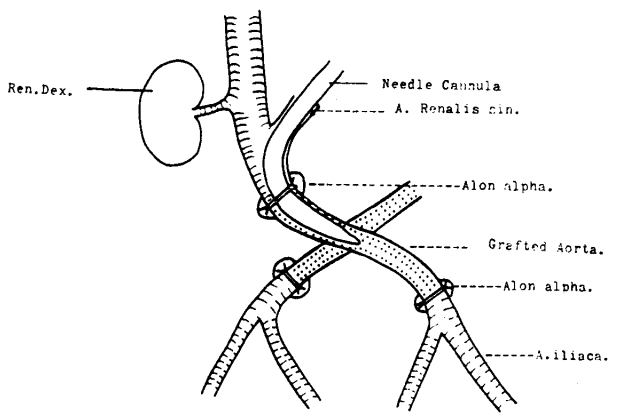

Fig. 4 Diagramatic picture of the anastomosis of the aorta of the parabionts by grafting a homologous thoracic aorta. The anastomosis is completed by removing needle core and ligating the left renal artery

described. Eventually the needle core was removed and the renal artery was ligated. In the same way another end of aorta was anastomosed with the proximal end of the partners aorta by means of another fresh aorta-graft. Covering the operated part of the aorta with the retroperitoneum, the muscle and skin were sutured. In this series of experiments all the animals died about one hour after the operation, probably because of the short length of grafted aorta, resulting in the serious disorientation of the aortas and attached vessels and organs. No thrombotic change was observed excepting the case of technical errors which resulted in the turning of the adventitial tissue into the inside of the anastomosed aorta.

B) *The Rats Jointed with Parallel Anastomosis of Aortas :* For this series of experiment about 70 animals were used forming more than 30 parabiotic couples.

The animals were anesthesized and operated as in the cases of those of group A, however, the animals were placed in the opposite direction. In this situation, the proximal end of the aorta of one animal came closer to the distal end of the aorta of the partner, making it easier to anastomose them, in spite of the limited length of the thoracic aortas to be grafted. After completion of the vascular conjugation, the two grafted aortas were put parallel in close contact, covered with the peritoneum, and the muscle and skin were sutured leaving only a narrow space for the grafted aortas so as to keep the intestine into the individual abdominal cavities, which is necessary to avoid accidental ileus seen occationally in coerio-parabiosis.

In this group, the animals survived throgh the operation for more than 3 weeks. And they died by developing symptoms of the so-called parabiotic intoxication (5, 13, 14, 15, 16) but not directly by the ill effect of vascular anastomosis itself.

C) Grafting of aorta : In a few animals the abdominal aortas were removed and grafted with homologous thoracic aorta by same method as those in groups A and B. The animals survived through the operation quite well. Observations over 30 days after operation proved successful transplantation without any rejecting reaction.

Through these observations, it seems that the parabiotic rats having parallel anastomosis of aorta serve as useful tools for the parabiosis experiment.

SUMMARY

1. For the purpose to obtain parabiotic rats having well maintained humoral circulation, the author observed parabionts having coerio-anastomosis and vascular anastomosis.

2. In the parabiotic rats having coerio-auastomosis when one of the parabionts was prevented from taking food and water by mouth sealing, the animals died within 5 to 6 days just as the control animals subjected to complete starvation, indicating that in coerio-anastomosis no appreciable humoral exchange was established between the two parabionts.

3. In vascular parabiosis having cross anastomosis of the aortas with polyethylene tubules, the animals died about 24 hours after the operation because of blocking thrombosis formed in the polyethylene tubules.

4. In the vascular parabiosis having cross anastomosis of the aortas by the homologous thoracic aorta animals did not survive through the operation but those having parallel anastomosis of the aortas survived after

the operation for 3 weeks at largest and they seem to serve as useful tool for the parabiosis experiment.

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