clones remained alive. Peters and Shio[16] have demonstrated the presence of D-valine amino acid oxidase in suspensions of pure epithelial cells from rat jejunum.

Despite the lengthy maintenance in culture, none of the original epithelioid colonies or strains were seen to form the expected characteristic columnar cells with distinct brush borders, as found in the differentiated mucosal epithelium. This absence of terminal differentiation in vitro may have been due to the presence of an inappropriate hormonal milieu[17], or, alternatively, as these cells grew in isolated colonies, to a lack of contact inhibition thought to be necessary for differentiation[18].

The present study indicates that axenic animals are suitable for the establishment of cultures of intestinal cells and that epithelioid cells can be isolated and selectively grown. The identification of these epithelioid cells as crypt cells remains to be established. However, subsequent to the original submission of this report, Quaroni et al.[19] have published a comparable method of culturing undifferentiated intestinal epithelial cells from germ-free rats. These latter workers have provided morphological and immunological evidence which suggests that these cells are originating in the crypts of the intestinal mucosa.

1 Supported by the Medical Research Council (Canada). The authors gratefully acknowledge the advice of Dr P. Rae of this institute.

2 G. Henle and F. Deinhardt, J. Immun. *79*, 54 (1957).
3 L. Lichtenberger, L.R. Miller, D.N. Erwin and L.R. Johnson, Gastroenterology *65*, 242 (1973).
4 C.F.A. Culling, P.E. Reid, L.S. Trueman and W.L. Dunn, Proc. Soc. exp. Biol. Med. *142*, 434 (1973).
5 A.G.D. Hoffman and A. Kuksis, Gastroenterology *72*, 1160a (1977).
6 C.M. Schiller, In Vitro *13*, 198 (1977).
7 F. Raul, M. Kedinger, P. Simon, J. Grenier and K. Haffen, Biol. cell. *33*, 163 (1978).
8 P. Pothier and J.S. Hugon, J. Cell Biol. *79*, 86a (1978).
9 A.G.D. Hoffman and A. Kuksis, manuscript submitted.
10 R.P. Spencer, The Intestinal Tract. Ryerson Press, Toronto 1960.
11 J.T. Emerson and D.R. Pitelka, In Vitro *13*, 316 (1977).
12 R.G. Ham, in: Tissue Culture - Methods and Applications, section V, chapter 3. Ed. P.F. Kruse, Jr, and M.R. Patterson, Jr. Academic Press, New York 1973.
13 S.F. Gilbert and B.R. Migeon, Cell *5*, 11 (1975).
14 D. Perlman, in: Cell Culture, section 7. Methods in Enzymology, vol. 58. Ed. W.B. Jakoby and I.H. Pastan. Academic Press, New York 1979.
15 L.R. Johnson, Gastroenterology *72*, 788 (1977).
16 T.J. Peters and H. Shio, Clin. Sci. molec. Med. *50*, 355 (1976).
17 A.M. Carriere, Anat. Rec. *156*, 423 (1966).
18 H. Green, Cell *11*, 405 (1977).
19 A. Quaroni, J. Wands, R. Trelstad and K.J. Isselbacher, J. Cell Biol. *80*, 248 (1979).

The effects of parabiotic union with a normal partner on the blood tissue of the b/b rat suffering from an inheritable anemia

P.N. Martinovitch, D. Pavić and N. Živković

Boris Kidrič Institute, P.O. Box 522, Yu-11000 Beograd (Yugoslavia), 7 May 1979

Summary. The parabiotic union of a b/b rat, suffering from a red blood cell anemia, with a normal partner will restore to normal the functioning of this type of blood cell. The effect of parabiotic union is evident for several weeks following the separation of the parabiotic partners. It would be logical to conclude that the normal partner supplies a factor absent in the anemic animal needed for normal functioning of the red cell.

In 1962 after exposing a number of 8-day-old female rats to a dose of 50 R of X-rays, in the second filial of one of the irradiated animals 2 males and 1 female with an inheritable anemia were born. This anemia, recessive in nature, is characterized, among other defects, by microcytosis of red blood cells and hypochromia. Attempts to determine whether the disease appeared spontaneously or was induced by X-rays have, so far, failed. A number of results obtained from the study of this inheritable syndrome have already been published[1-4].

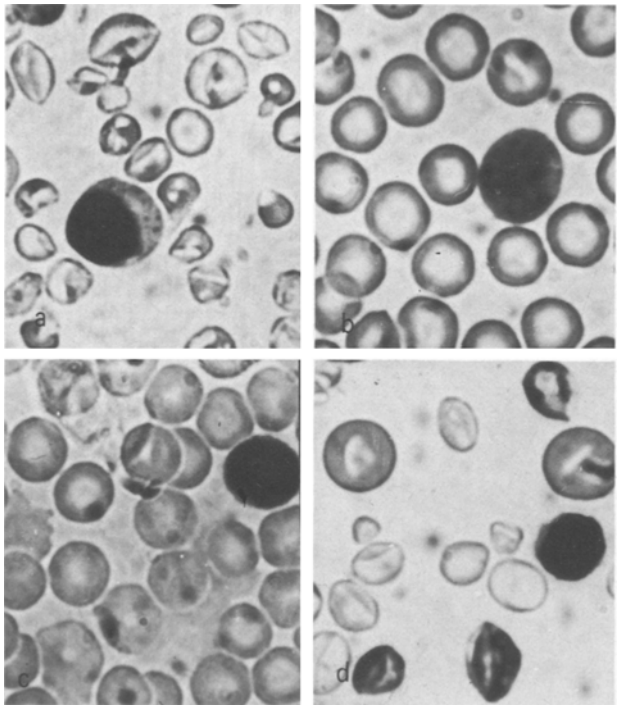
More recently, we have applied the standard technique of parabiosis between an adult normal rat and an anemic animal of the same age, keeping them in parabiotic union for up to 4 weeks. The technique of parabiosis applied by us involves skin union alone. From the normal rat a rectangular piece of tissue 2.5 cm in length and 2 cm in width starting from the blade-bone down is cut off. The wound left is covered by an equal piece of tissue supplied by the anemic partner. For suturing the wound we used the sterile catgut (Resorbierbarkeit normal 00, Veb Catgut, Markneukirchen), and for immobilizing the animals leucoplast No. 1517, a product of the pharmaceutic company 'Galenika', Beograd, which, allowing an intimate contact between the 2 partners, assured undisturbed healing of the wound. The operation was performed under semisterile conditions. Not 1 case of wound contamination has been recorded. 4 pairs of animals were used.

It was observed that, already after 3, 4 or 5 days of life in parabiotic union, both the peripheral blood and the bone marrow pictures of the anemic partner gave visible signs of uniform improvement. After 3-4 weeks, by applying the tests commonly used in the study of blood, no significant deviation from the standard values characteristic of the normal rat could be detected in the anemic partner (table), and the peripheral blood regained its normal appearance (figure, A and B). The capacity of even the shrunken and

Peripheral blood indices of anemic (b/b) and normal (+/+) rats before, during and after parabiotic union

	Animals	RBC ($\times 10^6$)	Hct (°/°)	Hb (g)	MCH (ng)	L ($\times 10^3$)	Range
Before	b/b	7.8	28	4.9	6	19	13-25
parabiosis	+/+	8.1	50	12.6	15	13	8-18
3 weeks in	b/b	7.1	44	10.8	15	15	8-22
parabiosis	+/+	7.1	44	10.5	14	14	12-17
3 weeks after	b/b	6.3	35	7.4	11	25	9-50
separation	+/+	8.6	50	12.4	14	11	8-16
14 weeks after	b/b	5.0	22	3.7	7	20	14-24
separation	+/+	8.5	47	12.6	16	13	8-18

Mean values for 4 pair of rats: RBC, red blood cells; Hct, hematocrit; Hb, hemoglobin per 100 ml of blood; MCH, mean corpuscular hemoglobin; L, leucocytes.



Red blood cells of b/b rat before, during and after parabiotic union with an normal partner. *A* Before parabiosis; *B* 4 weeks in parabiosis; *C* 5 weeks following separation; *D* 14 weeks following separation. × 2200.

shrivelled red blood cells to recover, and acquire a practically normal appearance, suggests that perhaps the structure of these cells is not irreparably damaged. The presence of red blood cells belonging to the anemic partner in the blood of the normal rat has been observed. The presence of red blood cells from the normal partner in the anemic animal cannot be easily established, as some normal looking red cells are always to be found in the peripheral blood of the anemic rat. From each pair of experimental rats closely comparable results were obtained. After separating the partners many of the peripheral red blood cells retained their normal appearance for 4–5 weeks (figure, C). With the further passage of time the circulating blood continued to undergo gradual but steady deterioration. 14 weeks following separation the peripheral blood returned to its original defective state (figure, D).

The effect which the parabiotic union had on the general health of the anemic rat was equally impressive. It is not without interest that in anemic rats very often the number of white blood cells may be considerably greater than in their normal controls (table), a fact totally overlooked in our previous studies.

1 D. Sladić-Simić, D. Pavić, N. Živković, D. Marinković and P.N. Martinovitch, J. Br. Radiol. *36*, 542 (1963).
2 D. Sladić-Simić, N. Živković, D. Pavić, D. Marinković, J. Martinović and P.N. Martinovitch, Genetics *53*, 1079 (1966).
3 D. Sladić-Simić, P.N. Martinovitch, N. Živković, D. Pavić, M. Kahn and H.M. Ranney, Ann. N.Y. Acad. Sci. *165*, 93 (1969).
4 D. Sladić-Simić, N. Živković and D. Pavić, J. Eur. clin. Biol. Res. *17*, 197 (1972).

Effects of loading density on catfish blood[1]

Sylvia A. Murray

Tennessee Valley Authority, Office of Natural Resources, Water Quality and Ecology Branch, Muscle Shoals (Alabama 35630, USA), 3 May 1979

Summary. Results from this study indicate that a fish density stress syndrome exists for red blood cell morphology. Smaller (by 3.5%) and rounder (by 0.6%) red blood cells were consistently found in intensive fish cultures.

Some effects of overstocking fish ponds include smaller size fish, behavioral changes in the fish, and even marked fish losses[2,3]. These effects may result in excessive cost to fisheries managers at fish hatcheries and farms, and also make assessments of bioassay data in the laboratory more difficult. Since fish are frequently used to test water quality, it is important to isolate the toxicant effect (at sublethal concentrations) from the effect of culturing practices.

There have been few studies on physiological effects of crowding stress. Hematological changes have been identified with crowding stress for salmon and goldfish[4–7]. This paper reports additional effects of intensive crowding on catfish hematology.

Materials and methods. Channel catfish (*Ictalurus punctatus* Rafinesque) were cultured at 4 different loading densities, ranging from 0.18 kg/m^3 for the controls to 224 kg/m^3, 337 kg/m^3, and 433 kg/m^3 for the intensive cultures. Fish ranged in age from 1 to 4 years. Dissolved oxygen was at saturation levels, and water temperatures were similar for all cultures.

Control fish were collected by angling from a commercial fishout pond in northwestern Alabama. The intensive cultures were kept in concrete raceways (15.2 × 1.2 × 1.1 m) at Gallatin, Tennessee. Water replacement time of the 20,000 liter raceways was about 25 min. Raceway fish were caught with a dip-net.

Fish used in this study were free of epizootics and ranged in size from 0.22 to 0.45 kg. Samples were collected from February to August 1973. Blood was collected by cardiac puncture, and blood smear slides were made. Wright's stain was used to differentiate cell types. Length and width measurements of red blood cells were taken from camera lucida drawings. Cell roundness was determined from the width/length ratios. Cell area was calculated by multiplying the width-length product by $\pi/4$.

10 fish from each of the 4 cultures, with 50 cell measurements per fish, were used to test for density differences. A nested 1-way analysis of variance test was used for the determination. The 95% least significant difference test was used to locate differences between the means[5]. In addition measurements from 20 control fish were used to determine differences in sample size. A t-test was used to compare the 2 groups: 50 measurements vs 250 measurements.

Results. The effects of intensive culture practices of catfish on red blood cell morphology are summarized in table 1. These results indicate 3 distinct groupings of the data for cell length, width, and area, viz the control density (0.18 kg/m^3), medium density (224 kg/m^3), and high densi-