not present in sufficient numbers during the summer months to inhibit the growth of *Cl. botulinum* type F, but since bacitracin production is apparently related to spore-formation in the organism (*6*), it is suspected that certain factors enhancing sporulation are present during winter but not summer months.

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7. Supported in part by the North Dakota Water Resources Research Institute with funds provided by the U.S. Department of Interior, Office of Water Resources Research under P.L. 88-379.
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31 October 1966

Erythrocyte Chimerism after Injection of Spleen Cells into Anemic Mice of the W-Series

Abstract. *Anemic mice* (W^vW^v) *when injected at birth with a mixture of isologous (genotype* W^vw) *and homologous spleen cells, showed an improvement in their peripheral blood picture when adult. Red blood counts, red cell size, and electrophoresis of the hemoglobins indicated that, in some cases, the homologous tissue had become implanted and, in others, the isologous tissue was functioning.*

After a lethal dose of irradiation to a mouse, death can be averted by the administration of spleen cells from a healthy donor (*1*). Subsequently, such animals become red-cell chimeras (*2*). Spleen is also commonly employed to create immunological tolerance, by administration at birth, which results in cellular chimerism. Donor cells have regularly been demonstrated in the host lymphoid system (*3*), and Wilson and Talmage (*4*) have provided immunological evidence that such animals are also red cell chimeras.

Mice of the genotype W^vW^v have a genetically determined lifelong macrocytic anemia. Hematopoietic tissue from normal animals will implant in these animals and transform the blood picture to normal. Bernstein and Russell (*5*) used isologous and Seller and Polani (*6*) used homologous fetal liver cells as the source of hematopoietic tissue. Seller (*7*) subsequently showed that when the donor's hemoglobin differs from that of the recipient, its presence can be detected in successfully treated animals a year or more after treatment.

In the work reported here anemic mice W^vW^v were treated with spleen cells as an alternative source of hematopoietic tissue. The W-series mice (*9*) were a mixed stock with CBA/Gr and C57BL/Gr as part of their background. The donor animals were a pure-line, CBA strain maintained at Guy's Hospital, London. In early experiments, it was found that skin grafts exchanged between CBA animals and mice of the W-series were rejected in 11 days.

Originally, a spleen cell suspension prepared from adult CBA mice was injected intravenously into the W^vW^v animals a few hours after birth. However, initial experiments showed that the recipients succumbed to runt disease at about 21 days of age. Only one animal survived to adulthood. Russell (*8*) showed how death from runt disease may be circumvented by the addition of isologous spleen cells to the homologous inoculum. Accordingly, spleen cells from animals of the genotype W^vw were also injected. This led to an increase in the survival rate, although there was some evidence of slight runting during the juvenile period in the survivors, with subsequent recovery in adulthood, although an occasional survivor did die.

The whole spleens were removed from one adult CBA and one adult W^vw mouse; they were cut into fragments, sieved through one layer of bolting silk (14N, St. Martins), and rinsed with Hanks balanced salt solution (BSS), *p*H 7.2. This treatment produced cell clumps, which were broken down to single cells by drawing them (approximately 20 times) through two layers of bolting silk contained in a filtering nozzle of a hypodermic syringe. The suspension was centrifuged at 1000 rev/min for 5 minutes and the cells were resuspended in 0.5 ml of BSS. The genotypically mixed cells (10 to 15 $\times$ 10^6) were injected into the anterior facial vein of newborn W^vW^v recipients.

At varying intervals during maturation the peripheral blood picture was examined. Blood was taken from the tail of the animal. Hematocrit values were estimated with the Hawksley microhematocrit centrifuge, but very small capillary tubes (3 cm by 1 mm) containing heparin were substituted for the usual tubes. Red blood counts were made by the conventional hemocytometer method with Hayem's fluid as the diluent.

Electrophoresis was performed on the hemoglobins when the mice were approximately 6 months old. Cellulose acetate paper was used as the supporting medium in a barbitone buffer (5,5-diethylbarbituric acid), *p*H 8.6, ionic strength 0.05, and the electrophoresis was conducted for 3 hours at 5 volt/cm. Afterward, the papers were stained with a 0.2 percent solution of Ponceau S in 3 percent trichloroacetic acid.

In all ten W^vW^v mice that survived to adulthood after treatment at birth with 10 to 15 $\times$ 10^6 CBA/W^vw spleen cells, the peripheral blood picture changed from the anemic type. The extent of the change was not the same in all the animals. While in some, the blood picture became that of a hematologically normal mouse—the criteria being red blood counts, hematocrit values, and mean corpuscular volumes—

Table 1. Red blood counts, hematocrit values, and mean corpuscular volumes of CBA, W^vW^v, and W^vw mice, and of W^vW^v mice treated at birth with a CBA/W^vw spleen cell mixture.

Animals (No.)	Red cells ($\times 10^6$/mm^3)		Hematocrit (%)		Mean corpuscular volume μ^3	
	Mean	Range	Mean	Range	Mean	Range
			W^vW^v			
15	7.20	5.48– 8.11	38.2	31.3–46.5	53.7	41.3–71.0
			CBA			
7	11.97	11.61–12.61	43.9	39.8–49.1	36.6	32.8–38.9
	W^vW^v treated with W^vw/CBA spleen, with CBA hemoglobin					
6	11.77	11.17–12.68	45.1	41.6–47.2	40.1	37.2–43.8
	W^vW^v treated with W^vw/CBA spleen, with W hemoglobin					
4	10.28	9.89–10.55	43.5	40.8–45.6	42.2	38.9–46.2
			W^vw			
11	9.89	9.16–10.80	42.5	34.1–47.1	43.1	35.6–50.9

in others the red blood cell count remained below normal. It appeared that in these animals, the transplantation had only been partially successful. However, a possible explanation suggested itself upon electrophoresis of the hemoglobins.

Mice of the W-series have a hemoglobin which on electrophoresis gives a single band. The hemoglobin pattern in CBA mice is diffuse and comprises two well-defined bands. The major, fast component of the hemoglobin corresponds to the single band of the W-series.

Electrophoresis of the hemoglobins of the ten mice revealed that the animals fell into two categories. Six mice had the characteristic two-banded CBA pattern, whereas four had the single W-type pattern (Fig. 1). When the two series of data were compared it was found that the six mice which had the CBA hemoglobin were the animals whose blood picture had become completely normal, and the four animals with the W-type hemoglobin were those in which the red blood count was lower (Table 1). There was no overlap between these two groups, the red blood counts being 11.17 to 12.68 $\times$ 10^6 for the CBA hemoglobin group and 9.89 to 10.55 $\times$ 10^6 for the W hemoglobin group. The difference between the two groups was significant ($P = .01$ to .001, by t-test). The red blood counts of the W-type hemoglobin group corresponded closely to those of W^vw mice—9.16 to 10.80 $\times$ 10^6 ($P = .3$ to .2).

The single animal that survived the CBA spleen alone also showed a permanent change in the peripheral blood picture to one typical of a normal mouse and had hemoglobin of the CBA electrophoretic mobility.

Thus the injected spleen cells have become implanted on the host animals,

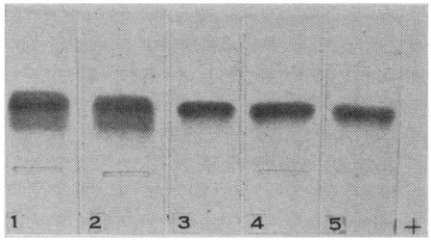

Fig. 1. Electrophoresis of hemoglobins of CBA strain, W^vW^v, and W^vw mice, and W^wW^w mice treated with CBA/W^vw spleen cell mixture. 1, CBA; 2, W^wW^w, treated with CBA/W^vw spleen; 3, W^wW^v; 4, W^wW^v, treated with CBA/W^vw spleen; 5, W^vw.

and the erythropoietic elements are functioning normally to produce erythrocytes. However, it appears that when a mixture of homologous CBA cells and isologous W^vw cells was offered, it was a random chance whether the CBA cells or the W^vw cells implanted. In the case of the animals which showed the CBA diffuse type of hemoglobin, it cannot be stated with certainty that there is no W^ww tissue functioning, but in the remaining four mice there is no doubt concerning the absence of CBA erythropoietic cells.

When Bernstein and Russell (*5*) first transplanted hematopoietic tissue into the W-series anemics, they suggested that the tissue implanted and continued to function according to its genotype. The present work may well provide evidence to support this hypothesis. The heterozygotes W^ww of the W-series mice have an anemia, although it is less severe than that possessed by the W^vW^w mice. They also have a very slight macrocytosis. The W^vW^w injected mice, which on electrophoretic evidence had W^vw tissue implanted, had a slightly anemic blood picture and slight macrocytosis of the red cells, when compared with the hematologically completely normal picture of the CBA-treated individuals. In these two cases, therefore, there seems to be readily observable proof of the transplanted cells functioning according to their genotype.

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31 October 1966

Biosynthesis of DNA by Isolated Mitochondria: Incorporation of Thymidine Triphosphate-2-C^{14}

Abstract. *Thymidine triphosphate-2-C^{14} and other deoxynucleoside triphosphates are incorporated into the DNA of isolated mitochondria from rat liver cells. The reaction is partially dependent on the other deoxynucleoside triphosphates and proceeds in the virtual absence of bacteria. The product has the properties of mitochondrial rather than nuclear DNA, and end-group studies indicate labeling of the interior of the molecule.*

The control of mitochondrial structure and function by cytoplasmic (*1*) as well as by nuclear (*2*) genes, the "nonconservative" replication of certain structural elements of mitochondria (*3*), and the presence in mitochondria of DNA (*4*), RNA (*5*), and a protein biosynthesis system (*6*) point to the semi-independence of these organelles. We now report on the incorporation of TTP-2-C^{14} (*7*) into the DNA of isolated mitochondria from rat liver, a reaction which may represent DNA replication.

The mitochondria were isolated according to the method of Schneider and Hogeboom (*8*) except that 0.001*M* EDTA, *p*H 7.0, was included in the isolation medium and all mitochondrial pellets were washed four times. In early experiments, mitochondria were further purified by centrifugation through 1*M* sucrose at 8500*g* for 30 minutes. In later experiments, sedimentation-velocity centrifugation in a continuous sucrose density gradient was used. In a gradient of 0.6 to 1.5*M* sucrose, sedimentation at 8500*g* for 20 minutes resulted in a somewhat diffuse band at a sucrose concentration averaging about 1.2*M*; in a gradient of 0.3 to 0.6*M* sucrose at 2543*g* for 20 minutes, banding occurred at about 0.5*M* sucrose. Immediately after the sedimentation, the mitochondrial band was carefully removed with a Pasteur pipette, and this material was diluted with water to a sucrose concentration of 0.25*M*. In the case of the more strongly hypertonic gradient, this dilution was performed as quickly as possible to minimize the period of contact of the mitochondria with the hypertonic sucrose. (Experiments done for another purpose showed that