alopathy. We suspect that in hepatic coma there is a derangement of cerebral glutamine metabolism involving either disruption of the enzyme linkage, or a decrease in ω-amidase activity.

It has been reported that certain pyrrolidone derivatives including 5-oxoproline, a dehydrated analog of 2-hydroxy-5-oxoproline, selectively antagonize the effects of the excitatory amino acid, L-glutamatic acid, on neural tissue (*20*). If α-KGM (cyclic) behaves similarly, its accumulation might impair neurologic function by competing for glutamic acid receptors in brain. Such a mechanism could account for the reported effectiveness of glutamate administration in the treatment of some patients in hepatic coma (*21*). However, before one seeks potential treatments to counteract α-KGM in hepatic coma, it must be more firmly established that the compound itself depresses the central nervous system.

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References and Notes

1. K. J. Breen and S. Schenker, in *Progress in Liver Diseases*, H. Popper and F. Schaffner, Eds. (Grune & Stratton, New York, 1972), vol. 4, p. 301.
2. M. Cole, R. B. Rutherford, F. O. Smith, *Arch. Neurol.* **26**, 130 (1972).
3. F. Plum, *Exp. Biol. Med.* **4**, 34 (1971).
4. S. P. Bessman and A. N. Bessman, *J. Clin. Invest.* **34**, 622 (1955).
5. J. Shorey, D. W. McCandless, S. Schenker, *Gastroenterology* **53**, 706 (1967); B. Hindfelt and B. K. Siesjö, *Scand. J. Clin. Lab. Invest.* **28**, 365 (1971); F. Vergara, T. E. Duffy, F. Plum, *Trans. Assoc. Am. Physicians Phila.*, in press.
6. K. S. Warren and S. Schenker, *J. Lab. Clin. Med.* **64**, 442 (1964).
7. B. Hindfelt, *Scand. J. Clin. Lab. Invest.* **31**, 289 (1973).
8. H. F. Bradford and H. McIlwain, *J. Neurochem.* **13**, 1163 (1966).
9. A. Meister, *J. Biol. Chem.* **200**, 571 (1953).
10. M. Sugiura, *Jap. J. Pharmacol.* **7**, 1 (1957); T. Yoshida, *Vitamins (Kyoto)* **35**, 227 (1967).
11. J. Folbergrová, J. V. Passonneau, O. H. Lowry, D. W. Schultz, *J. Neurochem.* **16**, 191 (1969); T. E. Duffy, S. R. Nelson, O. H. Lowry, *ibid.* **19**, 959 (1972). Glutamine was assayed as glutamate after incubation with *Escherichia coli* glutaminase. CSF extract (50 μl) was added to 100 μl of 100 m*M* citrate-phosphate buffer (*p*H 4.8) which contained glutaminase (0.2 mg/ml). The mixtures were incubated at 37°C for 1 hour, at which time the hydrolysis was complete. Portions (30 μl) of the mixtures were added to 1.0 ml of a reagent containing 50 m*M* tris-HCl (*p*H 8.5), 10 m*M* hydrazine, 0.5 m*M* NAD (nicotinamide adenine dinucleotide) and 0.1 m*M* ADP (adenosine diphosphate). The reaction was started by the addition of glutamate dehydrogenase (0.1 mg/ml), and followed fluorometrically. Glutamate, derived from glutamine, was calculated by difference from the independently measured glutamate concentration.
12. α-Ketoglutaramate (barium) was prepared according to Meister (*9*). Elemental analysis as calculated from the formula $C_5H_6NO_4Ba_{1/2}$ is C, 28.22 percent; H, 2.84 percent; N, 6.58 percent; Ba, 32.27 percent; the amounts found were C, 27.92 percent; H, 3.02 percent; N, 6.38 percent; Ba, 32.22 percent. One-dimensional thin-layer cellulose chromatography of the free acid in an ethanol, 25 percent ammonium hydroxide, water system (8 : 2 : 1) and an *n*-butanol, acetic acid, water system (10 : 2 : 6) gave a single spot to bromocresol green with R_F values of 0.51 and 0.45, respectively. Solutions of α-KGM were standardized by spectrophotometric measurement of ammonia released on hydrolysis at 98°C in 1*N* HCl, and by deamidation with ω-amidase and determination of α-ketoglutarate or ammonia.
13. L. B. Hersh, *Biochemistry* **10**, 2884 (1971). ω-Amidase was purified from fresh rat liver according to Hersh, up to and including fractionation on carboxymethyl-Sephadex. The enzyme preparation, essentially free of lactate dehydrogenase and NADH oxidase contamination, had an activity of 15.8 μmole/min per milligram of protein when assayed at 25°C in a reagent consisting of 100 m*M* tris-HCl (*p*H 8.5), 0.1 m*M* adenosine diphosphate, 20 m*M* α-ketoglutaramate, 40 m*M* ammonium acetate, 0.1 m*M* NADH, 1 m*M* dithiothreitol and 0.1 mg of glutamate dehydrogenase per milliliter. Glutamine α-ketoacid aminotransferase activity in the preparation was not detectable. The enzyme preparation was stored in a concentration of 0.8 mg/ml in 30 percent glycerol (suggestion of Dr. A. J. L. Cooper), and was stable for at least 6 months at 0°C.
14. C. J. van den Berg, in *Handbook of Neurochemistry*, A. Lajtha, Ed. (Plenum, New York, 1970), vol. 3, p. 355.
15. J. H. Walshe, *Q. J. Med.* **20**, 421 (1951); S. Brandstaetter and D. Barzilai, *Am. J. Digest. Dis.* **5**, 945 (1960).
16. A. Valero, G. Alroy, A. Stein, *Respiration* **28**, 137 (1971).
17. For perfusion studies, the sodium salt of α-KGM was prepared by titration of a 400 m*M* solution of α-KGM (barium) with a slight excess of Na_2SO_4 at 0°C. Precipitated $BaSO_4$ was removed by centrifugation at 0°C, and the supernatant was filtered through a sterile membrane (Swinnex-25, 0.45 μm). In view of the neurotoxicity of soluble barium salts, a group of rats were perfused for 30 minutes with 0.5 m*M* $BaCl_2$ in mock human cerebrospinal fluid. This treatment did not elicit any clinical changes in the animals. From the solubility of $BaSO_4$ in water at 25°C (0.246 mg/100 ml), the concentration of barium ion, infused as $BaCl_2$, is 200 times higher than the maximum expected concentration of residual barium in 100 m*M* solutions of α-KGM (sodium), prepared from the barium salt.
18. J. K. Merlis, *Am. J. Physiol.* **131**, 67 (1940).
19. V. Fencl, G. Koski, J. R. Pappenheimer, *J. Physiol.* **216**, 565 (1971).
20. I. L. Bonta, C. J. De Vos, H. Grijson, F. C. Hillen, E. L. Noach, A. W. Sim, *Br. J. Pharmacol.* **43**, 514 (1971); A. Van Harreveld and E. Fifkova, *J. Neurochem.* **18**, 2145 (1971); J. Davies and J. C. Watkins, *Nature (Lond.)* **238**, 61 (1972); D. R. Curtis, G. A. R. Johnston, C. J. A. Game, R. M. McCulloch, *Brain Res.* **49**, 467 (1973).
21. J. M. Walshe, *Lancet* **1953-I**, 1075 (1953); J. M. Baertl and G. J. Gabuzda, Jr., *Gastroenterology* **37**, 617 (1959).
22. C. A. Goodrich, B. Greehey, T. B. Miller, J. R. Pappenheimer, *J. Appl. Physiol.* **26**, 137 (1969).
23. We thank Dr. A. Meister for advice on the preparation and characterization of α-ketoglutaramate. This investigation was supported in part by PHS grants AM-16739 and NS-04928.

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Transplantation of Allogeneic Bone Marrow in Canine Cyclic Neutropenia

Abstract. *Transplantation of normal bone marrow cells to a gray collie dog with cyclic neutropenia resulted in normal granulocytopoiesis. The finding suggests that cyclic neutropenia occurs because the hematopoietic stem cells are defective. Because of the similarity of human and canine cyclic neutropenia, it also suggests that the human disease may be curable by marrow transplantation.*

Cyclic neutropenia occurs in both man and gray collie dogs. On the basis of marrow morphologic studies and the patterns of fluctuation of blood cell counts, it has been proposed that cycling occurs because of a regulatory defect affecting pluripotential stem cells in both species (*1*, *2*). There are several possible mechanisms for such a defect; the chief possibilities are that the marrow cells are defective or that some host environmental factor causes the marrow cell production to cycle. In order to further investigate the mechanism of cyclic neutropenia, as well as to explore a mode of therapy potentially applicable to the human disease, we have transplanted bone marrow cells from a normal collie to a gray collie. The marrow transplant resulted in normal granulocytopoiesis, ending the cyclic neutropenia.

The donor-recipient pair were a 10-month-old male collie with normal coat color and normal serial blood cell counts and an unrelated 1-year-old female sable-gray collie with well-documented cyclic neutropenia.

Blood cell counts were performed by standard techniques. DL-A typing of cells from the donor-recipient pair was performed by methods described (*3*). One-way mixed leukocyte culture responses were determined by the technique of Rudolph *et al.* (*4*) with minor modifications. The pair of dogs were DL-A identical and mixed leukocyte cultures were mutually nonstimulatory.

One day before transplantation, the recipient received 1000 rads from opposing cobalt-60 sources at 9 rad/min. Under sodium thiamylal and methoxyfluorane anesthesia, 5×10^9 bone marrow cells were aspirated with heparinized syringes from the proximal long bones of the four extremities of the donor; his recovery from this procedure was uneventful.

After filtration of the bone marrow

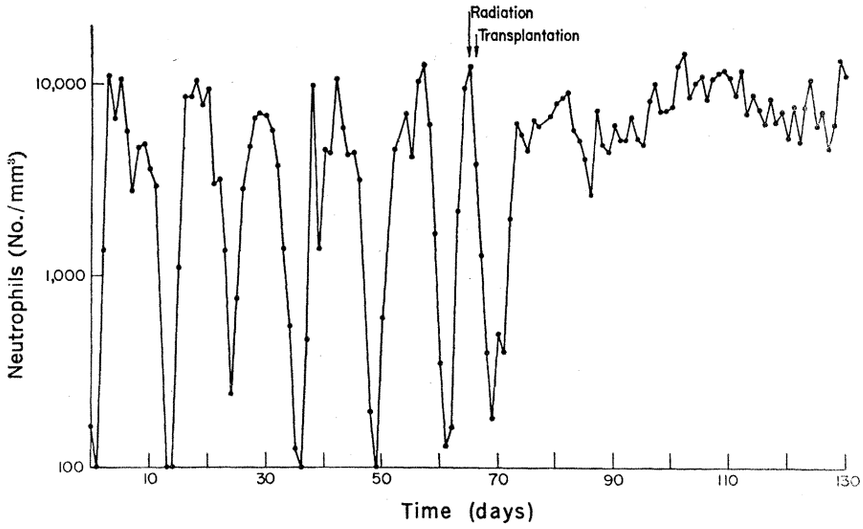

Fig. 1. Daily blood neutrophil counts of a gray collie dog before and after transplantation of normal bone marrow.

through several layers of coarse gauze and a 300-mesh stainless steel screen to remove bone spicules, the heparinized marrow suspension was administered intravenously to the gray collie (3.3×10^8 marrow cells per kilogram of body weight). The recipient was supported with parenteral fluids, antibiotics, and irradiated (2500 rads) leukocyte and platelet transfusions during the phase of radiation-induced diarrhea, leukopenia, and thrombocytopenia. No immunosuppressive drugs were administered.

Karyotype analyses of the bone marrow cells of the recipient dog were performed 7 and 19 days after transplantation (*5*).

Engraftment of the male bone marrow in the female recipient was observed 7 days after transplantation as determined by the initial rise in the blood leukocyte count and karyotype analyses of bone marrow cells. The pattern of the neutrophil fluctuations of the gray collie before and after transplantation is shown in Fig. 1. Prior to transplantation, neutropenia occurred every 12 days, which is characteristic for this disease (*6*). After transplantation there were no further periods of neutropenia. There was also a noticeable decrease in the chronic inflammation about the eyes, gum margins, and anus of the dog. From days 28 to 35 after transplantation, the dog developed mild graft-versus-host disease as manifest by mild conjunctivitis, rhinorrhea, and intermittent low-grade fever. The dog was quite well 80 days after the transplant and gave no evidence of chronic graft-versus-host disease.

The above experiment demonstrates that normal granulocytopoiesis results from engraftment of normal bone marrow cells into a lethally irradiated dog with cyclic neutropenia. This result suggests that cyclic neutropenia is a disease of the hematopoietic stem cells.

Previous studies on the mechanism of cyclic neutropenia in these dogs indicated that both erythropoietin (*7*) and colony stimulating factor varied cyclically with a period length of 12 days (*8*). The erythropoietin levels increased in the reticulocytopenic period and fell with the rise in reticulocytes. Colony stimulating factor, a substance found in urine and serum, which will stimulate bone marrow cells to divide and form granulocytic colonies in vitro (*9*), increased in the periods of neutropenia and monocytosis and fell during the phase of normal blood neutrophil counts. The cycling of these substances could be interpreted as the cause of cyclic blood cell production or as physiologic responses to some other factors causing the cell production to cycle. Radiation of an animal does not alter its capacity to produce erythropoietin or colony stimulating factor (*10*, *11*). Therefore, if the cyclic changes of these substances were causal, cyclic cell production by the transplanted marrow should have occurred. Since the marrow functioned normally we would conclude that the cyclic variations of erythropoietin and colony stimulating factor previously reported were normal physiologic responses in an animal with defective marrow cells.

Because we transplanted whole, unfractionated bone marrow, this experiment does not delineate the precise marrow element responsible for correcting the defect in cyclic neutropenia. The result suggests that it was the pluripotent stem cells which were defective, but it is also possible that some other critical marrow element was transplanted or that the irradiation prior to treatment eliminated some heretofore uncharacterized hematopoietic regulatory factor.

We have attempted to alter the cyclic fluctuations of the blood counts in these dogs by hypertransfusion, phlebotomy, repeated administrations of endotoxin, antiserum to neutrophils, testosterone, and normal and neutropenic dog plasma (*12*). Hypertransfusion and phlebotomy decreased and increased, respectively, the reticulocyte oscillations of the gray collie but did not alter the cyclic neutropenia (*7*). None of the other agents have altered the cycling (*12*). Similarly, nothing is known that will definitely alter the cyclic fluctuation of the blood counts in human cyclic neutropenia. Because of the similarity of human and canine cyclic neutropenia (*2*), the results of our experiment suggest that the human disease may also be cured by marrow transplantation.

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References and Notes

1. D. C. Dale, D. W. Alling, S. M. Wolff, *J. Clin. Invest.* **51**, 2197 (1972).
2. D. Guerry, D. C. Dale, M. Omine, S. Perry, S. M. Wolff, *Blood* **40**, 951 (1972).
3. F. T. Rapaport, T. Hanuka, T. Shimada, F. D. Cannon, J. W. Ferrebee, *J. Exp. Med.* **131**, 881 (1970).
4. R. H. Rudolph, B. Hered, R. B. Epstein, E. D. Thomas, *Transplantation* **8**, 141 (1969).
5. J. H. Tijo and J. Wang, *Stain Technol.* **37**, 17 (1962).
6. D. C. Dale, S. B. Ward, H. R. Kimball, S. M. Wolff, *J. Clin. Invest.* **51**, 2190 (1972).
7. J. W. Adamson, D. C. Dale, R. J. Elin, *ibid.* **52**, 1a (1973).
8. D. C. Dale, C. H. Brown, P. Carbone, S. M. Wolff, *Science* **173**, 152 (1971).
9. D. Metcalf and M. A. S. Moore, *Haemopoietic Cells* (North-Holland, Amsterdam, 1971), p. 37.
10. ———, *ibid.*, p. 114.
11. D. Metcalf, J. W. Sheridan, S. H. Chan, E. R. Stanley, in *In Vitro Culture of Hemopoietic Cells*, D. W. van Bekkum and K. A. Dicke, Eds. (Radiobiological Institute TNO, Rijswiji, Netherlands, 1972), p. 73.
12. D. C. Dale and S. M. Wolff, in preparation.
13. We thank E. Harvey for technical assistance, F. D. Cannon for DL-A typing, C. Graw for mixed leukocyte culture, T. Knutsen for karyotype analysis, and the Armed Forces Radiation Research Institute for the use of radiation facilities.

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