4 days later the animals were challenged with 5×10^5 tumor cells in 0.5 ml of Hanks-Simms solution injected s.c.
The evaluation of the tumour growth was made by measuring its diameters 30 days after challenge. Major and minor diameters were measured, and the value expressed in mm^2. Animals were killed 40 days after the tumor challenge and autopsy was made. Histopathological analysis included spleen, thymus and regional lymph nodes. Diffusion chambers were removed and cellular viability was analyzed.
Results and discussion. The results are given in the table. It can be seen that rats bearing a diffusion chamber containing allogeneic spleen cells developed smaller tumors ($\bar{x}$: 654 mm^2) than those of the control group ($\bar{x}$: 1301 mm^2; $p < 0.01$).
On the other hand, neither sarcoma E 100 cells nor kidney allogeneic cells had obvious effects on tumour development. Although the tumour size in both groups was slightly smaller than in controls, statistical comparison did not show any difference.
Cells contained in the diffusion chambers at the end of the experiment showed a 98% viability. This indicates that allogeneic cells kept their function throughout the experiment. The study did not show evident changes in spleen, thymus and regional nodes.
Data have been presented to demonstrate that allogeneic spleen cells introduced into a diffusion chamber are able to protect rats from developing progressively transplantable tumours. This effect could not be obtained either with non-immunological cells or with sarcoma E 100 cells carrying specific tumor antigens. The observation that allogeneic spleen cells have some significant role in protection against a rat sarcoma suggests that these cells are capable of stimulating the immune system. This enhancement of the immune response could be considered an expression of allogeneic inhibition. Since only soluble factors produced by the cells could leave the diffusion chamber, it seems possible that spleen cells may elaborate a mediator that enhances the immune response.
Katz et al.[3-5] reported that the mechanism of allogeneic inhibition was the result of a specific immunological attack of grafted donor cells on cells of the host. The authors speculated whether the nature of cellular events during this interaction was the result of a direct cell-to-cell interaction between the lymphocyte populations (T and B), or whether it was due to the release of nonspecific factors from T cells which influence B cells, or both[6,14]. Further experiments of Armerding and Katz demonstrated that a nonspecific mediator (allogeneic effect factor-AEF) could be produced by T lymphocytes activated in vitro with alloantigens. The AEF, a proteic factor, would be able to trigger B lymphocytes and to produce their subsequent differentiation and proliferation to antibody forming cells in vitro[15]. According to our results, it appears possible to confirm with an in vivo assay that the regulatory influences in the immune responses are exerted by a soluble factor produced by allogeneic lymphoid cells.

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Evidence for the existence of an agent in the serum of the cyclic hematopoietic dog which influences hemoglobin synthesis

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Summary. Serum samples collected through the cycle of a cyclic hematopoietic (CH) dog under reduced atmospheric conditions, were assayed for their ability to affect hemoglobin synthesis by normal canine bone marrow. Varying levels of hemoglobin synthesis in the presence of different serum samples suggest an agent cycles in the serum of CH dogs which influences hemoglobin synthesis.

Canine cyclic hematopoiesis (CH) is characterized by cyclic variations in the levels of neutrophils, platelets, monocytes, and reticulocytes[1,2]. Based on the observations that marrow transplanted from a CH dog into a normal dog will continue to display cyclic variations, and that transplantation of bone marrow from a normal dog into a CH dog abrogates the cycle, the conclusion has been drawn that the primary hematopoietic defect involves elements in the bone marrow, most likely the stem cell[3-6].
2 laboratories have demonstrated that erythropoietin levels also cycle in the CH dog, but only following an erythropoietic stress, such as phlebotomy[7], or hypoxia[8]. Since hemoglobin synthesis is an important aspect of erythroid maturation, this project was initiated to determine whether additional extramedullary agents cycled which might influence hemoglobin synthesis.
Methods. The dogs used in this study were part of a colony maintained at the University of Tennessee Memorial Research Center Animal Facility. Hematologic descriptions of these animals have been previously reported[8]. Bone marrow from the iliac crest of normal dogs were collected in heparinized syringes and then suspended in NCTC 135

(Grand Island Biological Co.). Marrow clumps were gently dispersed into homogeneous suspensions by passage through nylon mesh filters.

Final nucleated cell concentration was adjusted to 5×10^6 cells/ml. Normal or CH dog serum was added to each reaction to a final concentration of 5%. Serum samples for assay were obtained each day through a cycle while the CH dog was maintained in a vacuum chamber (0.5 atmosphere). Cells were preincubated for 30 min before the addition of radioactive precursor (^{3}H-leucine, 50 mCi/mM New England Nuclear Co.). All reactions were performed at 37° in sealed test tubes. Erythropoietin activity was determined by the exhypoxic mouse assay[9].

Results and discussion. The results in figure 1 demonstrate the effect of serum samples from the hypoxic CH dog upon hemoglobin synthesis by bone marrow of a normal dog. After a 30-min preincubation, ^{3}H-leucine was added and the reaction proceeded for 4 h. Hemoglobin synthesis was markedly increased in marrow samples incubated in the presence of serum obtained during the 4th to the 7th days of the hypoxic CH dog's cycle. Day 1 was considered to be the first day the absolute neutrophil count was less than 1600/mm^3.

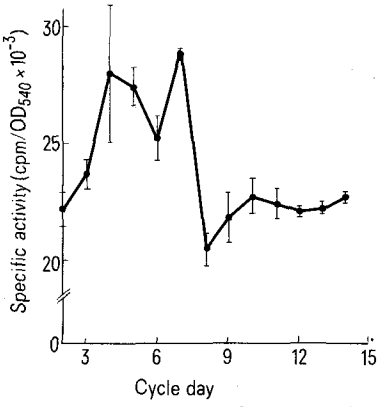

Fig. 1. Effect of serum from CH dog exposed to hypoxia on hemoglobin synthesis by normal canine bone marrow cells. The abcissa refers to the day in the CH dog's cycle on which the serum sample was collected. The 2nd day of the cycle was the 1st day in hypoxia. The cells were preincubated for 30 min, ^{3}H-leucine was added, and the reaction continued for 4 h at 37 °C. Each incubation was run in duplicate, and each point represents the mean and the range of variation.

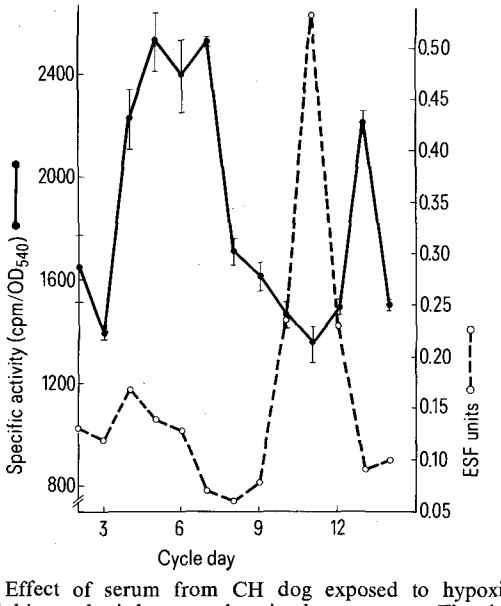

Fig. 2. Effect of serum from CH dog exposed to hypoxia on hemoglobin synthesis by normal canine bone marrow. The abscissa refers to the day in the CH dog's cycle on which the serum sample was collected. The 2nd day of the cycle is the 1st day in hypoxia. The cells were preincubated for 30 min, ^{3}H-leucine was added, and the reaction continued for 90 min at 37 °C. Each incubation was run in duplicate, and each point represents the mean and range of variation.

When the reaction time was shortened to 90 min after a 30-min preincubation, a comparative increase of hemoglobin synthesis was again apparent in serum samples obtained during the 4th to the 7th days of the cycle (figure 2). Peak values were almost twice the level observed with serum samples of lowest activity. In the same figure, the dashed line represents the erythropoietin activity of the serum samples. There is clearly non-identity between erythropoietin and the agent assayed by the in vitro bone marrow assay.

Similar activity was found in serum samples obtained from CH dogs not exposed to hypoxia. The magnitude of the variations between serum activity from days of high and low activity, however, were greatly reduced. This is consistent with the published reports[6,7] that erythropoietic cycling in the CH dog is of low magnitude, unless there is additional stress, such as hypoxia, or phlebotomy.

Leucine incorporation by normal canine bone marrow incubated in the presence of serum from normal dogs was generally intermediate between the high and low values observed with CH dog serum. This might suggest that the observations in this report are attributable to inappropriate modulation in the CH dog of a normal erythropoietic control mechanism. Further study, however, will be necessary to determine the nature of the agent.

From the data in figure 2 the agent which influenced hemoglobin synthesis is clearly not erythropoietin. The magnitude of the effect observed after only 90 min incubation is also not consistent with the expected time course for an erythropoietin stimulation. Experiments in this laboratory have found that erythropoietin stimulation of hemoglobin synthesis would not be appreciable until after 4 h of incubation. The rapidity of the effect would suggest that at least part of the effect is attributable to a translational influence, although serum iron in the present studies revealed no significant difference between serum from days of high and low activity. Therefore, it seems unlikely that globin synthesis was stimulated by increases in iron concentration with consequent stimulation of heme biosynthesis. Trivial effects due to variations in isotope dilution were eliminated when amino acid analyses showed there was no significant difference in leucine concentration between days with high and low activity.

The results reported in this paper must be considered as preliminary, but point strongly to the existence of a humoral agent which influences hemoglobin synthesis, at least at the level of translation.

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