Results and discussion. The table shows that the highest activity of CSF is localized in microsomes, lower activity was found in cytosol, but mitochondria and lysosomes had less activity than homogenate of the whole kidney. The only traces of activity were noted in the nuclei and membranes. The main part of CSF found in microsomes may be logically explained by the fact that in these structures protein biosynthesis takes place. Higher activity of CSF in cytosol is probably connected with a transportation route from the microsomes to out cell compartment. The other subcellular fraction, and mitochondria and lysosomes, containing this activity may be due to contamination by fractions rich in CSF or by absorption of the protein. From the literature on the presence of large amounts of CSF in urine, and the findings presented may be further evidence that the kidney is the organ of biosynthesis of CSF.

Activity of CSF in kidney subcellular fractions of mice

Fractions	Colonies/mg of protein
Homogenate	60 (49-70)
Nuclei and membranes	5 (4-9)
Mitochondria	51 (39-80)
Lysosomes	43 (32-57)
Microsomes	139 (125-160)
Cytosol	67 (52-96)

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Evidence for in vivo protection against a rat sarcoma by allogeneic spleen lymphocytes[1,2]

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Summary. The protective effect of normal allogeneic spleen cells against the growth of a transplantable rat sarcoma was studied.

The transfer of allogeneic lymphoid cells exerts a stimulating action on the immunologic system of the recipient animal[3-10]. This action, termed 'allogeneic effect', involves the development of a specific graft-versus-host reaction in the lympoid organs of the host[3].

At the same time, a rejection response is produced by the recipient. It has been established that this effect is the result of a specific immunologic influence of the donor cells on either the host's B or T cells[3,4,11].

In a previous study, it was observed that pretreatment with allogeneic spleen cells contained in diffusion chambers inserted i.p. in the recipients significantly diminished the number of succesful ovarian allografts (7/24; 29.1%) in contrast to controls not receiving spleen cells (23/32; 71.8%; $\rho < 0.01$) (unpublished data). In the present experiment, the effect of enhancing the immune system by allogeneic cells has been used in a rat tumour system.

Materials and methods. Adult inbred rats of both sexes (strain b)[12] were used as hosts. Normal spleen and kidney cells were obtained from another inbred strain (strain l). A spontaneous transplantable sarcoma (sarcoma E 100)[13] maintained by successive passages in several strains since 1955 s.c., (in this case in strain l) was used as a source of tumor cells. This tumour is an encapsulated fibrosarcoma giving no metastasis and leading to the animal's death 30-40 days after grafting.

Several cell suspensions were prepared: a) alogeneic spleen cells were obtained by gently teasing the fresh, whole organ in a Potter-Elvehjem flask with Hanks-Simms solution. The suspension was centrifuged at $120 \times g$ for 15 min, the supernatant was discarded and cells were resuspended in Hanks-Simms medium. This operation was repeated twice, then the viable cell number was determined; b) tumor cells were obtained by trypsinizing fresh pieces of tissue (Trypsin Difco 1:250) in sterile conditions with Hanks-Simms solution; c) allogeneic kidney cells were obtained in the same manner as tumor cells from fresh, whole organ of rats from strain l. All cell suspensions were adjusted to a concentration of 7.5×10^6/ml. Cellular viability was determined in every case by the trypan blue dye exclusion test.

Suspensions of 1.5×10^6 cells in 0.2 ml of Hanks-Simms solution were put into a diffusion chamber. This consisted of a plastic (lucite) ring covered on each side with a Sartorius filter of 0.2 µM pore size. The diffusion chambers containing the cells were introduced through an abdominal incision into the peritoneal cavity of the rats under ether anesthesia.

Rats were divided into 4 experimental groups with diffusion chambers containing either: 1. allogeneic spleen cells; 2. sarcoma E 100 cells; 3. allogeneic kidney cells (as non-immunological control cells); 4. Hanks-Simms medium.

Size of sarcoma E 100 at 30 days after challenge in diffusion chamber bearing rats

Diffusion chamber containing	$\bar{x} \pm SE$	P
Allogeneic spleen cells	654 ± 102	
	$n = 8$	< 0.01
Sarcoma E 100 cells	906 ± 162	
	$n = 12$	NS
Kidney allogeneic cells	806 ± 215	
	$n = 12$	NS
Hanks-Simms solution (controls)	1301 ± 235	
	$n = 13$	

$\bar{x} \pm SE$: Mean tumor size expressed in $mm^2 \pm SE$. n, number of rats. Each group was compared wit the controls. P, value was obtained by Student's t-test. NS, not significant.

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