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Serine Requirement of Bone Marrow Cells of Experimental Animals[1] (40513)

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Leukemic and normal human blood cells have a requirement for serine independent of cell concentration (1, 2), although this amino acid is generally considered nutritionally nonessential for human cells (3).

A serine antimetabolite (cyclohexylserine) that could possibly control the proliferation of certain serine-requiring leukemias has been synthesized and found to inhibit *in vitro* macromolecular synthesis in human blood cells (4). Such a compound, if used therapeutically, would directly affect serine-requiring leukemic cells and serine-requiring normal cells of the body. However, before this or any such compound can be tested clinically in humans, it must be shown effective in serine-requiring cells of experimental animals. In an effort to find a suitable *in vivo* test system, we have investigated the serine requirement of bone marrow cells from the dog, cat, rabbit, guinea pig, rat, and mouse. As in humans, if the normal bone marrow cells of the experimental animal require serine, we expect bone-marrow-derived leukemias from that species to also require serine.

Materials and methods. The animals used in these experiments were: three beagle dogs, two males and one female; one nondescript male house cat; two New Zealand white rabbits, one male and one female; one strain 13 guinea pig (Biological Systems, Toms River, NJ); two F-344 rats (Charles River Breeding Laboratories, Wilmington, MA), one male and one female; and CD1 mice (Charles River Breeding Laboratories, Wilmington, MA), males and females. All animals were mature and appeared to be normal and healthy.

The dogs, cat, and rabbits were sedated, and 1–2 ml of marrow was aspirated from the ends of long bones. The smaller animals were killed and marrow cells were flushed from the femoral cavity with Hanks' balanced salt solution. Marrow cells were washed twice in Hanks' balanced salt solution, counted, resuspended at a concentration of 5×10^5 cells/ml in Eagle's minimal essential medium (3) containing 10% dialyzed fetal calf serum, then seeded in 30 ml plastic tissue culture flasks. Additionally, appropriate nonessential amino acids (3) (as indicated in Table I) were added to cultures at a final concentration of 10^{-4} *M*.

The cells were incubated at 37° for 24 or 48 hr in the presence of [^{3}H]thymidine (1.9 Ci/mmole) at a concentration of 1 μCi/ml, [^{3}H]uridine (2 Ci/mmole) at a concentration of 1 μCi/ml or in the presence of [^{14}C]phenylalanine (522 mCi/mmole) at a concentration of 0.1 μCi/ml. The cells were then harvested, counted, and assayed for precursor uptake as previously described (1).

Standard errors were calculated from variation among animals of the same species and pooled over the two deficient media. The Student's *t* test was used to test the statistical significance of the change in precursor incorporation percentages observed in the deficient media. The standard errors and significant reductions are indicated in Table I.

Results. The results of these experiments are shown in Table I. A different animal was used for each of these experiments except for the mouse experiments, in which cells from several animals were pooled because of the limited number of cells that could be obtained from a single mouse.

Dog and rabbit marrow cells grown in deficient media (Medium Nos. 8 and 9) showed a striking reduction in radioactive precursor uptake compared with cells grown in the complete medium (Medium No. 1).

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TABLE I. PERCENT INCORPORATION OF RADIOACTIVE PRECURSORS INTO DNA, RNA, AND PROTEIN IN BONE MARROW CELLS GROWN IN MEDIA SUPPLEMENTED WITH EAGLE'S "NONESSENTIAL" AMINO ACIDS FOR 24 AND 48 HR.

		Precursor incorporation (%)					
		[^{3}H]Thymidine[b]		[^{3}H]Uridine[b]		[^{14}C]Phenyalanine[b]	
Experimental animal	Medium[a]	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Dog (n = 3)	1	100.0	100.0	100.0	100.0	100.0	100.0
	8	45.4[c]	43.1[c]	48.3[c]	40.6[c]	78.6[d]	78.0[d]
	9	36.6[c]	42.4[c]	38.0[c]	32.9[c]	75.4[d]	74.2[d]
	(SE[e])	(5.9)	(10.5)	(6.5)	(5.0)	(9.1)	(9.8)
Cat (n = 1)	1	100.0	100.0	100.0	100.0	100.0	100.0
	8	69.5	67.5	58.9	65.8	72.1	68.9
	9	83.7	84.8	61.9	70.3	75.4	64.8
Rabbit (n = 2)	1	100.0	100.0	100.0	100.0	100.0	100.0
	8	71.0[d]	59.5[c]	50.0[d]	31.9[c]	44.8[c]	38.9[c]
	9	61.7[d]	54.5[c]	63.0[d]	35.9[c]	46.8[c]	41.1[c]
	(SE[e])	(6.0)	(5.7)	(9.8)	(3.7)	(5.4)	(3.3)
Guinea Pig (n = 1)	1	100.0	100.0	100.0	100.0	100.0	100.0
	8	85.4	89.2	77.8	97.9	89.6	95.9
	9	66.3	81.5	88.0	77.5	84.8	101.0
Rat (n = 2)	1	100.0	100.0	100.0	100.0	100.0	100.0
	8	90.4[c]	96.5	84.5	87.0[d]	77.2[c]	83.7
	9	82.5[c]	95.2	83.3	88.6[d]	74.4[c]	87.4
	(SE[e])	(1.4)	(8.0)	(14.5)	(5.1)	(2.3)	(9.4)
Mouse (n = 1)	1	100.0	100.0	100.0	100.0	100.0	100.0
	8	103.7	112.9	79.5	78.9	101.4	89.5
	9	78.3	91.5	60.9	71.4	97.0	93.6

[a] Medium No. 1 contains all of the "nonessential" amino acids (alanine, aspartic acid, asparagine, glycine, glutamic acid, proline, and serine). Medium No. 8 contains all of the "nonessential" amino acids except serine. Medium No. 9 contains none of the "nonessential" amino acids.

[b] For each experiment medium No. 1, the control, was set equal to 100%.

[c] $P < 0.01$.

[d] $P < 0.05$.

[e] Standard error of the mean percent.

Moreover, the uptake values observed for the two serine-deficient media were quantitatively the same. These results indicate that extrinsic serine is required for the optimum *in vitro* macromolecular synthesis by dog and rabbit marrow cells. There was a suggestion of a serine requirement in some assays of rat cells, but the data for the rat did not consistently show this requirement. The cat, guinea pig, and mouse marrow cells did not show a serine requirement.

Discussion. The serine requirement observed in dog and rabbit marrow cells is comparable to that observed in human marrow cells (1), peripheral leukocytes (2), and certain leukemic cells (1).

The molecular basis for the human requirement is a reduced level of the enzymes of the phosphorylated pathway of serine synthesis (2). However, the molecular basis for the requirement in the dog and in the rabbit is yet to be determined. Nevertheless, it appears that dog and rabbit bone-marrow-derived leukemias would make suitable *in vivo* test systems for assessing the effectiveness of potential serine antimetabolites. Those found effective may be very helpful in controlling certain leukemias in crisis or acute situations by selectively inhibiting the proliferation of bone-marrow-derived blood cells.

We are not aware of any experimental bone-marrow-derived tumors from the dog or rabbit that could be used to test the effectiveness of serine antimetabolites *in vivo.* However, dog bone marrow leukemias are occasionally observed in veterinary clinic patients; the most commonly observed malignancy is a multicentric lymphosarcoma that involves both lymphoid tissue and bone marrow. Some of these dogs might be used in experimental chemotherapy programs to test the therapeutic effectiveness of serine antimetabolites.

Summary. In an effort to find animal models for serine antimetabolite studies, we examined the serine requirement of bone

marrow cells of the dog, cat, rabbit, guinea pig, rat, and mouse. From measurements of radioactive precursor uptake, we found that extrinsic serine is required for optimum *in vitro* macromolecular synthesis by dog and rabbit marrow cells. Marrow cells from the other animals did not require extrinsic serine. It appears that the dog and rabbit would make suitable *in vivo* test systems for serine antimetabolite studies.

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