organs of these four mice was approximately 1/2 to 1/4 as much per gram as the blood radioactivity. The specificity shown by these data indicates that the conjugated antibodies were stable and that free radiometal was not released in vivo. Studies of the half-life of the ^{111}In-DTPA–IgG's in normal mice support this conclusion (*15*).

We had determined the optimal time for tumor imaging after intravenous injection of ^{111}In-EDTA-103A (*5*). Although tumor targeting occurs immediately, more accurate imaging is obtained if sufficient time is allowed (5 to 6 hours) for extravascular distribution of the antibody and the consequent reduction of the radioactivity in the blood pool. Several parameters that must be considered for optimal tumor imaging are the half-lives of the isotopes used, the accelerated metabolism of the specifically bound antibody (*6a*, *14*), the slower rate of accumulation of nonspecific antibody (*14*) than of the targeted antibody, and the half-life of the particular monoclonal antibody used. Therefore, imaging immediately after antibody distribution is preferable.

Thus, rapid and specific tumor imaging without computer enhancement or subtraction techniques is possible with radioactive metal chelates conjugated to monoclonal antibodies. Erythroleukemic spleens of 200 to 300 mg were easily resolved, and the limits of resolution may be much lower. By analogy, it may be possible to detect small human tumors or metastases. The chelate-conjugated monoclonal antibodies are extremely versatile and can be used with a variety of isotopes of different half-lives and emission spectra to yield superior imaging and to accommodate to a particular tumor system. We have labeled both IgG's and IgM's with indium, gallium, and scandium. One advantage of the labeling with radioactive metal chelates is that if the chelate becomes unconjugated from the antibody during metabolism, the protected isotope is excreted immediately. This contrasts with the fate of the unprotected iodine isotopes (*6*, *6a*).

The chelate-conjugated monoclonal antibodies have, in addition, great therapeutic potential, since cytotoxic isotopes may also be chelated. The most powerful therapeutic isotopes are the alpha emitters because their immense energy of radiation is confined to an extremely small volume, about the size of a few cells. Theoretically, a single alpha emission in a tumor focus is likely to kill a cell (*16*) with minimum damage to normal tissues. Substitution of a short-lived alpha emitter for indium-111 in the targeting experiments (Fig. 2D) could have resulted in the delivery of several thousand rads of high linear energy transfer radiation to the leukemic spleen cells; this dose is vastly in excess of that necessary to kill every leukemic cell.

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Congenital Malformations Induced by Laetrile

Abstract. *Laetrile administered orally to pregnant hamsters caused skeletal malformations in the offspring, but intravenous laetrile failed to result in embryopathic effects. Oral laetrile significantly increased in situ cyanide concentrations, while intravenous laetrile did not. Thiosulfate administration protected embryos from the teratogenic effects of oral laetrile. The embryopathic effects of oral laetrile appear to be due to cyanide released by bacterial β-glucosidase activity.*

The term laetrile refers to a class of cyanogenic glycosides that occurs naturally in the pits of certain edible fruits and berries. Recent legislative activity has legalized the use of laetrile in the treatment of cancer in at least 23 states. The National Cancer Institute is sponsoring clinical studies on the efficacy of laetrile at the University of California (Los Angeles) Comprehensive Cancer Center, the Mayo Clinic, the Memorial Sloan-Kettering Cancer Center, and the University of Arizona Health Sciences Center.

Amygdalin (D-mandelonitrile-β-D-glucoside-6-β-D-glucoside) is the most common constituent of laetrile samples, but injectable preparations from Mexican laboratories have also contained substantial amounts of unknown pyrogens, visually apparent microbial growth, sucrose, phenol, diisopropylammonium iodide, and isopropyl alcohol (*1–4*). Oral preparations contain primarily D-amygdalin (*3*). The glycone moiety of the amygdalin molecule consists of two sugars that are glycosidically attached to mandelonitrile, which is the genin or aglycone portion of the molecule and the cyanohydrin of benzaldehyde (*5*). The β-glycosidic bond can be hydrolyzed by amygdalase to yield the secondary glycoside, prunasin, and glucose. Prunasin may be further degraded by prunase to yield glucose and mandelonitrile. Amygdalin can also be hydrolyzed to mandelonitrile and glucose by bacterial β-glucosidase in the gastrointestinal lumen. The mandelonitrile decomposes in vivo to yield free cyanide and benzaldehyde (*6*). The benzaldehyde formed is a weak anesthetic agent (*7*), but it is rapidly oxi-

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Table 1. Teratogenic effects of laetrile on hamster embryonic development. Pregnant hamsters received the indicated drugs on the morning of the eighth day of gestation. Abbreviations: po, orally; ip, intraperitoneally; iv, intravenously.

Treatment	Dams treated	Dams that died	Litters*	Implantation sites	Resorptions	Living fetuses								Fetal weight (g) (mean ± standard deviation)
						Normal		Abnormal		Abnormalities				
						N	Percent	*N*	Percent	Exencephaly	Encephalocele	Rib anomalies	Other†	
NaCl, 0.9 percent (0.5 ml, po)	5	0	5	61	0	61	100	0	0	0	0	0	0	1.08 ± 0.28
D,L-Amygdalin (200 mg/kg, po)	6	0	5	67	1	64	96	2	4	0	0	2	0	1.19 ± 0.18
D,L-Amygdalin (225 mg/kg, po)	6	0	6	79	3	73	95	4	5	0	0	0	4	0.89‡ ± 0.30
D,L-Amygdalin (250 mg/kg, po)	10	0	9	104	4	76	76	24	24	2	15	10	0	1.23‡ ± 0.14
D,L-Amygdalin (275 mg/kg, po)	12	1	10	106	6	68	68	32	32	4	15	16	1	0.92‡ ± 0.19
D-Amygdalin (300 mg/kg, po)	12	2	8	99	2	60	62	37	38	5	15	20	1	1.35‡ ± 0.15
D-Prunasin (177 mg/kg, po)	8	0	7	84	3	69	85	12	15	0	4	9	0	1.10 ± 0.15
D,L-Amygdalin (275 mg/kg, po) plus $Na_2S_2O_3$ (300 mg/kg, ip)	12	0	11	129	4	123	98	2§	2	0	1	0	1	1.19 ± 0.19
NaCl, 0.9 percent (0.5 ml, iv)	6	0	6	83	2	81	100	0	0	0	0	0	0	1.15 ± 0.19
D,L-Amygdalin (275 mg/kg, iv)	11	0	9	111	4	107	100	0	0	0	0	0	0	1.22† ± 0.21

*The difference between litter number, dead animals, and total treated represents nonpregnant animals. †Includes crooked tails, renal hypoplasia, anophthalmia, and polydactyly. ‡Significantly different from corresponding value for the respective saline-treated animals at $P < .05$ (unpaired Student's *t*-test). §Significantly different from corresponding value for animals treated with D,L-amygdalin (275 mg/kg) at $P < .001$ (chi-square test).

Table 2. Concentrations of cyanide and thiocyanate in tissues of hamsters treated with laetrile or saline. Determinations were made 2.5 hours after administration. Values represent means ± standard errors for four or five animals and are expressed as nanomoles per milliliter of blood or nanomoles per gram of organ (wet weight). See the legend to Table 1 for abbreviations.

Treatment	Whole blood		Liver		Brain		Kidney	
	Cyanide	Thiocyanate	Cyanide	Thiocyanate	Cyanide	Thiocyanate	Cyanide	Thiocyanate
NaCl, 0.9 percent (0.5 ml, po)	0.05 ± 0.03	13.2 ± 1.8	0.06 ± 0.02	34.9 ± 4.8	0.23 ± 0.14	2.5 ± 0.7	0.02 ± 0.02	33.4 ± 4.5
D,L-Amygdalin (275 mg/kg, po)	4.0* ± 1.1	244.7* ± 32.9	2.5* ± 0.6	155.6* ± 20.2	1.6* ± 0.3	21.2* ± 1.0	2.8* ± 0.9	229.5* ± 38.2
NaCl, 0.9 percent (0.5 ml, iv)	0.03 ± 0.03	16.3 ± 3.4	0.25 ± 0.12	28.1 ± 2.6	0.13 ± 0.08	7.5 ± 1.2	0.5 ± 0.3	57.1 ± 8.1
D,L-Amygdalin (275 mg/kg, iv)	0.06 ± 0.03	19.8 ± 2.5	0.14 ± 0.07	29.0 ± 2.7	0.14 ± 0.09	9.0 ± 1.5	0.1 ± 0.05	66.2 ± 6.3

*Significantly different from corresponding value for the respective saline-treated animals at $P < .05$ (unpaired Student's *t*-test).

dized to benzoic acid, which is pharmacologically inert (*1*).

Previous studies (*8*, *9*) linked the in vivo liberation of cyanide from certain nitriles to the production of severe birth defects in hamsters. The production of these disorders following treatment with nitriles was antagonized by injection of sodium thiosulfate, an inorganic cyanide antagonist (*10*). Slow, continuous infusion of inorganic cyanide into pregnant hamsters during the same stages of gestation also caused a variety of defects (*11*). Because laetrile is metabolically degraded to cyanide and a nitrile in the human (*1*, *12*), the drug was evaluated for teratogenic effects on the embryonic development of the hamster (*13*, *14*).

Timed matings of female Syrian golden hamsters (strain LVG; 100 to 140 g) were carried out at the Charles River Breeding Laboratories. The day after the evening of breeding was considered the first day of gestation. The hamsters were provided unrestricted access to tap water and laboratory feed and were given pine shavings for bedding.

Tablets of D-amygdalin (lot UI-77-114) containing magnesium stearate, FD&C yellow No. 5, Plasdone K29-32, and Explo tab—intended for oral administration in cancer patients—were obtained from the Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland. Sterile D,L-amygdalin (lot BV-80-208) containing HCl to adjust *p*H—intended for intravenous administration in cancer patients—was also obtained from the Division of Cancer Treatment. The amygdalin preparations were dissolved in sterile, nonpyrogenic 0.9 percent NaCl (Travenol).

On the morning of day 8 of gestation, the hamsters were divided into groups and treated as follows. Some groups received an oral dose of the D-amygdalin or D,L-amygdalin preparation (0.5 ml per 100 g of body weight). Other hamsters were anesthetized with an intraperitoneal injection of sodium pentobarbital (6.5 mg/ml per 100 g) and then given a single intravenous injection (*13*) of D,L-amygdalin. Another group received an intraperitoneal injection of 300 mg of sodium thiosulfate (reagent grade; Mallinckrodt) per kilogram (1.0 ml per 100 g) and a teratogenic dose of D,L-amygdalin by intubation. Additional thiosulfate injections were given every 120 minutes for 10 hours after the intubation with D,L-amygdalin. Still other groups received an equivalent volume of saline by intubation or intravenous injection or received an identical series of thiosulfate injections alone. Finally, a group was treated orally with crystalline D-prunasin (D-

mandelonitrile-β-D-glucoside; Sigma) dissolved in saline.

All the pregnant hamsters were killed with excess CO_2 on the morning of day 14. The fetuses were removed by cesarean section and the resorption sites and living fetuses were counted. The living fetuses were blotted dry, weighed, and examined under a binocular dissecting microscope for gross developmental malformations. Rib anomalies were noted by examination through the translucent skin. The fetuses were either placed into Bouin's fluid and dissected or were fixed in ethanol for staining of the skeletons with alizarin red S.

To study the in vivo metabolism of laetrile, females (100 to 140 g) were given an oral or intravenous dose of D,L-amygdalin dissolved in saline as before. Control groups were given an equivalent volume of the vehicle. The animals were killed with excess CO_2 2.5 hours later. The blood was collected by cardiac puncture and immediately delivered to an aeration apparatus (*15*) for determination of cyanide content. Selected tissues were quickly excised, frozen on dry ice, and homogenized in ice-cold saline, and the concentrations of cyanide and thiocyanate were determined (*15*).

Saline administered orally during the primitive streak stage of gestation (*13, 14*) affected neither maternal well-being nor embryonic development (Table 1). Conversely, two fetuses with fused ribs were found in a litter carried by a mother treated with 200 mg of D,L-amygdalin per kilogram, and four offspring showing renal hypoplasia were found in a litter whose mother had been treated with 225 mg of D,L-amygdalin per kilogram. Three of ten mothers dosed with D,L-amygdalin at 250 mg/kg exhibited hyperpnea, dyspnea, ataxia, and tremors. Two of the three suffered convulsions, and their offspring were severely malformed. Seven of the ten litters were affected, with the offspring showing exencephaly, encephalocele, and rib fusions or bifurcations.

Five of 12 mothers treated orally with D,L-amygdalin at 275 mg/kg showed signs of intoxication, and one died. Seven of the ten litters were affected, and the offspring exhibited axial skeletal disorders, rib anomalies, or crooked tails. An increase in the percentage of abnormal fetuses was associated with an increase in the dose of D,L-amygdalin (Table 1).

An oral dose of 300 mg of D-amygdalin per kilogram caused identical malformations. Of the 12 mothers treated with the laetrile tablets, two were dyspneic and died. Six of the eight litters were affected. One fetus had exencephaly and bilateral anophthalmia. That a somewhat larger dose of the D-amygdalin preparation was required to produce frank terata (as compared with the doses of crystalline D,L-amygdalin) probably can be attributed to the fact that the D-amygdalin tablets contained not only the cyanogenic glycoside but numerous coloring and binding agents as well.

When seven pregnant hamsters of equivalent gestational age were given an equimolar, oral dose of crystalline D-prunasin, they did not exhibit signs of poisoning, but some of their offspring showed encephalocele or rib fusions (Table 1). Five of the seven litters whose mothers had been treated with prunasin contained one or more malformed fetuses. Although there were significant changes in fetal body weight within the laetrile-treated groups (Table 1), the changes were not dependent on dosage or on the particular laetrile studied. (The importance of the changes in fetal body weight is not clear.)

Intravenous injection of saline or D,L-amygdalin (275 mg/kg) did not affect maternal well-being, and the offspring were normal (Table 1). Multiple intraperitoneal injections of thiosulfate did not affect embryonic development, and an identical thiosulfate regimen protected all dams from toxicity and provided significant protection against the teratogenic effects associated with oral D,L-amygdalin (Table 1). Oral or intravenous administration of the saline solution was associated with small concentrations of cyanide in situ (Table 2). Intravenous injection of D,L-amygdalin (275 mg/kg) did not cause a significant increase in the concentration of cyanide or thiocyanate, but an equivalent oral dose of the drug resulted in significant elevations in cyanide and thiocyanate (Table 2). These data concerning biotransformation patterns of laetrile in hamsters parallel those obtained from studies of laetrile in cancer patients (*12*).

Clearly, oral administration of laetrile represents a toxic and teratogenic threat, whereas intravenous injection of an identical dose fails to have deleterious effects. This suggests that the parent drug itself is not responsible for interference with the rapid cell multiplication that is characteristic of embryonic growth. In addition, the observation that an equimolar, oral dose of prunasin is teratogenic indicates that this activity is not an exclusive property of the parent amygdalin molecule. Doses of amygdalin from 1 to 10 g have been given parenterally in humans without toxic effect, suggesting that there is little, if any, in vivo biotransformation of the intact, injected glycoside (*1*). Indeed, cyanide was essentially undetectable in whole blood taken from cancer patients receiving intravenous injections of amygdalin; the drug was rapidly cleared from the blood and excreted largely unchanged in the urine (*12*). Other researchers (*1, 12*) have shown that oral laetrile has manifest toxic effects, and they suggested that the overt toxicity is a consequence of free cyanide released by β-glucosidase activity in the gut. The elevated concentration of cyanide in tissues following oral, but not intravenous, administration of laetrile (Table 2) is further evidence that the laetrile molecule requires microflora-mediated bioactivation in order to have deleterious effects. The fact that thiosulfate protected embryos from the teratogenic effects of orally administered laetrile also implicates metabolically liberated cyanide as a teratogenic metabolite.

At least one human has been treated with laetrile during pregnancy (*16*). The woman started daily intramuscular injections of Mexican laetrile during the seventh month of pregnancy. Cyanide and thiocyanate in maternal and umbilical blood were at or below normal concentrations. The intramuscular route of administration may have prevented cyanide poisoning of the fetus.

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