be acting as an immunosuppressant in the usual sense, either by destroying cells in the irradiated hosts responsible for a residual host-versus-graft effect, or by destroying cells in the graft responsible for graft-versus-host reactions. Alternatively, the serum could be acting by some mechanism other than the destruction of immunocompetent cells. Certain features of the repression effect itself lead us to favor the latter hypothesis. Neither host-versus-graft nor graft-versus-host reactions provide a satisfactory explanation for repression (*5, 7, 8, 11*). A host-versus-graft reaction would require the presence on the parental C57BL/6 marrow cells of transplanation antigens that appear foreign to the irradiated F1 hybrid host. This is not consistent with the usual findings in inbred mice, but could occur, for example, through an allelic interaction at an H-2 linked locus that results in lack of expression of one or more isoantigens in the F1 hybrids (*2*). However, a search for such an immunological reaction of hybrid hosts to parental antigens was unsuccessful (*7*), and it is unlikely that a response too weak to be detected would survive the 900 rad of radiation given to the F1 hybrid hosts in our experiments. A graft-versus-host reaction also seems unlikely as an explanation of the repression phenomenon for the following reasons. Although graft-versus-host effects should be greater when C57BL/6 cells are transferred into homologous C3H hosts than into F1 hybrid hosts, the repression effect is reduced, not increased, in these recipients (*1*). The C57BL spleen cells are repressed to the same degree as marrow or fetal liver cells, although the former usually incite a more severe graft-versus-host reaction than the latter (*1*). Finally, repression was lessened rather than increased in experiments in which F1 recipients that had experienced graft-versus-host reaction prior to irradiation and transplantation of C57BL cells were used (*11*). If evidence of this kind is correctly interpreted to mean that CFU repression cannot be attributed to immunological mechanisms of graft-host interactions, neither can its elimination by active ALS be explained by usual mechanisms of immunosuppression. Instead, our experiments may have revealed an action of ALS on some other mechanism, perhaps one responsible for normal regulation of stem cell functions in the hemopoietic system (*8*).

Fisher *et al.* (*12*) have reported that daily injections of antiserum to mouse lymphocytes will also abrogate the inhibition of growth of a spontaneous C3H mammary carcinoma in C3H × DBA/2 F1 hybrid hosts. Although their results are in agreement with our findings, their experiments differed from ours in two important ways. The recipients were not irradiated, and the transplanted tumor cells used could easily have been carrying new antigens not represented in the F1 hybrid recipients. Thus, although Fisher *et al.* discuss the two alternative mechanisms outlined above, simple immunosuppression provides a more likely explanation for their results than for ours.

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Teratogenicity of Vitamin B_6 Deficiency: Omphalocele, Skeletal and Neural Defects, and Splenic Hypoplasia

Abstract. *Vitamin B_6 deficiency was induced in pregnant rats with a deficient diet and with 4-deoxypyridoxine, a B_6 antagonist. Treated animals developed typical skin changes of B_6 deficiency. Fetuses were small and appeared anemic. Major fetal malformations were omphalocele, exencephaly, cleft palate, micrognathia, digital defects, and splenic hypoplasia. This teratologic system was developed as a model for human syndromes that exhibit combined immunologic and neurologic or skeletal defects.*

One of the challenging observations in clinical medicine is that immunologic deficiency is often associated with neurologic or skeletal abnormalities. For example, ataxia telangiectasia is a syndrome of immunologic deficiency, cerebellar ataxia, and telangiectasias of the conjunctiva and skin (*1*). A second example is hereditary lymphopenic agammaglobulinemia associated with short-limbed dwarfism. These patients have a severe deficiency of immunologic function, a hypoplastic thymus, an unusual type of dwarfism, and ectodermal dysplasia (*2*).

Stimulated by these clinical observations, we have developed a new experimental model. Vitamin B_6 deficiency was chosen as the method because it causes convulsions in newborn infants (*3*) and impairs immunologic response in experimental animals (*4*). Pregnant animals were studied because it has been established that interference with the normal development of lymphoid organs leads to permanent immunologic

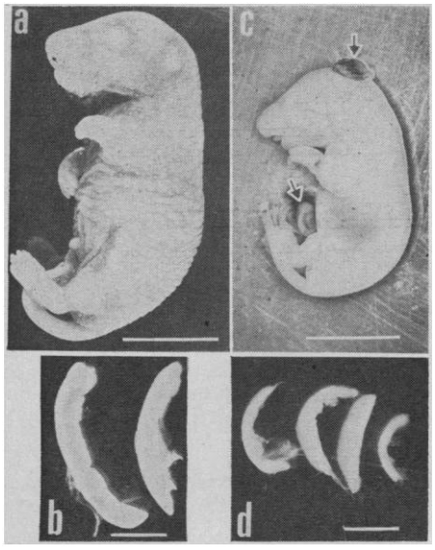

Fig. 1. Twenty-day-old rat fetuses. (a) Typical control fetus. Fetal size and appearance are normal for gestational age. (b) Spleens from control fetuses. (c) Vitamin B_6-deficient fetus with exencephaly and omphalocele (arrows). Fetus is near the mean in weight and length for the B_6-deficient group. (d) Spleens from B_6-deficient rat fetuses. Scales (a) and (c), 1 cm; (b) and (d), 0.2 cm.

deficiency in many species (see *5*).

Virgin Sprague-Dawley rats weighing 238 to 288 g were mated at night, and the following morning was counted as the beginning of day 0 (*6*). At this time the B_6-deficient group of animals was placed on a B_6-deficient diet, which was freely available, and was given drinking water containing 0.1 mg of 4-deoxypyridoxine HCl per milliliter (*7*). Control rats were given the same diet and water, except that their water was supplemented with 1.0 mg of pyridoxine per milliliter. Thus the only difference in the diet between the B_6-deficient group and the control group was that pyridoxine was added to the drinking water of the control group.

Pregnant does were killed by cervical dislocation at 9:00 a.m. on day 20. The number of living and dead fetuses and the number of resorptions were noted. Litters were weighed, measured, examined for external anomalies, and then placed in Lillie's alcoholic fixative or 10 percent neutral formalin. Organ weights were determined on several litters after 14 days of fixation in formalin.

Fifteen does were in the B_6-deficient group. Dietary intake was measured in seven and found to average 12.3 g/day. These animals consumed an average of 2.3 mg of 4-deoxypyridoxine. All rats in the treated group developed typical skin changes of B_6 deficiency. The control group consumed an average of 19.4 g of diet, 24.5 mg of pyridoxine, and 2.5 mg of deoxypyridoxine per day.

Results of the studies are summarized in Table 1. The B_6-deficient fetuses were small (Fig. 1) and many appeared edematous. The gross anomalies observed do not occur spontaneously in our laboratory in this strain of rat, and we have previously shown that simple starvation does not cause anomalies in this strain (*6*).

The abdominal defect in the omphaloceles usually measured 3 to 5 mm in diameter, and various portions of the viscera protruded through it. The mild defects contained portions of jejunum and ileum and proximal colon. The most severe defects contained a major portion of the liver, the stomach, pancreas, spleen, and all the small intestine and proximal colon. Extruded viscera were contained by a thin membrane.

Most common forms of digital defects were partial or total fusion of two digits on one or more extremities. One fetus had an extra first digit on each forepaw, and one had the fourth and fifth digits missing entirely. Many fetuses had more subtle degrees of digital fusion.

Several of the fetuses with cleft palates also had a short mandible (2 mm shorter than normal), although only three of the eight fetuses with micrognathia had cleft palates. One fetus had virtually no visible mandible. The exencephalic defects measured 2 to 5 mm.

Fetal spleens were strikingly small (Fig. 1), but fetal thymuses appeared to be normal. Both organs were smaller in the treated than in the control group, when compared by *t*-test ($P < .001$). Fetal kidneys did not differ significantly in size ($P > .3$).

Teratogenicity of B_6 deficiency appears not to have been reported before, although studies have been done on pregnant animals (*8*). This is apparently the first study in which treatment was started on the first day of pregnancy, and all treated animals developed frank clinical signs of B_6 deficiency during pregnancy. Earlier investigators used much smaller doses of the inhibitor 4-deoxypyridoxine. This is apparently the first experimental model in which omphalocele can be produced in a significant frequency.

Table 1. Comparison of B_6-deficient and control rats.

	Treated group	Control group
Pregnant rats	15	5
Mean weight gain (g)	8.7	102
Implantations	189	63
Resorptions	81	2
Live fetuses	108	61
Mean fetal weight (g)	1.63	3.24
Mean fetal length (mm)	28.3	37.0
Gross fetal anomalies		
Digital defects	48	0
Cleft palate	20	0
Omphalocele	12	0
Micrognathia	8	0
Exencephaly	6	0
Fetal organ weights		
Fetuses examined	21	26
Kidney*	0.88 ± 0.12	0.85 ± 0.07
Thymus*	.19 ± .03	.24 ± .04
Spleen*	.03 ± .02	.14 ± .03

* Mean weights and standard deviations expressed as grams per 100 g of fetal weight.

Vitamin B_6 deficiency inhibits both cellular and humoral immunity in experimental animals (*4*). We had expected that B_6 deficiency in fetal rats would cause severe hypoplasia of all lymphoid tissue; we were surprised to find that the thymus appears grossly normal and is only slightly smaller than in the controls. We anticipate that fetal B_6 deficiency will cause a permanent immunologic defect.

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