cent of children from normal pregnancies had various congenital defects, with 10 percent requiring special medical and educational services. Sixty-eight children in our series are 2 years of age or older and are, therefore, at greater risk for the recognition of a congenital defect, yet only two children required long-term medical care, one for tetralogy of Fallot and one for bilateral nerve deafness. Ekelund *et al.* (*5*) found an incidence of minor malformations of 9.6 percent and of major malformations of 3.3 percent in a recent prospective study of over 6200 children followed to age 1 year.

It is interesting to speculate on the apparent absence of any significant increased risk to mother or fetus in this study. The human fetus is most susceptible to teratogenic agents such as rubella, toxoplasmosis, irradiation, and other agents or forces during the first trimester. None of our patients was treated while pregnant, and pregnancy was proscribed for one full year following exposure to these agents.

There are many data that would suggest that methotrexate and actinomycin-D are most effective during cellular DNA synthesis. Cells in a "resting stage" are relatively resistant to these agents. The 1-year period of forced contraception may allow the more mature and possibly defective ova to be wasted. It is also possible that only oocytes in a developing follicle are active enough metabolically to be damaged and that a year of contraception allows only previously inactive and immature oocytes and follicles to persist and produce the gametes for subsequent reproduction.

There is a possibility that mutations due to chemotherapy were present but undetected in the products of the pregnancies of our patients. Most mutations are recessive and, therefore, are not easily detected unless the trait is sex-linked and thus occurs with greater frequency in male offspring. Observations over several generations would be required to answer this question.

The aborted fetuses in our series were not reported as abnormal, but no systematic examinations were undertaken.

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6. We thank Mrs. Lynn McMahan for her effort in obtaining this information, maintaining the records, and typing the manuscript.

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Repression of Colony Formation Reversed by Antiserum to Mouse Thymocytes

Abstract. *Marrow cells derived from C57BL/6 mice form many fewer splenic colonies in irradiated C57BL/6 × C3H F1 hybrid recipients than in irradiated C57BL/6 recipients (repression of colony formation). This effect is reversed by treatment of the hybrid recipients with active antiserum to mouse thymocytes. The repression phenomenon cannot readily be explained in immunological terms; hence the effect of the antilymphocyte serum on this phenomenon may not result from immunosuppression in the usual sense.*

Normal (*1, 2, 3*) or malignant (*4*) cells are known to grow less well in F1 hybrid recipients than in isologous hosts. This phenomenon has been called CFU (colony forming unit) repression (*1*), hybrid resistance (*2*), or allogeneic inhibition (*5*). Cudkowicz and Stimpfling (*2, 6*) have provided evidence that the phenomenon can result from heterozygosity at a genetic site closely linked to the D subregion of the histocompatibility-2 (H-2) locus. The mechanism of the repression effect is unknown, but attempts to explain it in terms of immunological phenomena associated with transplantation, such as ordinary host-versus-graft reactions, have not been widely accepted (*1, 5, 7*). We have suggested that the defective growth of marrow transplants in this situation may be a reflection of failure of a control mechanism that normally regulates cellular proliferation through gene products existing as cell surface components (*8*). To test this hypothesis, we have studied the effects of a number of agents on the formation of splenic colonies by C57BL/6 marrow stem cells injected into irradiated C57BL/6 × C3H F1 hybrid mice. An active horse antiserum to mouse thymocytes (ALS; antilymphocyte serum) is highly effective in improving the efficiency of colony formation in this donor-host combination.

Two different preparations of horse antiserum to mouse thymocytes were used, one nonactive (ALS-A) and one active (ALS-B) in tests for immunosuppressive effect (*9*). Female (C57BL/6 × C3H F1) mice received 0.5 ml of either ALS-A or ALS-B intraperitoneally. One day later they were given 900 rad of ^{137}Cs gamma radiation followed by intravenous injection of known numbers of C57BL/6 marrow cells. The animals were killed 9 days later, their spleens were removed and fixed in Bouin's solution, and the number of macroscopic splenic colonies was determined (*10*).

When C57BL/6 marrow cells are injected into heavily irradiated C57BL/6 × C3H F1 mice, the efficiency of colony formation is only 1 to 10 percent of that observed when the same cells are transplanted into isologous irradiated C57BL/6 hosts (*1*) (Fig. 1). When varying numbers of C57BL/6 marrow cells were injected into irradiated female C57BL/6 × C3H F1 hosts 24 hours after treatment with nonactive ALS-A, full repression of colony formation was still seen, similar to that observed in irradiated C57BL/6 × C3H F1 mice that received no serum (Fig. 1). In contrast, complete elimination of the repression effect was achieved by treatment of the irradiated C57BL/6 × C3H F1 hosts with ALS-B 1 day prior to transplantation of C57BL/6 marrow cells. Not only was the efficiency of colony formation in the hosts treated with active serum similar to that observed in isologous hosts (Fig. 1), but the size of the individual colonies in the spleens of hosts treated with active serum was much larger than that in hosts treated with inactive serum (Fig. 1). Colonies from recipients treated with active ALS-B when examined histologically contained erythropoietic and granulopoietic cells similar

Table 1. Effects of various treatments on the repression phenomenon. Materials to be tested were given intraperitoneally in 0.5 ml to C57BL/6 × C3H F1 hybrid mice 24 hours prior to irradiation with 900 rad of total-body radiation and transplantation of 5×10^5 nucleated C57BL/6 marrow cells.

Treatments	Experiments (No.)	Total spleens examined	Colonies per spleen (mean)	Standard error
ALS-B	6	26*	>30†	
ALS-B fractions				
7*S*	2	39*	>30†	
19*S*	2	42	2.6	0.5
4.5*S*	2	42	6.5	0.6
Absorbed ALS-B‡	1	15	23.3	1.5
ALS-A	7	120	1.9	0.3
Horse γG to tetanus toxin	1	16	7.4§	1.7
Horse γG to human thymocyte membranes	2	29	5.1	0.8
Horse antiserum to insulin	1	15	0.4	0.2
Methyl cellulose	2	25	13.2	1.5
S. typosa endotoxin	1	20	4.8	0.8
Marrow cells alone	8	112	2.0	0.3
Irradiation, no marrow cells	4	59	0.12	0.04
Irradiation, no cells, + ALS-B	1	7	4.0	1.4

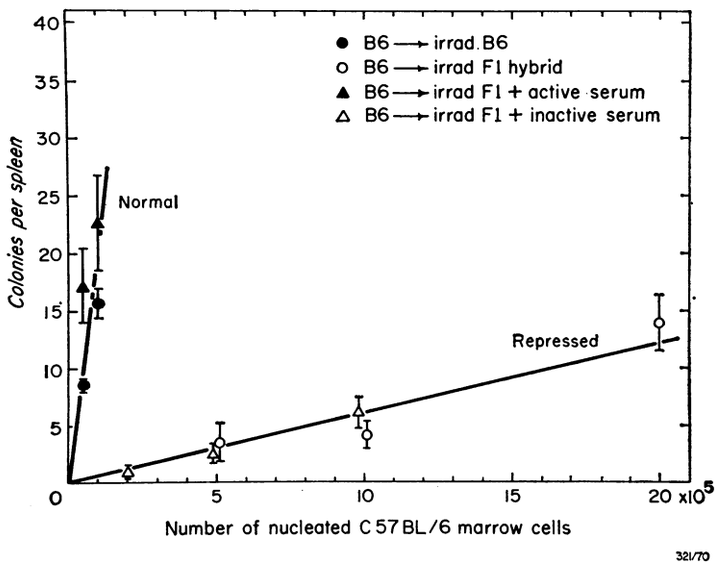
* Only 13 percent of the animals given unfractionated ALS-B survived the 10-day duration of the experiments. This toxicity of ALS-B was not seen with the 7*S* fraction, where 41 of 43 treated mice survived. † These spleens usually contained too many colonies to obtain a reliable count. Countable colonies were obtained when the number of C57BL/6 marrow cells given to hosts treated with ALS-B was below 10^5 cells per mouse (Fig. 1). ‡ ALS-B absorbed with 0.2 volume of mouse erythrocytes. After absorption, the thymocytoxicity was unchanged at 1/2048, the hemagglutinin titer had decreased from 1 : 256 to 1 : 4, and the hemolytic titer had decreased from 1 : 16 to less than 1 : 2. § This group received 10^6 C57BL/6 marrow cells.

to those in colonies derived from isologous hosts (*10*).

As a first step toward characterization of the active material, a mixture of 75 ml of ALS-B and 5 ml of horse antiserum to tetanus toxin was applied to the top of a G200 Sephadex column (78 by 8 cm; the void volume was 1380 ml), equilibrated with phosphate-buffered saline *p*H 8.0 and eluted with the same buffer. The fractions were concentrated and reconstituted in phosphate-buffered saline to the original volume of the ALS-B (75 ml). Although the 19*S* macroglobulin fraction eluting between 1380 and 1570 ml contained most of the lymphocytotoxic and hemagglutinating activities, it had no ability to reverse CFU repression, and the major CFU-promoting activity was located in the 7*S* fraction eluting between 1830 and 2200 ml (Table 1). This result is consistent with the active material being a 7*S* immunoglobulin.

The activity of the 4.5*S* fraction was similar in magnitude to that of a variety of other materials that exhibited some effect on the repression phenomenon (Table 1), including horse γ-globulin to human thymocyte membranes (Connaught Medical Research Laboratories, lot 9-1), methyl cellulose (Dow, 0.5 ml of a 1.3 percent solution in cell-culture medium), or *Salmonella typhosa* endotoxin (Difco, 25 μg to each mouse, in physiological saline). These results indicate that the 7*S* component in ALS-B had a specific ability to reverse the repression phenomenon in excess of the nonspecific effects observed with inactive serum, inactive serum fractions, or materials such as methyl cellulose or endotoxin. The activity in ALS-B was not the result of antibodies directed against mouse red cells, since ALS-B absorbed with mouse erythrocytes retained ability to reverse repression, even though the hemagglutinin and hemolysin titers were greatly reduced (Table 1). It seems unlikely that natural antibodies were responsible for the effect, since none of six other horses tested (the three that provided the pooled ALS-A, and the individual horses that provided the γ-globulin to the tetanus toxin, the γ-globulin to human thymocyte membranes and the antiserum to insulin) possessed the activity.

There are two alternative explanations for the observed specific effect of active antiserum to mouse thymocytes on the growth of parental C57BL/6 marrow cells in irradiated C57BL/6 × C3H F1 hybrid mice. The serum could



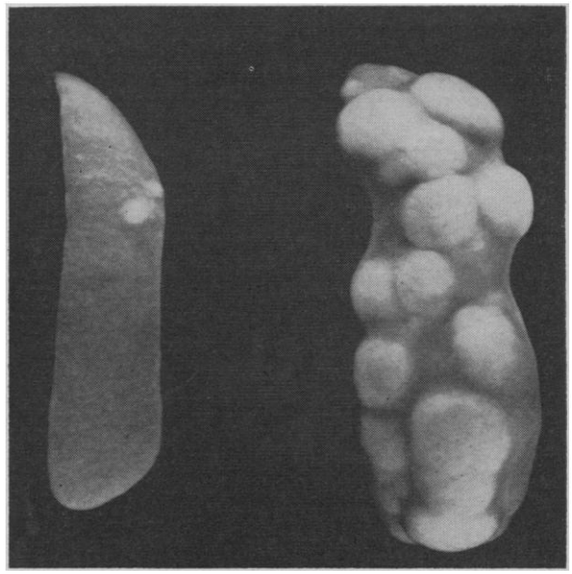


Fig. 1 (left). Comparison of the effects of active (ALS-B) and inactive (ALS-A) antiserum to mouse thymocytes on colony formation by C57BL/6 (B6) marrow cells in irradiated (900 rad) C57BL/6 × C3H F1 hybrid mice. Limits indicated are standard errors of the colony counts. Fig. 2 (right). Splenic colonies in irradiated C57BL/6 × C3H hybrid mice given transplants of 5×10^5 nucleated C57BL/6 marrow cells. (Left) Spleen of a mouse treated with nonactive ALS-A, 24 hours prior to irradiation and marrow transplantation. (Right) Spleen of a mouse treated with active serum (ALS-B).

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