In contrast to the heme effect, changing the oxygen tension after 10 minutes of incubation at 37°C changes the rate of protein synthesis (Fig. 2). This rate change occurs both in the presence and absence of heme, and it indicates that oxygen exerts some effect on the actual rate of protein synthesis independent of the action of heme. The fact that changes in oxygen tension affect the synthesis of globin after the first incubation, even when heme no longer is effective, indicates that the protective effect of heme in preventing peroxide accumulation cannot be exerted through its catalase activity. The fact that heme relieves completely the inhibition by oxygen during the early minutes of incubation, returning the rate of protein synthesis to that during hypoxia, which is maximal for heme synthesis, supports the regulatory mechanism proposed, namely that oxygen inhibits globin synthesis by inhibiting the formation of heme.

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Virus-induced Murine Leukemia: Its Inhibition and Suppression by Serum Containing Erythropoietin

Abstract. *Mice infected at 2 days of age with a virus that induces reticulum cell sarcoma and myeloerythroleukemia were treated with erythropoietin-containing serum obtained from rabbits that had been treated with acetylphenylhydrazine. A marked inhibition of tumor induction occurred, particularly in females, when treatment was begun early. Delayed treatment resulted in instances of regression of overt neoplasia.*

Certain experimental findings, of our own and others, suggested to us the advisability of investigating the effect of erythropoietin on a virus-induced leukemia which we described a few years ago (*1*). Foremost among these findings was that abnormal erythroid proliferation is a prominent feature of this leukemia; this finding suggested that the disease could be due, in part, to a disturbance in erythropoiesis. A second finding was that the disease is sex-oriented, males being at least twice as resistant as females. Moreover, castration diminished the natural resistance of males; in a study involving 95 male MABA mice, the incidence of disease in castrates was more than twice that in intact animals. Further, administration of testosterone altered the natural susceptibility of females; in a study involving 72 female MABA mice, the incidence of disease in untreated females was nearly twice that in treated ones. These findings concerned with our disease, together with two reports in the literature concerned with erythropoiesis, focused our attention on the possibility that the difference in susceptibility of the sexes to the disease could have resulted from a difference in their normal levels of erythropoietin. The experiments in the literature were those of Fried, De Gowin, and Gurney (*2*), who discussed the erythropoietic effect of testosterone in mice, and those of Mirand, Gordon, and Wenig (*3*), who attributed the effect of testosterone to stimulation of erythropoietin.

Erythropoietin-containing serum (ERS) was prepared as follows: New Zealand White rabbits, in groups of six, were inoculated subcutaneously with a 2½ percent solution of 1-acetyl-2-phenylhydrazine, 0.35 ml per pound, one dose daily for four consecutive days. On the 5th day surviving animals were exsanguinated. Serums from rabbits with hematocrits of 15 percent by volume, or less, were pooled and frozen at −20°C until use. Two such pools were assayed for erythropoietin by an established procedure (*4*). One pool, assayed after 6 months in storage, was estimated to contain 8 to 10 units per milliliter of serum; a more recent pool assayed 5.6 units per milliliter. When normal rabbit serum (NRS) was required, it was obtained from rabbits which had not, to our knowledge, been previously bled.

In our experiments inbred BALB/c mice maintained in our colony were randomly bred. First-generation (F_1) mice were inoculated both subcutaneously and intraperitoneally at 2 days of age with an extract of 10 percent neoplastic spleen containing virus; 0.05 ml was given at each site. The animals were weaned at 5 to 6 weeks of age, at which time weekly examinations were begun to determine the progress of their disease. The examination consisted primarily of palpation of the spleen and inguinal lymph nodes. Numbers from "0" to "3.5" were assigned to the mice, corresponding to the development of disease. Number "2" represented, in an adult mouse, a spleen of approximately 500 mg (normally about 125 mg) and enlarged lymph nodes. These were considered to be unequivocal signs of neoplastic disease. Number "3" represented an advanced case in which the spleen weighed 1000 mg or more. The disease in an animal was considered to have regressed if, after progressing to at least the stage "2", it regressed to "1" or less. In most instances, these limits were exceeded.

In the first experiment, treatment of mice (primarily BALB/c females) with ERS commenced at weaning. It consisted of the subcutaneous inoculation of 0.5 ml of serum per mouse weekly for 8 weeks. At first the results seemed disappointing, for we anticipated some effect of the treatment on the incidence of leukemia rather than on the disease after it had become overt. Indeed, the incidence (number neoplastic/number inoculated) was initially somewhat higher (22/25) and the latency somewhat reduced (107 days) in the treated mice compared with the controls (18/25, 126 days), but in the course of subsequent weekly examinations it became evident that palpable lesions in treated mice had regressed in ten instances. Five mice with regressed lesions were killed at 207 days. One had been a "3" at 120, had regressed to "1" at 183 days, and had become a "2" again; two had been "2.5" at 110 days, regressing to "0" at 161 and 187 days, respectively, where they remained until sacrifice;

and two had been "2" at 112 days, regressing to "0" and "1" at 196 and 207 days, respectively. At autopsy, the white blood cell counts were within normal limits; the spleens of four were somewhat enlarged, averaging 300 mg; the other organs were grossly normal. The spleen of one weighed 520 mg, and its thymus and lymph nodes were enlarged. This mouse was, however, a "2" at sacrifice and, therefore, was considered to have relapsed after remission.

Three mice with regressed lesions died before 207 days from unidentified causes. On necropsy, their organs were considered normal from the standpoint of gross neoplasia. Two additional "regressed" mice progressed again to advanced neoplastic disease and died. One of these was "2" at 104 days, "0" at 148 days, and "3" at 162 days. Another was "2" at 104, "1" at 139, and "3" at 180 days.

In our experience, untreated mice exhibiting unequivocal signs of disease usually progressed rapidly to termination. For example, mice with unregressed neoplasms in the above series died at an average of 19 days after first exhibiting these signs of disease, whereas mice with regressed neoplasms died or were killed 87 days after they had reached this point. The life-span of the treated mice was thus prolonged.

Although less extensive data are available for males, regression was also observed in them. Thus, neoplasms regressed in three of seven treated males, but none regressed in four untreated males. Moreover, the mice with unregressed neoplasms died or were killed with advanced disease at an average of 15 days after positive diagnosis; for mice with regressed neoplasms the average was 73 days.

It cannot be said at this time that the disease was eradicated in any instance by the particular regimen employed. Indeed, in three of the cases detailed above it clearly was not. A histological and hematological examination of mice with regressed neoplasms is in progress.

Two preliminary observations are of interest. One was the prominence of cells resembling tissue basophils in the spleens. The other was that the peripheral blood and bone marrow displayed no evidence of acute leukemia; the circulating white cells were within normal limits, and blood and bone marrow differentials suggested a mild leukemoid reaction.

Table 1. Inhibitory effect of ERS on the induction of viral leukemia in BALB/c mice. The data in this table were calcuated on the basis of an experimental period of 119 days after infection, at which time approximately 50 percent of the untreated control mice had developed neoplastic disease. Values in parentheses are percentages. Treatments are shown in italic type.

No. neoplastic/No. inoculated (%)		
Totals	Male	Female
	Untreated	
25/48 (52)	8/27 (30)	17/21 (81)
	*Normal rabbit serum**	
21/45 (47)	11/28 (39)	10/17 (59)
	*Erythropoietin-containing serum**	
3/28 (11)	2/11 (18)	1/17 (6)

* See text for regimen employed.

In the second experiment, mice were treated at 1, 3, and 7 days after birth with 0.1, 0.2, and 0.3 ml of serum at the respective times, then weekly with 0.5 ml for four doses and 1 ml for three doses, at which time treatment was terminated. As shown in Table 1, a substantial reduction in incidence of leukemia occurred within the experimental period; only 11 percent of the ERS-treated mice developed leukemia ($P < 0.001$) at a time when 52 percent of the untreated and 47 percent of the treated controls exhibited neoplastic disease.

The effect of the ERS was particularly striking when the sex of the mice was taken into account. Thus, the incidence of disease in females was reduced from 81 to 6 percent ($P < 0.001$) whereas that in males was reduced only from 30 to 18 percent ($P < 0.8$). This result is compatible with the idea that the higher susceptibility of untreated females to induction of this viral leukemia (81/30, or 2.7:1, at 119 days) could have been due to a lower level of erythropoietin in this sex. The data also indicate that the females were considerably more sensitive than the males to the inhibitory effect of exogeneous erythropoietin on the induction of overt neoplasia. It appears from Table 1 that the normal rabbit serum could have exerted a minor salutary effect on the incidence of disease in females ($P < 0.3$), a result not entirely unexpected, inasmuch as serum from untreated rabbits should contain some normally low level of erythropoietin.

The difference in incidence of disease in the variously treated female mice decreased with time; at 5 months the incidence was 86, 71, and 53 percent for untreated, NRS-treated, and ERS-treated mice, respectively; and at 6 months, at which time the mice were killed, it was 91, 88, and 71 percent, respectively. The disease was thus not eradicated by the particular program of treatment employed. Nevertheless, disease-free life was considerably prolonged. This is reflected by the mean latency—that is, the time to onset of unequivocal signs of disease—determined at 6 months, the close of the experiment. Latency was 107, 114, and 154 days ($P < 0.001$ with respect to the untreated mice) for untreated, NRS-treated, and ERS-treated mice, respectively. Males showed a similar trend, but, because the differences between the ERS-treated and other groups were much smaller, larger numbers would have to be studied to obtain statistically significant data.

If the active principle in the erythropoietin-containing serum was, indeed, erythropoietin, these experiments support the hypothesis that this leukemia results from a disturbance of erythropoiesis. In any event, they demonstrate that the onset of this virus-induced disease can be blocked, its course can be modified, and life can be prolonged by specific physiologic intervention.

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