endotoxin in patients with liver diseases strongly suggests an abnormality of clearance by liver macrophages. Hence, with respect to the chronic character of these disorders, endotoxinemia in these patients resembles the situation in experimental animals in which endotoxin tolerance takes place following repeated administration of endotoxin.

Thus, the complex relationship of stem cell activation and defence against bacterial infection in man appears similar to that in mice. Since however the term 'CSA' comprises various factors differing in biological and chemical qualities, the direct assessment of these may still exhibit some correlations with certain pathological conditions.

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Dose-related enhancement of erythropoiesis by sulfhydryl compounds and its reversal with a thiol inhibitor

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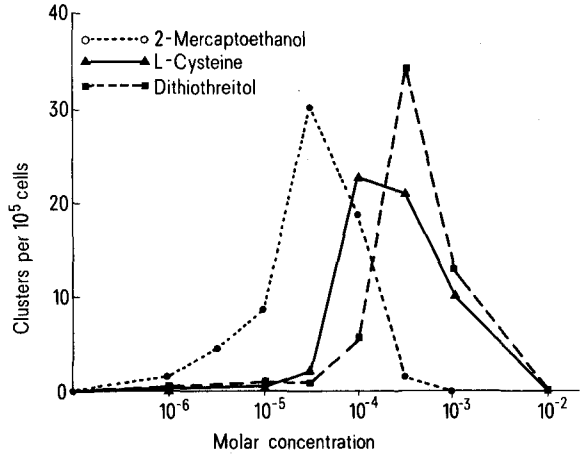
Summary. Erythropoietin-mediated erythrocyte development from bone marrow of hypertransfused rats was significantly greater when the culture medium contained an optimal dose of certain sulfhydryls. This stimulatory action was attributed to the presence of SH groups because erythroblast numbers fell to control levels when the culture contained the thiol inhibitor, p-hydroxymercuribenzoate.

Bone marrow of rats rendered polycythemic by hypertransfusion is practically devoid of morphologically identifiable erythroblasts[2,3]. We have previously reported that by using such polycythemic marrow we were able to follow the orderly progression of erythroid proliferation and maturation[4]. We described the development of erythroid cells which formed tight aggregates of quite homogeneous composition and which could be easily harvested for cytological examination because of the liquid system. These observations on the amplification of erythropoiesis have been the subject of a further report[5].

In the original paper, we mentioned that the culture medium contained 2-mercaptoethanol and L-cysteine. Although the use of thiol additives to promote erythropoiesis in culture is not new[6,7], insufficient emphasis has yet been placed on the necessity of SH protection for erythrocytic development. The present report provides dose-response curves for three thiols, 2-mercaptoethanol (2-ME), L-cysteine (L-CY) and dithiothreitol (DTT), in the polycythemic bone marrow system in vitro and shows that their stimulatory action on erythroid cell cluster formation is reversed by thiol inhibition.

Materials and methods. Femoral bone marrow was taken 5 days after hypertransfusion (packed red blood cells) from 8- to 12-week-old female Wistar rats (hematocrit > 70%). The marrow was mechanically dissociated into a single cell suspension and plated 10^5 nucleated cells/ml in a modified NCTC medium[8] containing 30% fetal bovine serum, and when desired 0.2 IU erythropoietin (Connaught Step III), and microliter doses of 2-ME, L-CY, DTT, or the thiol inhibitor, p-hydroxymercuribenzoate (p-HMB). After 48 h of incubation at 37 °C, the cell suspension was harvested onto glass slides by cytocentrifugation and stained by May-Grünwald-Giemsa or Benzidine. The erythroid groups were counted and classed according to size and to stage of maturation.

Results and discussion. At the time of plating, the bone marrow suspension contained less than 5% nucleated erythroid cells. After 48 h of culture without erythropoietin, regardless of the presence of thiol, erythroid development was nil. With erythropoietin and without thiol additive (EPO controls), a mean number of 28.7 erythroid cell clusters formed. In the experimental groups, containing erythropoietin and an optimal concentration of one of the 3 thiols, erythroblastic differentiation and proliferation resulted in a 10-fold increase in the number of clusters and in the total number of erythroblasts with 2-ME or DTT, and a 5-fold increase with L-CY (table). In paired observations with corresponding erythropoietin controls, the thiol cultures showed significant erythrocytic cluster formation ($p \leq 0.01$ for 2-ME and L-CY and $p \leq 0.05$ for DTT). t-test showed significant erythrocytic development in the 3 thiol groups over the corresponding EPO controls ($p \leq 0.01$). Dose-response curves for the 3 thiols (figure) showed that for erythroid development 2-ME was most effective at



Relationship between the dose of sulfhydryl compounds and the number of erythroid clusters formed from 10^5 bone marrow cells after 48 h in the presence of erythropoietin. Each point is the mean count of erythrocytic groups in thiol-containing medium divided by the corresponding erythropoietin control in 3 to 6 individual experiments. In paired observations with the corresponding erythropoietin control p-values were found to be: $p \leq 0.05$ for 2-mercaptoethanol at the concentration of 5×10^{-5} M, and for dithiothreitol at 5×10^{-4} M; $p \leq 0.01$ for L-cysteine at 5×10^{-4}.

Thiol regulation of erythrocytic development in vitro

Conditions of incubation	Number of experiments	Number of clusters (2 cells or more)	Number of clusters (4 cells or more)	Total erythroblasts	Erythroblasts per cluster
Without EPO, without thiol	7	6.2± 9.1	1.1± 2.6	18± 29.2	1.4±1.4
Without EPO, with thiol	5	14 ± 21	2.0± 2.8	33± 50.1	1.8±1.0
With EPO, without thiol (EPO control)	9	29 ± 26	13 ±13.4	120± 119	3.9±0.9
With EPO, with 2-ME (2-ME+EPO control)	9	290 ±216*	148 ±120*	1500±1140*	5.4±1.8**
With EPO, with L-CY	8	170 ±141**	78 ± 37	800± 440*	5.7±2.6
With EPO, with DTT	4	290 ±135	150 ± 98	1500±1050	4.9±1.8
With EPO, 2-ME and pHMB 5×10^{-6} M	4	22 ± 20***	7 ± 5.1	70± 61	3.4±0.5
With EPO, 2-ME and pHMB 10^{-5} M	4	8.5± 11.6	3.5± 4.4	30± 35	2.8±0.9

EPO = erythropoietin 0.2 IU/ml; 2-ME = 2-mercaptoethanol; L-CY = L-cysteine; DTT = dithiothreitol; pHMB = p-hydroxymercuribenzoate of sodium. * p-values significantly higher than corresponding EPO control in paired observations ($p \leq 0.01$). ** p-values significantly higher than corresponding EPO control in paired observations ($p \leq 0.05$). *** p-values significantly lower than 2-ME+EPO control in paired observations ($p \leq 0.05$). t-test showed significant differences between erythropoietin controls and the 3 thiol groups ($p \leq 0.01$). In the 4 experiments in which pHMB was incorporated into thiol-containing media erythrocytic development was not significantly different from EPO controls ($p \simeq 0.50$).

5×10^{-5} M concentration, L-CY at either 10^{-4} M or 5×10^{-4} M and DTT at 5×10^{-4} M. Another thiol, glutathione, and a diol, ethylene glycol, were ineffective at doses of 10^{-6} to 10^{-3} M.

In 4 experiments the thiol inhibitor, p-HMB, was incorporated into media containing EPO and 5×10^{-5} M 2-ME and incubated at 37 °C for 30 min before adding the bone marrow cells. At concentrations of 10^{-7} or 10^{-6} M, the number of erythroid clusters was similar to that of 2-ME + EPO controls. However, at the concentrations of 5×10^{-6} and 10^{-5} M erythropoiesis fell to EPO control levels ($p \simeq 0.50$) with no adverse effect on granulocytic proliferation. Nonspecific toxicity had to be ruled out because growth with p-HMB + EPO without thiols was found not to be significantly different from EPO controls (t-test leads to a p-value = 0.325).

These data provide evidence that certain SH compounds enhance the in vitro response of erythropoietin-sensitive precursors from polycythemic rat bone marrow. Preliminary studies show that this is also the case for normal or anemic rat bone marrow (unpublished observations, N. P-S). This enhancement of erythropoiesis is dose-related and can be reversed by specific thiol inhibition using p-HMB, indicating that it is effectively the SH moiety that is active in the in vitro system. Ohmiya and Nakai[9] have recently provided evidence that p-HMB inhibits membrane SH in human erythrocytes (50%) with minimal hemolysis (15%). It is interesting to note that another sulfhydryl inhibitor, iodoacetate, administered to mice causes a dose-related increase in the number of CFU-c but no observable effect on CFU-s[10]. More detailed studies will be required to confirm the importance of membrane SH in hemopoiesis, and particularly to determine whether these in vitro findings have any correlation with the in vivo situation.

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Alterations in bone-marrow cellularity following thymectomy in rats[1]

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Summary. The number of nucleated marrow cells was decreased following neonatal thymectomy in rats, and was corrected by administration of syngeneic lymphoid cells, or by implantation of a syngeneic testis. These results suggest that, in the rat, as has been shown previously in the mouse, lymphoid cells exert parital control over bone marrow cellularity and this effect may be further modulated by sex steroids.

It has been known for several years that the addition of thymocytes enhances both the growth of transplanted bone marrow cells in the mouse[2,3] and the size of granulocyte progenitor colonies in (CFC-GM) agar[4], but the mechanism of this effect is uncertain. It has also been shown that the number of nucleated marrow cells is decreased following neonatal thymectomy, and that thymectomy reduces the ability of bone marrow cells (CFU-s) to produce spleen colonies in the Till and McCulloch assay[5]. This latter effect, possibly due to an effect on the growth fraction of CFU-s, can be corrected by incubation of the bone marrow cells with thymic humoral factor[6]. It has been suggested that a thymus-derived cell sensitive to cytotoxic killing with anti-theta (Thy-1) serum plus complement is involved in the