also blocked the CFU-E enhancement. The present study provides the first demonstration that BFU-E proliferation is enhanced by beta-2 adrenergic drugs. Of perhaps greater importance is the observation that the BFU-E compartment appears to be more sensitive to beta-2 agonists than the CFU-E compartment as demonstrated with our dose response data for salbutamol. Byron's report[7] that the pluripotent stem cell (CFU-S) can be triggered into DNA synthesis with concentrations of isoproterenol as low as 10^{-14} M suggests that the CFU-S may be exquisitely sensitive to catecholamines, as well as to drugs such as prostaglandins, carbamylcholine, and certain steroids. Gregory[4] recently provided evidence that a decline in proliferative capacity of cells may be associated with a progressive increase in erythropoietin responsiveness as primitive erythroid progenitors move from a position close to pluripotent stem cells through several differentiation steps of the erythroid progenitor cell compartment until they reach a stage just prior to the onset of hemoglobin synthesis. On the basis of the data presented here and the findings of Byron[7,10] and Gregory[3,4], it is postulated that pluripotent stem cells have a variety of hormone and drug receptors on their membrane surfaces which when triggered can determine the future pathway of their differentiation. The possibility that the concentration of a ligand can regulate the number and/or binding properties of its own receptors on the surface of target cells has gained considerable support in recent years[17]. Our present data suggest that beta-2 receptors may decrease during maturation while those receptors necessary for further differentiation increase, i.e., erythropoietin receptors. The observed increases in both BFU-E and CFU-E seen with salbutamol suggest that an increase in recruitment of erythropoietin responsive cells can be mediated by a beta-2 adrenergic mechanism. These data may be useful in designing therapy in certain clinical refractory anemias.

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Increased incidence of lymphomas in survivors of the host-versus-graft syndrome[1]

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Summary. When $(SB)F_1$ spleen cells were injected into perinatal parental B strain mice a lethal runting syndrome was induced. The survivors showed a significantly increased incidence of lymphomas in old age. The tumors occurred much later and less frequently than in the reverse reaction, $B \rightarrow (SB)F_1$ GVHD.

When F_1 lymphoid cells are injected into neonatal mice of the parental (P) strain, a lethal runting syndrome ensues in certain combinations of strains[2]. Recently I reported an increased incidence of lymphomas in a mild sublethal form of this disease, designated low dose host-versus-graft disease (HVGD)[3]. The present report confirms and extends this finding by providing evidence of the maximal tumor induction potential of this model, by use of a highly vigorous form of this disease (high dose HVGD).

Spleen cells from young adult (SJL/J X C57BL/1)F_1 ($(SB)F_1$) were injected i.p. into nursing parental B strain mice. 2 or 3 doses of cells were given at weekly intervals, with the 1st dose within a few days of birth (see table, footnote). Each cell dose consisted of 30–50 million cells. The mortality from HVGD in this group of 94 mice up to 90 days of age was 64%. Thereafter gradual recovery occurred. The 34 surviving mice form the basis of this report. These mice were observed 3 times weekly up to 18 months of age for tumor development. Mice which were moribund from tumor were autopsied. At 18 months, all remaining mice were sacrificed. Control B mice which received the same dose of B strain spleen cells or which were left uninjected were also sacrificed at 18 months.

The results are summarized in the table. In low dose HVGD, cumulative lymphoma incidence was 12 of 23 or 52%. In high dose HVGD, the incidence was 22 of 34 or 65%. The difference is not significant. In both groups tumor incidence was significantly higher than in $B \rightarrow B$ ($p < 0.05$, $p < 0.001$ respectively) and in uninjected normal B controls ($p < 0.001$ for both). The tumors were reticulum cell sarcomas or lymphosarcomas or a mixture of these. In low dose HVGD 6 of 12 tumors were detected clinically up to 18 months, the 1st one at 12.5 months. In high dose HVGD, 8 of 22 tumors were detected clinically up to 18 months, 3 of the 8 in the 1st year, at ages 7.5, 9 and 11 months. Thus, despite maximally vigorous HVGD, tumors appeared much later and in lower incidence than in the reverse model, $B \rightarrow (SB)F_1$ GVHD (see Cornelius[3], table 4). The tumor induction potential of HVGD therefore appears more limited than in the reverse model, GVHD. In contrast to low dose HVGD, in which injection of the initial dose of $(SB)F_1$ cells into perinatal B mice within 24 h of birth was essential for tumor development, in high dose HVGD an initial injection of $(SB)F_1$ cells into B mice at 24–48 h of age was as effective in inducing tumors (9 tumors in 15 mice) as an initial injection in the period 0–24 h (9 tumors in 14 mice).

6 lymphomas were transplanted into 3-week-old B, $(SB)F_1$, S, and AKR mice. 1 tumor did not grow in any hosts. 5 tumors grew in B and $(SB)F_1$ hosts but not in S and AKR

hosts. These tumors therefore were phenotypically of host type.

The immunologic mechanisms of HVGD are complex and beyond the scope of this paper. I suggested originally that bidirectional immune reactions, $P \rightarrow F_1$ and $F_1 \rightarrow P$, were probably involved[3]. Further experiments have however provided more evidence that the $F_1 \rightarrow P$ reaction is the crucial reaction[4]. The immunogenetic basis of this reaction has not been clarified but certain features resemble hybrid resistance and its in vitro counterpart, F_1 antiparent cell mediated lympholysis[5].

Tumor incidence in low and high dose $(SB)F_1 \rightarrow B$ HVGD and in $B \rightarrow B$ and B controls

Group	Cell donor	No. mice with tumors (%)[a] / No. mice autopsied	p[b]
Low dose HVGD[c]			
1	$(SB)F_1$	12/23 (52.2)	
2	B	4/20 (20.0)	0.05
High dose HVGD[d]			
1	$(SB)F_1$	22/34 (64.7)	
2	B	1/18 (5.6)	0.001
Normal B mice	–	3/53 (5.7)	0.001[e]

[a] Cumulative histologic tumor incidence up to 18 months of age. [b] χ^2 test. [c] Weekly × 20 cell dosage according to age at time of initial cell dose as follows: 0–48 h, 10 million; 48–72 h, 20 million; 72–192 h, 30 million. There was no mortality. [d] Weekly × 2–3 cell dosage according to age at time of initial cell injection as follows: 0–48 h, 30–50 million; of 82 injected mice, 53 died of acute HVGD and 29 survived. At age 48–72 h a single dose of 75 million cells killed 1 of 5 mice and 6 weekly doses of 75 million cells beginning at this age killed 6 of 7 mice. Thus in high dose HVGD, of 94 B mice injected with $(SB)F_1$ cells, 34 survived for long-term observation. [e] Compared to both low and high dose HVGD mice.

Since in high dose HVGD F_1 cell injection into P hosts on the 2nd day of life was tumorigenic, whereas in low dose HVGD it was not, some change must have occurred in the P host mouse by the 2nd day which could be overcome by a larger dose of F_1 cells. If one assumes that HVGD is due primarily to $F_1 \rightarrow P$ reactivity[4], which is opposed by classical H-2 barrier $P \rightarrow F_1$ reactivity[3], one may be dealing with the ontogeny of the latter reaction which is as yet not detectable by GVH assay. (GVH assay of nursing B mice of various ages showed that in the B strain significant anti-F_1 reactivity developed between the 4th and 5th days of life; it increased gradually in the 1st month, and compared to 3.5 month old mice, had declined significantly at 16 months (unpublished observations).) The very late development of most lymphomas in HVGD mice may therefore bear some relation to decline in immune function of the host mouse in old age.

Although the mechanism of tumor induction remains to be elucidated, HVGD can already be added to the list of immunologic syndromes found to be lymphomagenic, e.g. the GVHR[6] and the post-thymectomy state[7]. If further research should confirm the strong suspicion of the basic similarity of HVGD and HR, it would mean that a natural lymphoma-leukemia surveillance mechanism[5] can by manipulation become tumorigenic. This is relevant to clinical bone marrow transplantation.

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Permeability of fresh and stored human erythrocytes to glycerol and its acylated derivatives[1]

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Summary. The effect of copper ions on the permeation of glycerol and its mono-, di-, and triacetate derivatives was studied in fresh and stored erythrocytes. Permeability was unchanged with storage. Acylated glycerols permeate the cells mainly by non-facilitated mechanism, as their transport is almost unaffected by copper ions.

Glycerol permeability has been studied extensively, as a parameter for membrane function in erythrocytes of different species. Human erythrocytes have high permeability to glycerol, like those of rats, rabbits and guinea-pigs, whereas those of pigs, dogs and cats have low permeability[4,5], and those of the camel have extremely low permeability[6].

Recently, Carlsen and Wieth[7] have demonstrated by ^{14}C-glycerol exchange that glycerol transport is performed by 2 mechanisms: facilitated diffusion[8] susceptible to inhibition, and an unspecific pathway for individual molecules. In this study we investigated the permeability patterns after substitution of hydrophilic hydroxyl groups by hydrophobic moieties[9–11]. Permeability studies were also performed using these derivatives after storage, when the lipid composition and other membrane characteristics are changed[12–14].

Materials and methods. The experiments were performed on fresh erythrocytes or on cells taken out of blood units, after different periods of storage in ACD and CPD media. Glycerol lysis time (GLT) and GLT 50 were determined for glycerol and its acylated derivatives as reported previously[6,15,16]. The rate of erythrocyte lysis was followed by recording the fall in the density of the reaction mixture in a Gilford Microsample Spectrophotometer 300 – N (GLT_{50} corresponds to the time required for the OD to fall to half the initial value[15]). The determinations were performed in room temperature, pH 6.8, with or without copper ions (10^{-4} M and 10^{-5} M $CuCl_2$). Cells of fresh units were incubated for 2 h at 37 °C with or without 5 mM sodium fluoride. ATP levels and glycerol permeability were examined before and after incubation, as described above.

Results and discussion. The figure 1 illustrates the typical kinetics of glycerol transport and that of glycerol derivatives in the presence and absence of copper ions. On the left we see the rapid lysis curves with glycerol. The GLT_{50}-values of fresh cells varied between 30 and 60 sec[15]. The