treated with total body irradiation alone survived and recovered (unpublished data). However some precautions are necessary. In preliminary studies we saw that an infusion of more than 10 ml leads to acute right heart failure in the rabbit. If the total amount is split into single portions not containing more than 10 ml of volume, no such side effect is seen. A maximum number of cells is desirable, since the duration of aplasia is directly related to the number of bone marrow cells reinfused[6]. This was the reason that in vitro and in vivo studies were performed in separate animals. Our study also shows that with this freezing technique, which is commonly used in clinical studies[5], not all hematopoietic precursors are ideally preserved. The aim of our study was not to find the optimal freezing technique for rabbit bone marrow but to test an established technique for its use in the rabbit. Using this technique erythroid precursors survive better. They grow in vitro as well as before cryopreservation and reticulocytes recover first in vivo (figures 1 and 2). Growth of myeloid colonies, however, is clearly impaired in vitro. The fact that others have described normal recovery of CFU_C after cryopreservation is not surprising[9]; they adjusted their technique for optimal recovery of CFU_C but did not control the recovery of erythroid precursors. This clearly shows that assessing just one function can lead to erroneous results and does not necessarily represent total bone marrow function. In conclusion we can say that the rabbit is a useful model for assessing the use of cryopreservation of bone marrow and that with the established technique the bone marrow will repopulate the empty autologous bone marrow and will grow in vitro. Further studies are needed to evaluate how erythroid and myeloid precursors can both be optimally preserved.

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Effects of retinoic acid on the fibrinolytic activity of HL 60 human promyelocytic leukemia cells

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Summary. Fibrinolytic activity of HL 60 human leukemic cells was found to increase in parallel with myeloid differentiation following retinoic acid but not dimethylsulfoxide treatment. However, both retinoic acid and dimethylsulfoxide produced an increase in acid phosphatase and a decrease in muramidase.

Retinoic acid (all-trans-retinoic acid) and retinol are essential factors for the normal differentiation of epithelial cells[1]. They also seem to be active in preventing chemical induction and development of epithelial tumors as well as in promoting in vitro and in vivo differentiation of some neoplastic epithelial cells[2-6]. The ability of retinoic acid to promote differentiation does not seem to be limited to epithelial cells, since myeloid leukemic cells of murine origin are also induced to differntiate in vitro, exhibiting an increased lysosomal enzyme activity[7]. More recently Breitman et al.[8] have demonstrated that the treatment of a human promyelocytic leukemia cell line (HL 60) with retinoic acid increases the percentage of cells with the ability to reduce nitroblue tetrazolium salts (NBT), a marker of myeloid differentiation. In the present study we report the effects of retinoic acid on the fibrinolytic activity of HL 60 cells. Since cellular fibrinolytic activity is dependent on lysosomal enzymes, acid phosphatase and muramidase, 2 markers of lysosomal activity, were also studied.

Materials and methods. HL 60 cells (a gift from Dr Rovera, Wistar Institute) were grown in RPMI 1640 supplemented with 12% fetal calf serum (FCS) in 5% CO_2 incubator. Retinoic acid (Sigma, USA) was dissolved in 98% ethanol and used at the final concentration of 1×10^{-6} M; dimethylsulfoxide (DMSO) Sigma, USA) at the final concentration of 1.2%. NBT (Sigma, USA) reduction was assayed as described by Collins et al.[9]. The percentage of differentiated cells was determined on cytocentrifuge slides stained with May Grünwald-Giemsa. Fibrinolytic activity was assayed by the ^{125}I-fibrin plate method of Unkeless et al.[10]: fibrinogen (Kabi, Sweden) was dissolved in water at the concentration of 100 µg/ml and mixed with ^{125}I-fibrinogen (Sorin, Italy) to obtain 60,000 cpm/ml, and placed in 35 mm diameter plastic dishes at the concentration of 10 µg/cm^2. Fibrin was obtained by incubating the plates at 37 °C for 2 h with acid-treated 5% FCS. 2×10^5 cells were resuspended in 1 ml of 0.15 M tris buffer pH 7.8, plated on fibrin plates and incubated for 4 h at 37 °C. Fibrinolysis was evaluated by measuring the radioactivity of the supernatant in a γ-counter. Addition of retinoic acid and DMSO at the same concentrations used in the cellular cultures to the fibrin plates did not produce lysis of fibrin nor affected exogenous urokinase fibrinolytic activity. Plasminogen-free fibrin plates obtained by the method of Lassen[11] allowed us to evaluate the proportion of non plasminogen activation mediated fibrinolytic activity, which was found to be about 50% of the total activity. For the determination of acid

Fibrinolysis, acid phosphatase and muramidase activities of HL 60 cells untreated and following a 4-day treatment with retinoic acid and DMSO

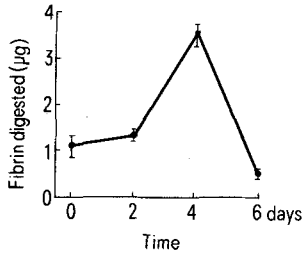
	Fibrinolytic activity*	Acid phosphatase**	Muramidase***
HL 60	1.17 ± 0.2503	107 ± 9.90	3.90 ± 0.1414
HL 60 + retinoic acid	3.51 ± 0.2691	255 ± 7.07	1.75 ± 0.2121
HL 60 + DMSO	0.65 ± 0.1813	188 ± 8.48	1.52 ± 0.1061

* µg of fibrin digested in 4 h at 37 °C/plate; ** pM of p-nitrophenyl phosphate hydrolyzed/min/1×10^6 cells; *** ng of egg white muramidase/1×10^6 cells.

phosphatase (EC 3.1.3.2) and muramidase (EC 3.2.1.17) activities 1×10^7 cells were resuspended in 2 ml of 0.2% sodium deoxycholate in 1 mM tris buffer pH 7.4. The methods described by Nagata et al.[12] and by Prockop and Davidson[13] were used for the acid phosphatase and muramidase assays respectively.

Results and discussion. Following the addition of retinoic acid, HL 60 cells, which are predominantly promyelocytes, begin to differentiate into more mature myeloid cells as judged by their morphology and NBT reduction. Our data, not shown in the text, confirm those reported by Breitman et al.[8].

The effects of retinoic acid on the fibrinolytic activity of HL 60 cells are shown in the figure. The lytic activity was tested 2, 4, and 6 days following retinoic acid addition. Increase of the lytic activity was already present on the 2nd day; on the 4th day, when maximal morphological differentiation occurred, fibrinolytic activity also showed the highest level, while on the 6th day it had already dropped. Fibrinolytic activity was also tested, as shown in the table, following treatment for 4 days with DMSO, another inducer of myeloid differentiation in the HL 60 cell line[14]. No increase was found following the use of this compound. In the table the activities of acid phosphatase and muramidase of untreated cells and of cells treated for 4 days with retinoic acid and DMSO are also reported: both the inducers were found to produce an increase of acid phosphatase and a decrease of muramidase activity. Retinoic acid is known to increase the production of plasminogen activator of mouse teratocarcinoma cells[5]. Our data show that a similar effect is produced by this compound in HL 60 cells. In normal myeloid cells the fibrinolytic activity is known to increase in parallel to the maturation process, reaching a maximum at the metamyelocyte and granulocyte stages[15,16]. Thus the fibrinolytic activity can also be used as a parameter to evaluate myeloid differentiation. In our experiment we found that retinoic acid, but not DMSO, enhanced the fibrinolysis of human leukemic cells. On the contrary other parameters such as the morphology, the ability to reduce NBT and the activities of some lysosomal enzymes were affected in the same manner by retinoic acid and DMSO. Our findings suggest that retinoic acid provides a more reliable model for myeloid differentiation of the HL 60 cell line than DMSO.



Changes of fibrinolytic activity of HL 60 cells at different times following retinoic acid addition. µg of fibrin digested in 4 h at 37 °C/plate. (Mean values ± SD).

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Androgen levels in the rete testis fluid during sexual development[1]

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Summary. The concentrations of testosterone (T) and 5α-dihydrotestosterone were measured in fluid collected from the rete testis of immature and adult rats. The results indicate that adult levels of T are attained in the seminiferous tubules much earlier than in the peripheral circulation.

Production of spermatozoa in the adult male is thought to depend primarily on the locally high concentration of testosterone (T) in the testis. In the adult rat, concentration of T in the fluid collected from the rete testis (rete testis fluid, RTF) is approximately 20–30 times higher than in peripheral plasma[2]. Even though concentration of T in RTF is lower than in lumenal fluid collected from the seminiferous tubules[3], it correlates very closely with efficiency of spermatogenesis under a variety of experimental conditions[2,4] and thus appears to provide a valid estimate of T levels in the spermatogenic compartment of the testis. During sexual maturation of the male rat, plasma T levels increase sharply after the age of 30 days and reach adult values at 50–60 days of age[5]. However, spermatocytes already appear in the testes at 10 days of age and meiotic figures are abundant at 20 days of age[6]. It was therefore of interest to examine changes in androgen levels in the RTF of developing rats. To this end, concentrations of T and 5α-dihydrotestosterone (DHT) were measured in RTF from rats between 30 and 130 days of age. Younger animals were not included, because their testes secrete little if any fluid into the rete[7].

Male CD rats were purchased from Charles River Breeding Laboratories and samples of RTF were collected with glass