tant role in the clearance of bacteria and endotoxin[8]. From this point of view a better manifestation of the protective effectiveness of glucan at higher radiation doses can be understood. At these doses autointoxication and infectious consequences of intestinal damage can be expected to play a leading role in the death of the organism. The decreased efficacy of postirradiational treatment with glucan, as compared with the preirradiational one, may be due to a reduction of available effector cells.

Glucan thus seems to have good radioprotective potencies. However, the proper dosage, time and route of administration have yet to be studied.

1 Wooles, W.R., and DiLuzio, N.R., Science *142* (1963) 1078.
2 Morrow, S.H., and DiLuzio, N.R., Proc. Soc. exp. Biol. Med. *119* (1965) 647.
3 Burgaleta, C., and Golde, D.W., Cancer Res. *37* (1977) 1739.
4 Patchen, M.L., and Lotzová, E., Exp. Hemat. *8* (1980) 409.
5 Kokoshis, P.L., Williams, D.L., Cook, J.A., and DiLuzio, N.R., Science *199* (1978) 1340.
6 Hassid, W.Z., Joslyn, M.A., and McCready, R.M., J. Am. chem. Soc. *63* (1941) 295.
7 Suzuki, M., Yokota, K., and Sakagucki, O., Tohoku Yakka Daigaku Kenkyu Nempo *14* (1967) 7; Chem. abstr. *71* (1969) 109981n.
8 Walker, R.I., Exp. Hemat. *6* (1978) 172.

A rapid method of following the spontaneous regression of experimental leukemias

I. Ember, J. Szöllösi, V. Thomázy, I. Arany, P. Rády and P. Kertai

Institute of Hygiene and Epidemiology, Institute of Biophysics, and Institute of Pathology, University Medical School, H-4012 Debrecen (Hungary), 9 February 1982

Summary. Transplantation of virus and chemically induced leukemias from C3H/He-mg × AKR/F_1 hybrid mice into C3H/He-mg males induced leukemias in the latter, which was followed by a spontaneous regression of the disease within a few days. The regression of leukemia could easily be followed by measuring the changes in the pyruvate kinase activity of para-aortic lymph node cells.

Relatively few reports have been published so far on chemically induced leukemia characterized by a spontaneous regression[1-7]. The degree of regression has been studied mainly by laborious morphological methods. It has earlier been observed in our laboratory that transplantation of Gross-virus leukemia and of a chemically induced leukemia from C3H/He-mg × AKR/F_1H-2^k hybrid mice into male C3H/He-mg mice identical at the H-2^k locus induces leukemia in the recipients. It will however, regress spontaneously within a few days. According to Weber, pyruvate kinase is a good marker of malignancy in experimental tumors[8]. The activity of pyruvate kinase also increases in the lymph nodes and in the spleen with both Gross-virus leukemia[9], and chemically induced leukemia[10]. Hence, the changes in pyruvate kinase activity, which can be determined by a simple and rapid method, can serve as a marker of the spontaneous regression of leukemia. In the present communication the changes in pyruvate kinase activity in para-aortic lymph nodes were compared with the results of histological and flow cytometric studies.

Materials and methods. 6-8-week-old male and female AKR and C3H/He-mg inbred mice were purchased from the Laboratory Animal Breeding Center of Hungary (Gödöllö). To obtain hybrid mice, pairs of the 2 strains were cross-bred in our own animal breeding house. Gross-virus leukemia was originally obtained from an AKR mouse, and has been maintained in our laboratory in AKR/Lati × C3H/He-mg/Lati/F_1 hybrid mice by serial passages since 1975. Chemically induced leukemia was originally observed in our laboratory in AKR/Lati × C3H/He-mg/Lati/F_1 hybrid female mice after a simultaneous treatment with urethane and diethylstilboestrol[11]. This form of leukemia has since been maintained in these hybrids by serial passages. Both forms of leukemia were transplanted by i.p. injection of 10^6 viable spleen cells[12]. The animals with Gross-virus leukemia were killed by cervical dislocation 3, 6, 9 or 14 days after injection.

Histological sections, prepared from fragments of different organs fixed in formalin and embedded in paraffin, were stained with haematoxilin-eosin. The activity of pyruvate kinase from para-aortic lymph nodes was measured according to Guttman and Bernt[13]. The size of cells from mesenteric lymph nodes was determined with a Becton-Dickinson FACS-III flow cytometer on the basis of the scattered light intensity of the cells[14,15]. Similar studies were also carried out in the case of chemically induced and transplanted leukemia except that the experiments were performed on the 9th, 14th and 21st (and also, in certain cases, on the 6th, 7th and 28th) days after injection with leukemic spleen cells because of the longer duration of the disease.

Results and discussion. Histological studies indicate that Gross-virus leukemia both in male and in female mice, and also chemically induced leukemia in C3H/He-mg male mice, are accompanied by the gradual formation of diffuse blastic infiltration which destroys the original structure of the lymph nodes spleen and thymus. Then residual proliferating foci are present during regression and finally, only the loose structures indicate the previous leukemic state. The phase of regression is characterized by a considerable increase in macrophage activity.

The size distribution of lymphocytes prepared from the mesenteric lymph nodes of C3H/He-mg and AKR mice is presented in figures 1 and 2, respectively as was determined

Pyruvate kinase activity/mU/mg/in para-aortic lymph node cells

	Gross-virus-induced leukemia	Chemically induced transplantable leukemia
Control	227.4 ± 19.6	
3rd day	351.11 ± 25.4	301.11 ± 24.5
6th day	632.21 ± 22.8[a]	663.90 ± 25.2[a]
9th day	259.22 ± 30.2	494.19 ± 26.8[a]
14th day	222.23 ± 20.0	261.38 ± 29.9
21st day	220.31 ± 18.1	257.12 ± 20.6
28th day	223.22 ± 19.3	285.50 ± 27.3

[a] $p < 0.001$. Each value represents the mean of 12 determinations ± SE.

at different times after injection with Gross-virus or chemically induced leukemia. The 1st peak of the scattered light intensity histograms indicates small normal cells, and the 2nd one corresponds to large blastic cells. The changes in the ratio of normal to leukemic lymphocytes also corroborate the results of histological studies. Both Gross-virus leukemia and chemically induced leukemia kill both the male and female mice when transplanted back into AKR mice, one of the parental strains. Gross-virus leukemia shows a regression in C3H/He-mg mice, both in males and in females, whereas chemically induced leukemia kills the C3H/He-mg females; however, in the males it regresses similary to Gross-virus leukemia.

The activity of pyruvate kinase in para-aortic lymph nodes of mice with Gross-virus or chemically induced leukemia is presented in the table. In both cases the highest activity values were measured on the 6th day. The activity of pyruvate kinase reached a normal value on the 14th day in Gross-virus leukemia, whereas in chemically induced it became normal only on the 21st day. Our results correlate well with the results of much more laborious morphological studies as well as with those obtained by the more sophisti-

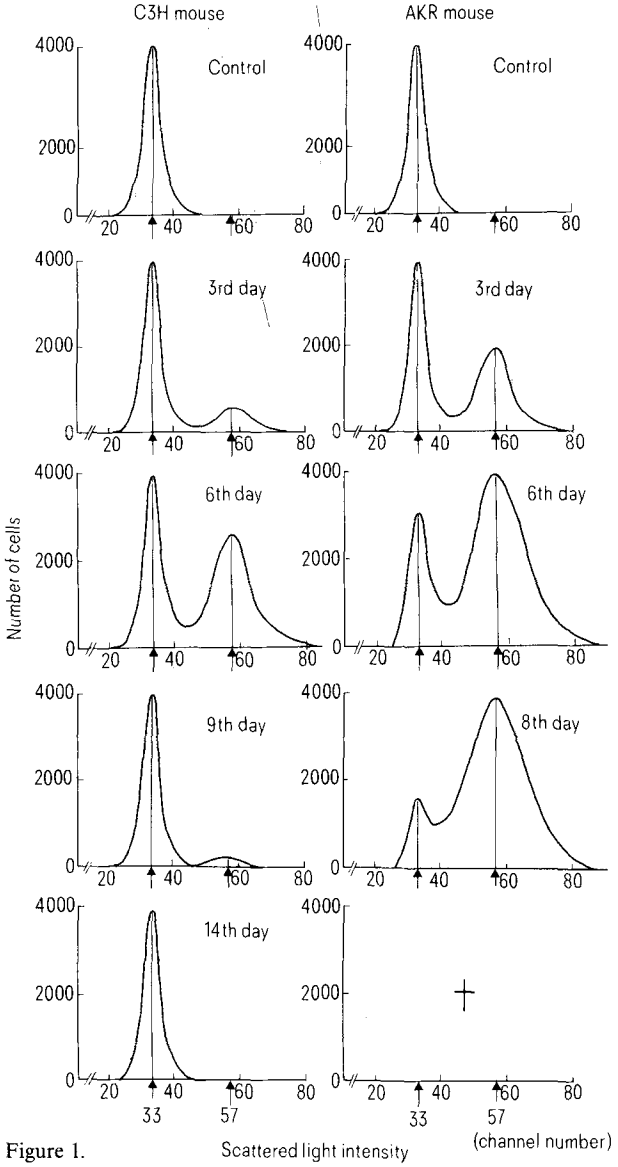

Figure 1.

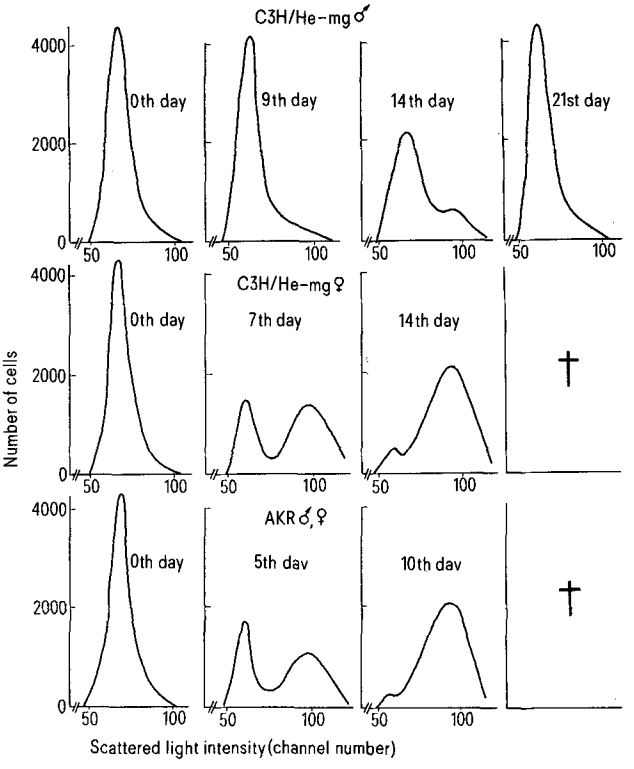

Figure 2.

cated flow cytometric methods. Therefore, this simple and rapid procedure may be suitable for the detection of the progression or regression of leukemia and for the study of the mechanism of spontaneous regression[16-19] as well as for testing chemotherapeutic agents.

1 Herberman, R.B., and Oren, M.E., J. natl Cancer Inst. *46* (1971) 391.
2 Oren, M.E., and Herberman, R.B., J. natl Cancer Inst. *46* (1971) 621.
3 Rich, M.A., and Clymer, R., Cancer Res. *31* (1971) 803.
4 Rich, M.A., Siegler, R., Karl, S., and Clymer, R., J. natl Cancer Inst. *42* (1969) 559.
5 Tóth, F.D., Gomba, Sz., Váczi, L., Kása, M., and Jakó, J., Neoplasma *23* (1976) 771.
6 Rice, J.M., and Davidson, J.K., Cancer Res. *31* (1971) 2008.
7 Ember, I., Arany, I., Rády, P., Szöllösi, J., Tatár-Kiss, Zs., Thomázy, V., and Kertai, P., Kisérl. Orvostud. *33* (1981) 101 (in Hungarian).
8 Weber, G., New Engl. J. Med. *296* (1977) 486 and 541.
9 Arany, I., Rády, P., and Kertai, P., Br. J. Cancer *43* (1981) 804.
10 Ember, I., Arany, I., Szöllösi, J., Thomázy, V., and Tatár-Kiss, Zs., 6th Meet. Eur. Ass. Cancer Res., Budapest 1981, abstr. No. 168.
11 Boján, F., and Kertai, P., unpublished results.
12 Kiss, A., Jakó, J., Telek, B., Rády, P., Rák, K., and Kertai, P., Magy. Onkol. *24* (1980) 243 (in Hungarian).
13 Guttman, J., and Bernt, E., in: Methoden der enzymatischen Analyse, p. 800. H.V. Bergmeyer. Verlag Chemie, Weinheim 1974.
14 Arndt-Jovin, D.J., and Jovin, T.M., FEBS Lett. *44* (1974) 247.
15 Jovin, T.M., Morris, J., Stiker, G., Schultens, H.A., Disweed, M., and Arndt-Jovin, D.J., J. Histochem. Cytochem. *24* (1976) 269.
16 Boiocchi, M., Pierotti, M.A., Ménard, S., Miotti, S., and Colnaghi, M.J., Tumori *65* (1979) 435.
17 Slettenmark, B., and Klein, E., Cancer Res. *22* (1962) 977.
18 Rich, M.A., Karl, S., and Clymer, R., J. Immun. *106* (1971) 1488.
19 Landazuri, M.O., de Kedar, E., and Fahey, J.L., J. natl Cancer Inst. *52* (1974) 147.