MORPHOLOGICAL BEHAVIOUR OF LYMPHO-MYELOID ORGANS AT EXPERIMENTAL STIMULATION WITH LEUKOTROPHINE

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Summary. The influence of leukotrophine on the behaviour of lymphomyeloid organs was studied in male Swiss mice aged 2 months. In the four comparatively studied groups it was shown that leukopoiesis and immune maturation was slightly stimulated by leucotrophine or protected by thiola, a radioprotective substance; the administration of both substances, leukotrophine + thiola, enhances the two actions, myelo-lymphoid leukopoiesis and cellular pyroninophilia, almost doubling them. Histochemical studies were supplemented with histoenzymatic and cytogenetic investigations.

Key words: leukotrophine, thiole, leukopoiesis, immune competence.

Leukotrophine is a thymic extract which has been considered an important leukopoietic stimulant and an efficient modulator of immunocompetent cells (Comșa, 1964; Takada et al., 1969; Călușer, 1974; White, 1975). In order to verify these two potential actions of leukotrophine we have imagined an experimental model which simulates, on animal organisms, the situation of leukodepressed humans.

MATERIAL AND METHODS

The experiment was carried out in four groups of animals, each consisting of six male Swiss mice, aged two months, as shown in Table 1.

Fragments of femoral bone marrow were used for smears; pieces of spleen, liver, thymus, colon and mesenteric lymph nodes were processed after embedding them in paraffin or freezing and cutting them in a cryostat. Bone marrow smears were stained with May Grünwald-Giemsa. Paraffin embedded slides were stained with hematoxylin-eosin, PAS and methyl-green-pyronin. Succinate dehydrogenase ATP-ase, acid and alkaline phosphatases were also investigated.

Similarly treated animals were sacrificed for cytogenetic studies.

Table 1

Experimental groups and methods

group	irradiation	day, intervals, substances	sacrificing term after irradiation
M(controls)	1 dose 400 rads	–	9th day
L (leukotrophine)	idem	days 1–6, 2 ml leukotrophine	9th day
T (hiola)	idem	days 1–6, 200 mg thiola/kg body weight	9th day
LT (leukotrophine + thiola	idem	days 1–6, 2 ml leukotrophine + 200 mg thiola/kg body weight	9th day

RESULTS AND DISCUSSION

The examination of femoral bone marrow smears revealed more numerous granulocytes and lymphocytes in groups T and LT, while megakaryocytes were denser only in group LT (Fig. 1).

Histopathologically a marked depletion of lymphoid cells in the spleen (Fig. 2) and lymph nodes of groups M, L and T (Fig. 3), was obvious and a spectacular regeneration of lymphocytes in the red pulp of the spleen (Fig. 4) and of the lymphoid follicles of lymph nodes in group LT. Lymph node follicles tend to be restored in the T group, too (Fig. 5). The thymus shows a slight medullary depopulation of lymphocytes in groups M and T, and a clear repopulation in groups L and LT, i.e. where leukotrophine had been used.

The number of megakaryocytes is normal in group M, but the nuclei show dystrophic changes, while in group L megakaryocytes are very numerous and their trophicity is preserved, suggesting a possible thrombopoietic role of leukotrophine (Fig. 6).

The immune competence of cells, as revealed by pyroninophilic affinity of cells, is similar in the lymphoid organs, however there are also quantitative differences between the four experimental groups. Pyroninophilia is reduced in group M, slightly diminished in group L, and well represented in groups T and LT (Fig. 7).

In order to verify the action of mice organisms we also studied the behaviour of the liver. Nuclear pyknosis and hepatocytic necrosis were seen in all groups; there are, however, striking differences concerning the mitotic activity in hepatocytes; the most numerous mitoses are seen in group M; they are less frequent in groups L and T and almost disappear in group LT (Fig. 8).

Cytogenetic studies included the frequency of micronuclei in erythrocytes (Table 2) and the incidence of metaphases with chromosomal abnormalities in the bone marrow cell population (Table 3).

Table 2

Incidence of micronuclei (mean values)

group M	28‰
group L	27.7‰
group T	23.7‰
group LT	24.2‰

Table 3

Incidence of cells with chromosomal abnormalities (mean values)

group M	58%
group L	61%
group T	53%
group LT	51%

The frequency of micronuclei and the incidence of cells with chromosomal abnormalities are similar in controls and in group L; both T and LT groups show lower values, indicating a slight radioprotective effect of thiola.

Histoenzymatic studies did not reveal conspicuous changes. Thus, succinate dehydrogenase was more evident in colonic and hepatic cells of the group LT, indirectly revealing the protection of animals; similarly, the ATP-ase in the spleen and liver cells was more manifest in the same

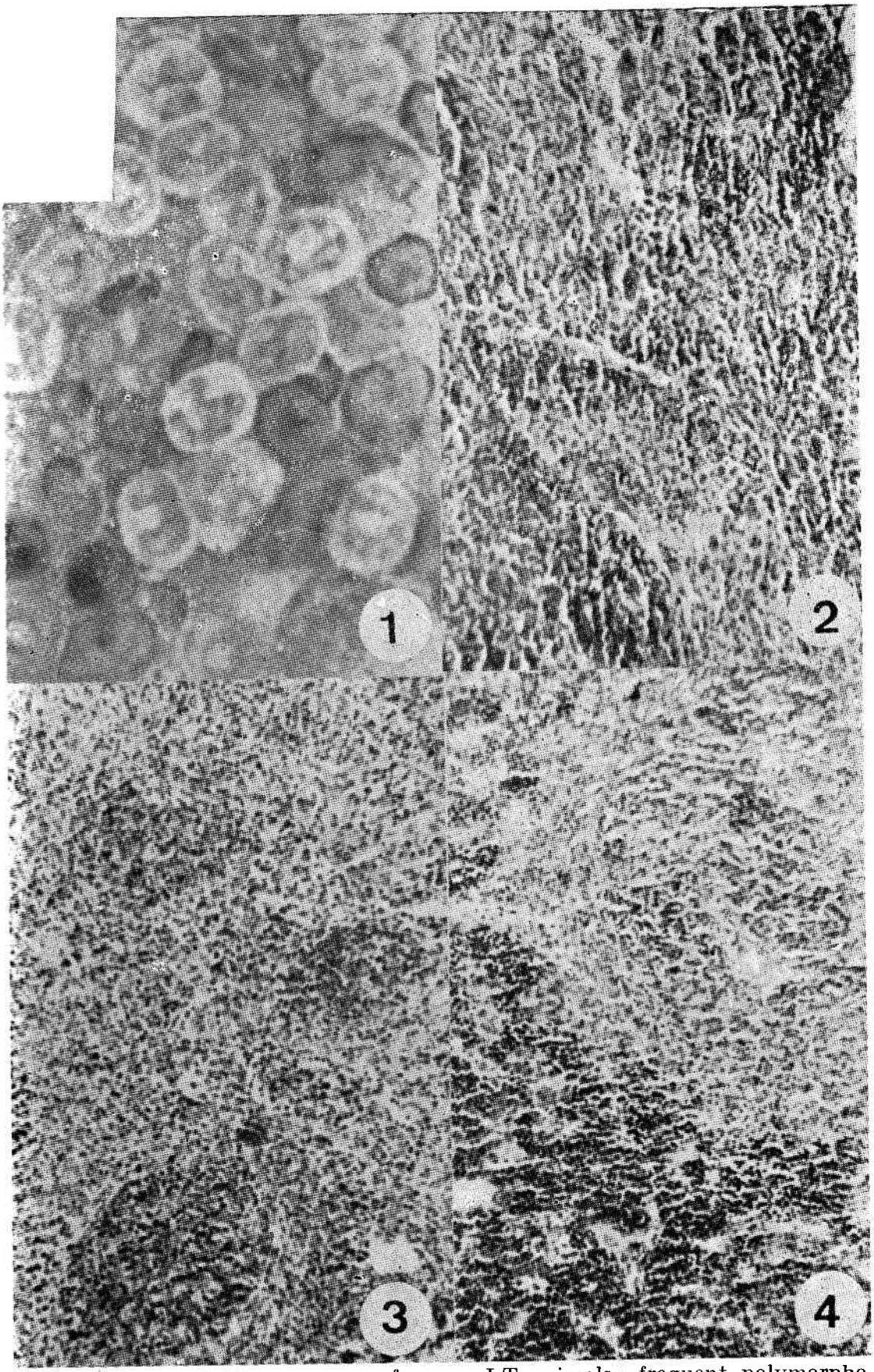

Fig. 1. — Femoral bone marrow of group LT animals : frequent polymorphonuclears and lymphocytes (x 400).

Fig. 2. — Moderate lymphoid depletion in spleen of groups M, L and T (x 140).

Fig. 3. — Marked lymphoid depopulation in lymph nodes of groups M. L and T (x 140).

Fig. 4. — Conspicuous lymphoid regeneration in the red pulp of spleen of LT animals (x 140).

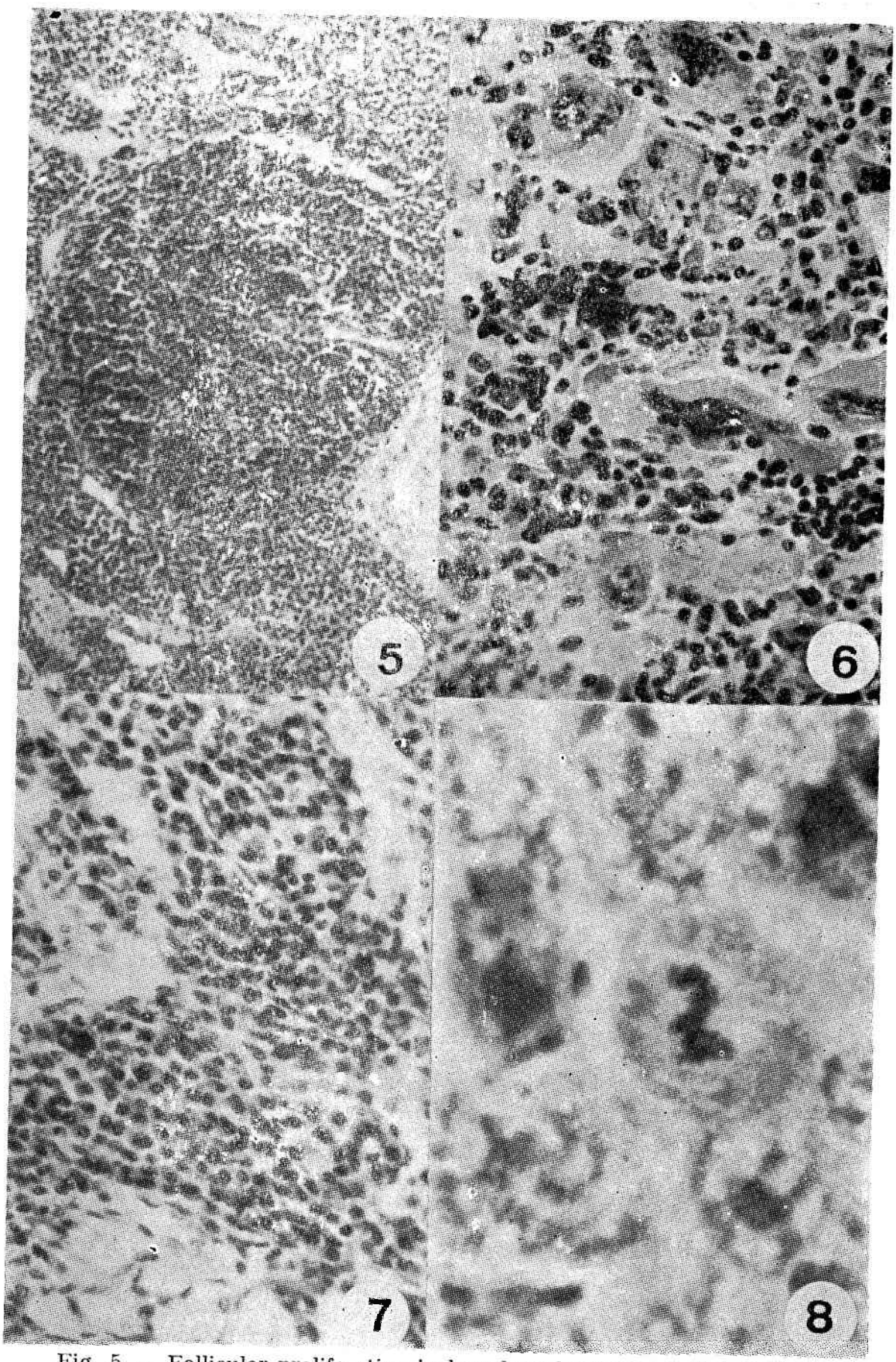

Fig. 5. – Follicular proliferation in lymph nodes of group T (x 140).
Fig. 6. – Dense megakaryocytic population in spleen of L group animals (x 140).
Fig. 7. – Numerous intensely pyroninophilic cells in lymph nodes of L group animals (x 240).
Fig. 8. – Dystrophic changes and numerous mitoses in the liver of M group animals (x 400).

group. Alkaline phosphatase was more obvious in the spleen and medulla of lymph nodes in groups L, T and LT than in controls, while acid phosphatase did not exhibit significant changes.

CONCLUSIONS

The analysis of results shows that the administration of leukotrophine after irradiation has leukopoietic effects (Goodman and Grubs, 1970); Sudo et al., 1970; Vavrova et al., 1975 a,b; Uray et al., 1979 a, b) and thrombopoietic effects (Căluşer, 1974), enhanced by the association of a radioprotective agent like thiola. This effect also bears positively on the immunocompetence of lymphoid cells. The positive and additive effect of these two substances is also proved by the enzymic behaviour of nonlymphoid organs and by the cytogenetic studies on femoral bone marrow cells.

The study reveals the practical importance of obtaining a compound with protective effects on irradiated organisms.

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