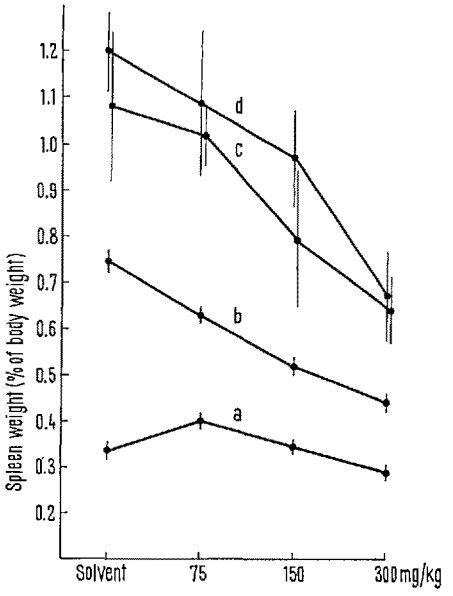

Effect of ovalicin on spleen weight in (albino × DBA/2)F_1 male mice. The spleens were weighed on day 7, 4 h after the last drug application. Ovalicin was given i.p. to all 4 groups on days 5, 6 and 7. Curve a, drug only. Curve b, SRBC i.v. on day 4. Curve c, 10^6 L-1210 cells on day 0. Curve d, L-1210 cells on day 0 and SRBC on day 4. Each dot represents the mean ± S.E.M. of 4–6 animals.

becomes also evident from comparison of the mitotic index in the spleen with that in the jejunum (Table IV) and from the Figure; similarly, there seems to be no inhibition of proliferation in the hematopoietic system, since rats[1] and rhesus monkeys[6] showed normal leucocyte values after treatment with immunosuppressive doses of ovalicin.

Zusammenfassung. Ovalicin, isoliert aus dem Kulturfiltrat von *Pseudeurotium ovalis*, hemmt die Bildung von Antikörpern schon nach einmaliger Applikation. Es kommt zur Ausbildung einer partiellen immunologischen Toleranz. Die Abstossungszeit von homologen Hauttransplantaten wird bei Mäusen durch eine einmalige Ovalicinbehandlung signifikant verlängert. Die Substanz hemmt den Anstieg des Milzgewichtes bei Mäusen, die mit Schaferythrozyten immunisiert und/oder mit lebenden Leukämie-L-1210-Zellen geimpft wurden; letzteres gilt auch für den isologen Wirt (L-1210-Zellen in DBA/2-Mäusen). Die Hemmung des Milzgewichtanstieges durch Ovalicin geht parallel mit einer Reduktion des Mitoseindex in der Milz von immunologisch stimulierten Tieren; die Mitosenzahl im Darmepithel wird hingegen nicht beeinflusst.

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[6] S. Lazáry, unpublished results.

Hypodiploidy of Bone Marrow in Hypertransfused Mice Stimulated with Erythropoietin

The karyological examination of the bone marrow of persons carrying body burdens of ^{90}Sr and ^{226}Ra revealed, apart from other abnormalities, hypodiploidy of marrow elements[1]. However, the question of the mechanism by which the ionizing radiation induces the decrease of ploidy still remains unexplained. In this connection, attention was paid to some earlier findings[2,3] which gave suggestive evidence of the fact that the loss of chromosomes takes place in the course of maturation of erythroid precursors. Providing this assumption be confirmed, varying representation of haemopoietic series and maturation stages in the bone marrow could participate in the change of ploidy after irradiation.

The model of the bone marrow with varying proportions of individual generations of red precursors was prepared by using hypertransfused female C 57 Bl mice in which the bone marrow was examined at the intervals of 24, 48 and 72 h after the administration of erythropoietin. The mice were injected i.p. on 2 successive days with 1 ml of 80% suspension of isogeneic donor red cells. It was assumed that on the sixth day after the first transfusion the bone marrow had been cleared from differentiated erythroid precursors.[4,5] At that time erythropoietin was administered in the amount of 16 U in one experiment and of 6 U in the other, both levels representing the submaximum stimulation, however. Intact animals and hypertransfused ones not treated with erythropoietin were investigated as well. At the time of sacrifice haematocrit was checked and in all animals the values were well above the critical level,[6,7] being at least 60%.

The mice were injected i.p. 2 h prior to sacrifice with colchicine (1×10^{-5} g/g body weight) and the bone marrow obtained from the femur was immediately treated cytologically. After hypotonic treatment and fixation, the smears were prepared, dried over the spirit flame and stained with Giemsa. Chromosomes in 100 metaphases from each animal were counted by drawing from microfilm. The metaphases with non-reproducible count and/or without distinct cytoplasmatic area were discarded. The cells with chromosomal counts $2n = 40$ were classified as euploid. The chromosomal count in our study ranged from 5–47. In Figure 1 the hypodiploid and euploid fractions are included. The first pair of columns shows the situation in the intact controls. The second pair of columns depicts the decrease of hypodiploidy in hypertransfused mice. The next 3 pairs of columns represent the values obtained at

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[5] K. Hughes, J. Anim. Techns Ass. *15*, 1 (1964).
[6] R. Alexanian, D. D. Porteous and L. G. Lajtha, Int. J. Radiat. Biol. *7*, 87 (1963).
[7] D. D. Porteous, S. C. Tso, K. Hirashima and L. G. Lajtha, Nature *206*, 204 (1965).

the early, medium and late stage of the wave of erythropoiesis in the bone marrow[4]. The increase in the hypodiploid fraction can be seen as early as 24 h after the administration of erythropoietin. Figure 2 shows the distribution of chromosomal counts in intact animals, hypertransfused mice, and in mice 72 h after the erythropoietic stimulation.

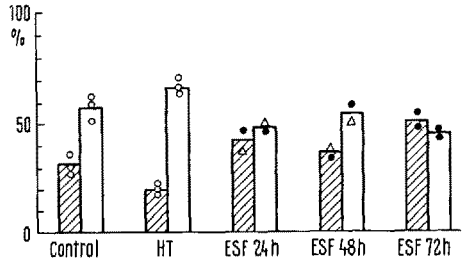

Fig. 1. Fraction of hypodiploid and euploid cells. Dark columns represent the hypodiploid fraction in % of all metaphases evaluated. White columns show the euploid fraction. Counts of chromosomes higher than diploid ($2n$) have been omitted for the sake of simplicity. The sequence of pairs of columns: (1) intact controls; (2) mice 6 days after hypertransfusion (HT); (3) hypertransfused mice, 24 h; (4) 48 h; (5) 72 h after erythropoietic stimulus. Different symbols are used for 2 levels of erythropoietin (ESF) administered. • 6U; △ 16 U.

The bone marrow of intact controls represent the mixed population of blood precursors, which corresponds to the physiological conditions. In the group of mice hypertransfused only, the bone marrow is cleared from erythroid precursors so that the remaining pool of marrow cells is represented by myeloid and lymphoid cells including the stem cells. This was the situation under which the decreased hypodiploid fraction was found. In the hypertransfused mice stimulated with erythropoietin, the increased hypodiploidy was observed at all time intervals. It is not possible, however, to ascribe the degree of hypodiploidy found to the defined generation of erythroid precursors owing to the admixture of elements belonging to other hematopoietic series. Supposing that the increase of hypodiploidy reflects the enrichment of marrow population with differentiated erythroid precursors, the actual fraction of hypodiploid elements in pure erythroid population must be higher than our values.

Summarizing our data from karyological examination, we may conclude that in mice bone marrow enriched with erythroid precursors shows the decrease of chromosomal counts. The question remains to be explored of whether the process of chromosomal loss during differentiation and maturation is of more general biological significance.[8]

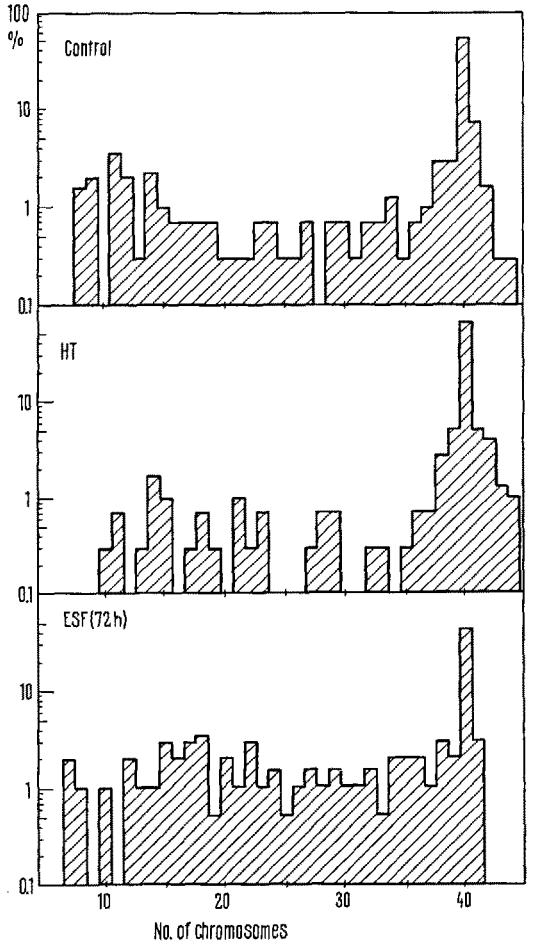

Fig. 2. Distribution of the chromosome count in bone marrow cells in % of total metaphases evaluated in each group of mice. Above: intact controls. In the middle: mice hypertransfused only (HT). Below: mice 72 h after erythropoietic stimulus (ESF).

No. of animals and evaluated metaphases in parenthesis

Strain	Control	Hyper-transfused only	Units of ESF adminis-tered	Time after administration 24 h	48 h	72 h
C 57 Bl		1 (100)	16 U	1 (100)	1 (100)	
C 57 Bl	3 (300)	2 (200)	6 U	1 (100)	1 (100)	2 (200)

Zusammenfassung. Bei Mäusen führte eine Hypertransfusion zur Abnahme hypodiploider Zellen im Knochenmark. Nach Verabreichung von Erythropoietin wurden höhere Fraktionen der hypodiploiden Zellen als bei unbehandelten Kontrollen gefunden.

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Institute of Radiation Hygiene, Praha (Czechoslovakia), 6 May 1968.

[8] We are indebted to Dr. J. MÜLLER for his interest in this work and for his valuable comments and criticism. The erythropoietin (of urinary origin) used in this work had been supplied by the courtesy of Dr. TAKAKU from the Tokyo University.