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In Vivo Uptake of ^{57}Co, ^{54}Mn and ^{65}Zn by Peripheral Lymphocytes, Tumor and Various Organs of Rats Bearing Walker 256 Carcinosarcoma

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Abstract—*The* in vivo *uptake of nearly carrier free* ^{57}Co, ^{54}Mn *and* ^{65}Zn *in peripheral lymphocytes and various organs of Walker* 256 *carcinosarcoma bearing rats was measured* 2 hr *after i.v. administration of the radioactive tracers. Highly significant differences in plasma uptake of* ^{57}Co *and* ^{65}Zn *as well as peripheral lymphocyte uptake of* ^{57}Co *and* ^{54}Mn *were detected between tumor bearing animals and control groups. The amount of* ^{57}Co *and* ^{65}Zn *activity in plasma steadily decreases starting on the fourth day after transplantation.* ^{57}Co *and* ^{54}Mn *show a* 60–70% *enchanced accumulation in the lymphocytes only during the third day after transplantation. This increased uptake of* ^{57}Co *and* ^{54}Mn *is caused by macrophages. The decrease of tracer activities in plasma corresponds to the decreasing content of* Co *and* Zn *in the plasma of Walker* 256 *carcinosarcoma bearing rats.*

INTRODUCTION

RECENTLY the role of trace elements, especially of Zn, during transformation of white blood cells is of particular interest to many authors. Rühl *et al.* [1] found that Zn^{2+} stimulated DNA synthesis of cultured lymphocytes. Similar results are reported by Berger *et al.* [2], who found a similar effect of Hg^{2+} on DNA synthesis, while Mn^{2+}, Co^{2+}, Cu^{2+} and Ni^{2+} added to the culture medium, had an inhibitory effect. Phillips [3] observed a decreased Zn-transferrin uptake by leukemic lymphocytes after stimulation with poly-L-ornithine in comparison to lymphocytes from healthy volunteers. Zn content of granulocytes in normal subjects was significantly higher than that in patients suffering from neoplastic disease [4]. Phagocytic capacity of peritoneal macrophages in mice was shown to be dependent on the Zn content of the administered diet [5].

In a previous study [6] we determined the concentrations of 7 trace elements (Co, Cu, K, Mn, Rb, Se, Zn) in lymphocytes of Walker 256 carcinosarcoma bearing rats by means of neutron activation analysis. No differences in the lymphocyte trace element content between tumor bearing animals and controls could be detected, although the amounts of Co and Zn in plasma of tumor bearing animals strongly decreased.

The present work investigates the *in vivo* behaviour of i.v. administered radioactive Co, Mn and Zn isotopes in order to determine whether the rate of exchange or uptake of these isotopes in peripheral lymphocytes is influenced by a rapidly growing tumor.

MATERIALS AND METHODS

Male Sprague–Dawley rats (280–300g, obtained from Mus Rattus, Brunnthal, Munich) were injected subcutaneously with 10^7 Walker 256 carcinosarcoma ascites cells in 0.5 ml of Hanks' balanced salt solution. Animals were kept in Macrolon cages and received a standard diet (Altromin, Altrogge, Lage, Lippe) and water *ad libitum*.

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On each of seven consecutive days, starting with the first day after transplantation, six tumor bearing animals and six controls were injected i.v. with a mixture of 23.7 μCi ^{57}Co, 2.44 μCi ^{54}Mn and 7.8 μCi ^{65}Zn in 0.5 ml saline solution of pH 6.5. The dose corresponded to 0.003 μg Co, less than 0.001 μg Mn, and 0.07 μg Zn. Two hours after administration of activity animals received 0.2 ml (1000 i.u.) of Heparin i.v., were etherized and sacrificed by heart puncture. Tumor, liver, kidney, thymus and spleen were removed and weighed. The plasma was isolated by centrifuging at 3000 rev/min for 20 min. Leucocytes from whole blood were counted with a Coulter Counter model ZBI (Coulter Electronics, Dunstable, Bedfordshire, GB).

Lymphocytes were separated from heparinized blood via Ficoll-Isopaque gradient centrifugation at 400 ***g*** as described by Böyum [7]. The cells were then washed three times with 25 ml of a tissue culture medium (TCM 199 Difco), containing 625 i.u. heparin, and then centrifuged at 160 ***g*** to remove platelets. The final cell pellet was resuspended in 3 ml TCM from which 2 ml were taken for gamma counting. The amount of cells per ml was determined from another aliquot with a Coulter Counter. In general (12–15)$\times 10^6$ cells should be isolated from 7.5 ml of blood.

In a subsequent experiment uptake of ^{57}Co in macrophages was determined. Seven of fourteen male Sprague–Dawley rats (200–230g) were transplanted with 10^7 ascites cells. Three days after tumor transplantation animals received 23 μCi ^{57}Co i.v. and were sacrified 2 hr later by heart puncture as described above.

The separation of peripheral lymphocytes was somewhat modified. Six millilitres of blood were diluted with 6 ml of Eagle's MEM (minimum essential medium with glutamine and Hepes, Flow Laboratories, U.K.) containing 150 i.u. heparin. This mixture was layered onto 12 ml of a dextran solution (21.6 g dextran T500, Pharmacia, Uppsala, 80 ml sodium metrizoate 75%, w/v, Nyeguaard, Oslo and 460 ml H_2O) and kept for 2 hr at 37°C in an incubator. After centrifugation of the supernatant, which was extracted from the bulk of erythrocytes, at 3000 rev/min for 10 min, the resulting cell pellet was resuspended in 10 ml of Eagle's MEM and separated via a Ficoll–Ispaque gradient according to Böyum [7]. Cells accumulated near the boundary between Eagle's MEM and Ficoll–Isopaque solution were pipetted off and washed 4 times with Eagle's MEM solution at 3500, 2000, 1500 and 1200 rev/min. The final cell pellet was then resuspended in 4 ml of RPMI 1640 medium with glutamine and Hepes (Gibco Biocult, Glasgow). One aliquot was taken for gamma counting, another for cell counting in a Coulter Counter and a third was transfered to culture dishes 2cm in diameter (Falcon Plastics, Oxnard, U.S.A. The culture dishes were then allowed to stand 1 hr in an incubator at 37°C. Subsequently the cell suspension was decanted and again centrifuged at 1500 rev/min for 10 min. Supernatant was taken for gamma counting, the cell pellet was resuspended in Eagle's MEM and aliquots were taken for cell and gamma counting. The culture dishes were carefully washed twice with Eagle's MEM. Macrophages were then detached and lysed with 2 ml of a 0.2% solution of sodium dodecylsulphate and were measured in a gamma counter.

Gamma counting of all samples was done in a 5 × 6 in. NaI/Tl well detector coupled with a computerized multichannel analyser. The amount of radioactivity of ^{57}Co ($t_{\frac{1}{2}}$ 270 days, 0.122 and 0.136 MeV), of ^{54}Mn ($t_{\frac{1}{2}}$ 312 days, 0.835 MeV) and of ^{65}Zn ($t_{\frac{1}{2}}$ 244 days, 0.511 and 1.115 MeV) in each sample was computed by applying a multi-least square fit of known standard spectra to the sample spectrum.

RESULTS AND DISCUSSION

The increase of leucocytes in whole blood as well as tumor weight during the seven days after tumor transplantation of 10^7 Walker 256 carcinosarcoma ascites cells is given in Table 1. Figures 1 and 2 show the distribution of ^{57}Co, ^{54}Mn and ^{65}Zn in lymphocytes, blood, plasma, liver, tumor, kidney, thymus and spleen of tumor bearing and control animals 2 hr after injection of radioactive tracers.

As indicated by the 2-fold variance analysis the amounts of ^{57}Co and ^{65}Zn are significantly lower in plasma of tumor bearing animals. This is in agreement with the 30% decreased Co and 50% decreased Zn content of plasma in Walker carcinosarcoma bearing rats as determined by neutron activation analysis [6]. A possible explanation could be a substitution of Co^{2+} and Zn^{2+} from their plasma binding sites by Ca^{2+}, because growth of a Walker 256 carcinosarcoma causes hypercalcaemia and soft tissue calcification in the host animal [8]. This hypothesis, that the high loss of Co and Zn in plasma of Walker tumor bearing rats is not caused by tumor growth

Table 1. Tumor growth, increase of leucocytes in blood and yield of peripheral lymphocyte preparations in male Sprague–Dawley rats (280–300 g) *after transplantation of* 10^7 *ascites cells of the Walker* 256 *carcinosarcoma*

Days after transplantation	No. of leucocytes ± S.D. × 10^6/ml blood		No. of peripheral lymphocytes ± S.D. × 10^6 isolated/ml blood		Tumor weight (g ± S.D.)
	Tumor*	Control*	Tumor*	Control*	
1	11.7 ± 2.3	12.0 ± 3.4	2.0 ± 0.6 (17.1)†	2.3 ± 0.3 (19.2)†	—
2	13.7 ± 1.4	11.9 ± 2.0	2.1 ± 0.4 (15.3)	1.9 ± 0.8 (16.0)	—
3	14.3 ± 1.7	11.1 ± 2.1	2.6 ± 0.3 (18.2)	2.5 ± 0.7 (22.5)	0.18 ± 0.15
4	17.7 ± 2.0	11.6 ± 1.0	2.8 ± 0.6 (15.8)	2.4 ± 0.5 (20.7)	0.32 ± 0.19
5	16.0 ± 1.7	12.7 ± 2.1	2.6 ± 0.2 (16.3)	2.9 ± 0.8 (22.8)	1.04 ± 0.78
6	19.7 ± 4.4	12.5 ± 3.4	2.7 ± 0.8 (13.7)	2.1 ± 0.8 (16.8)	2.78 ± 0.80
7	22.7 ± 3.6	11.8 ± 2.2	2.2 ± 0.5 (9.7)	2.4 ± 0.5 (20.3)	8.59 ± 2.27

*$n = 6$

†No. of peripheral lymphocytes expressed as per cent of leucocytes.

The increased leucocyte number as compared to the experiment in Table 2 is due to the advanced age of the animals.

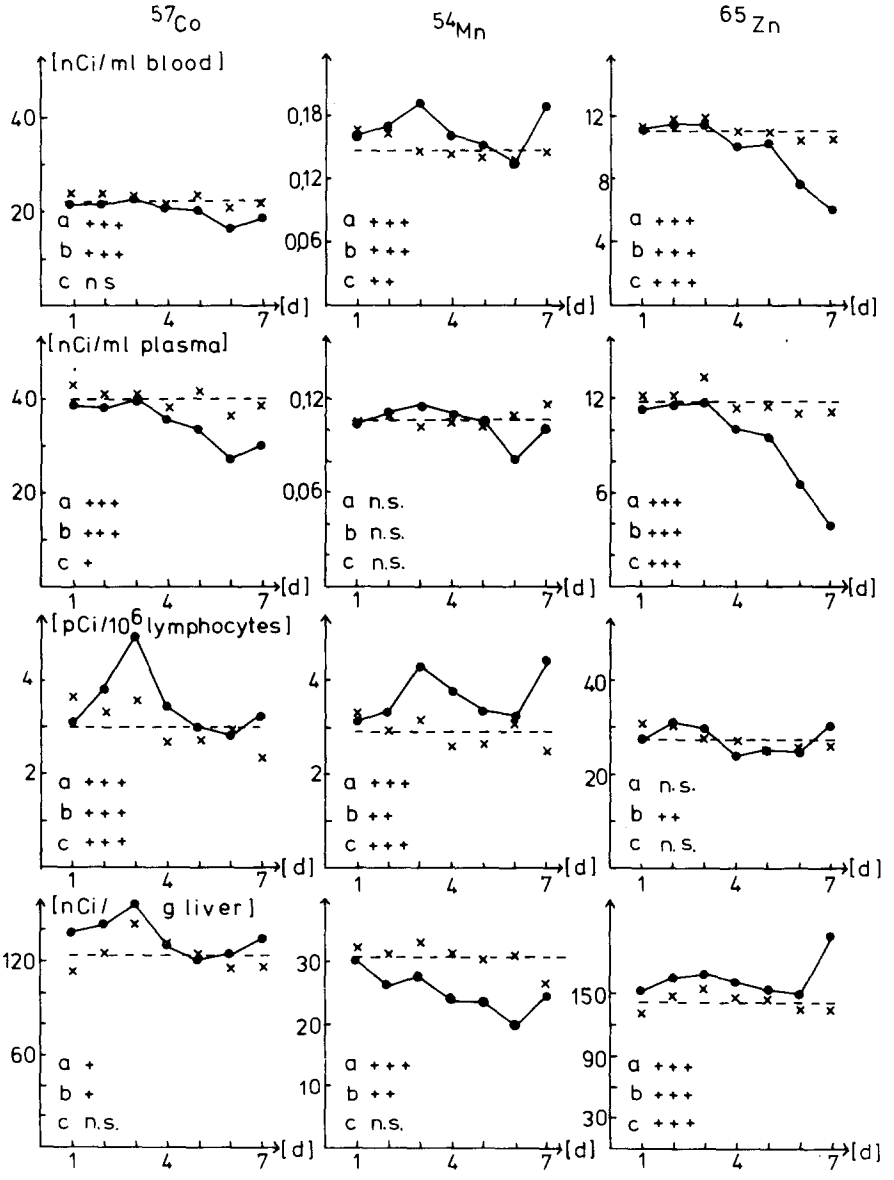

Fig. 1.

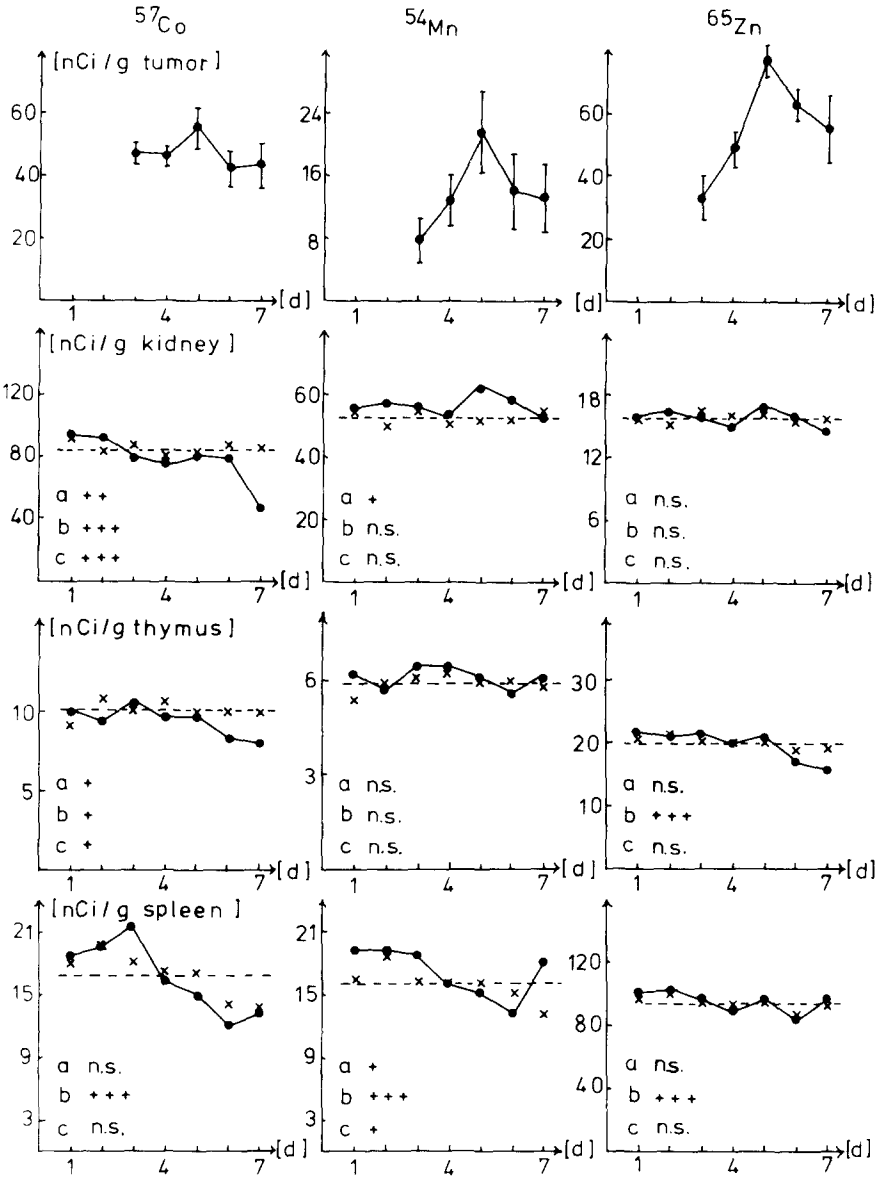

Fig. 2.

Figs. 1 and 2. Activity distribution in lymphocytes, blood, plasma, tumor and various organs of male Sprague–Dawley rats (280–300g) *2 hr after administration of* 23.7 μCi ^{57}Co, 2.44 μCi ^{54}Mn *and* 7.8 μCi ^{65}Zn.

Abscissa: days after transplantation of Walker 256 carcinosarcoma. Straight line, shaded circles = tumor bearing animals. Stars = controls. Each point mean value of 6 animals. Dashed line = mean value of the 7 control groups.

Two-way variance analysis: a = difference between tumor bearing animals and controls, b = time response, c = difference in time response between tumor bearing animals and controls.

Degree of significance: $+++P<0.001$, $++P<0.01$, $+P<0.05$. *n.s. = not significant*

alone, is supported by an investigation of Co and Zn content in plasma of male Sprague-Dawley rats bearing a solid neurosarcoma [9]. Even 4 weeks after transplantation of these neurosarcomas, which had developed an average weight of 25 g, only a 10% decrease in Zn content (accompanied by no difference in the Co content) was observed. The decrease of ^{57}Co and ^{65}Zn in blood and plasma is strongly correlated ($r=0.958$ and $r=0.976$). The decrease of Co and Zn in blood is only half in magnitude, thus indicating no difference in Co and Zn uptake by erythrocytes between tumor bearing animals and controls.

Distribution of ^{54}Mn could be disturbed by the high Mn content of heparin.

Uptake of ^{57}Co and ^{54}Mn in lymphocytes of Walker tumor bearing rats show a maximum on the third day after transplantation, at which time the tumors are for the first time palpable. With increasing tumor weight the uptake returned to control level. This suggests that the increased uptake is an enchanced macrophage activity.

This assumption was affirmed by an additional experiment undertaken to measure the ^{57}Co uptake by macrophages on the third day after tumor transplantation. As can be demonstrated by the results given in Table 2, the difference between ^{57}Co uptake of peripheral lymphocytes in Walker tumor bearing rats and controls (after separation on a Ficoll–Isopaque gradient) vanishes after plating the cells for 1 hr in culture dishes. From all peripheral lymphocytes isolated by the above given procedure (B-lymphocytes, T-lymphocytes, monocytes and macrophages) only macrophages adhere to the culture dishes. The ^{57}Co activity of macrophage fractions extracted from the culture dishes, in which peripheral lymphocytes from tumor bearing animals were plated, was 5 times higher than in the control group. Whether this difference is caused by an increased number or an increased stimulation of macrophages could not be elucidated. ^{65}Zn uptake of lymphocytes was not affected by tumor growth. (Liver uptake of ^{54}Mn was lower from the second day on, and returned to normal values on day seven). ^{65}Zn uptake was strongly increased in the final stage of tumor growth. These findings correspond to Mn and Zn content of the liver of Walker carcinosarcoma bearing animals as investigated in a previous work [10].

Uptake of ^{54}Mn and ^{65}Zn in tumors was highly correlated ($r=0.832$) and showed a pronounced maximum on the fifth day after transplantation and then slowly decreased. ^{3}H-thymidine incorporation per unit weight into DNA of Walker carcinosarcoma growing in Sprague–Dawley rats displayed the same temporal behaviour [11] suggesting a correlation between uptake of Mn and Zn to DNA synthesis.

Investigation of kidney, thymus and spleen resulted in the same uptake of the tracers in tumor bearing animals and controls, with exception of ^{57}Co. Accumulation in kidneys of tumor bearing animals was decreased by 50% on the seventh day after tumor inoculation.

Reviewing these data of radioisotope distributions in Walker 256 carcinosarcoma bearing rats for any diagnostic value, the following conclusions may be drawn. Differences in organ distributions mainly occur at the final stage of tumor growth when animals are in a failing condition. The strong decrease of ^{57}Co and ^{65}Zn in plasma of tumor bearing animals at an earlier stage of tumor growth seems to be a peculiarity of the Walker 256 carcinosarcoma and cannot be generalized to other tumors. The remaining point of interest is the enchanced uptake of ^{57}Co and ^{54}Mn in macrophages. Whether these findings are specific for a neoplastic disease or if they express a general immunological reaction against inoculated cells has to be further investigated.

Table 2. Number of leucocytes per ml *blood and yield of peripheral lymphocyte preparations of male Sprague–Dawley rats* (200–230 g) *on the third day after transplantation of* 10^7 *Walker* 256 *carcinosarcoma ascites cells.* ^{57}Co *activity of peripheral lymphocytes before and after plating, culture medium used for plating and macrophages; measured* 2 hr *after i.v. administration of* 23 μCi ^{57}Co

	No. of leucocytes ± S.D. × 10^6/ml blood	No. of peripheral lymphocytes isolated ± S.D. × 10^6/ml blood	pCi ^{57}Co ± S.D. 10^6 peripheral lymphocytes before plating
Control*	4.16 ± 0.90	0.69 ± 0.22 (16.6%)†	10.97 ± 1.31
Tumor*	6.35 ± 0.74	0.73 ± 0.20 (11.5%)†	14.63 ± 1.12
	pCi ^{57}Co ± S.D. 10^6 peripheral lymphocytes after plating	pCi ^{57}Co ± S.D. in RPMI 1640 culture medium corresponding to 10^6 plated lymphocytes	pCi ^{57}Co ± S.D. in macrophages corresponding to 10^6 plated lymphocytes
Control*	9.39 ± 1.19	1.84 ± 0.11	0.22 ± 0.11
Tumor*	10.16 ± 2.14	1.86 ± 0.33	1.26 ± 0.17

*$n=7$.

†Number of peripheral lymphocytes isolated expressed in percentage of leucocyte number.

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