RADIATION EFFECTS ON BONE MARROW FUNCTION

— The Relative Biological Effectiveness of 200-kvp X-rays, Cobalt-60 Gamma Rays, and 15-Mevp X-rays —

By

Yosimasa Tanaka and Masatomo Hashi
Department of Radiology, Faculty of Medicine, Kyoto University, Kyoto, Japan.
(Director: Prof. M. Fukuda)

骨髄機能に及ぼす放射線の影響

— 200kVp X線, ^{60}Co γ線, 15MeV X線の RBE —

京都大学医学部放射線医学教室（指導：福田正教授）
田 中 敬 正　吐 師 正 知

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近年来広い分野に高エネルギー放射線が用いられる様になり，之等の生体に及ぼす作用につき種々検討が加えられている． 我々は照射後の ^{59}Fe 摂取率の抑制，血漿放射性鉄消失時間測定等により，高エネルギー放射線の骨髄に及ぼす影響を求め， 200kVp X線を base line として，15MeV X線，^{60}Co γ線のＲＢＥを測定した．

実験動物は，ウイスター系ラッテを用いた．base line のX線は， 200kVp, 20mA, 0.9Cu＋0.5Al の Filter を用いた．15MeV X線は，焦点動物間距離は１ｍとし，11.5cmを一辺とするアクリライトのフアントーム内に，Victoreen Chamber を入れて測定した．

“γ”単位の rad への換算はすべて軟組織に対するものを使用した．

^{59}Fe 摂取率測定には， 照射24時間後に ^{59}Fe citrate 約1μc を注入し，５日後に採血し，ウエル型シンチレーションカウンターで測定した．又 Huff 等の方法に従つて， 放射性鉄消失曲線より血漿鉄50％消失時間を求めた．

赤血球の ^{59}Fe 摂取率は，線量の増加に対し，50～ 350rads の範囲で exponential に低下することが認められた． 200kVp X線を Base line radiation とした時，^{60}Co γ線は，0.75，15MeV X線は0.72の値を得た

血漿鉄交代時間の場合は ^{60}Coは 0.88， 15MeV は0.90であつた．

他の生物学的指標を用いた時の高エネルギーのX線，γ 線のＲＢＥは１より少い事が報ぜられているが，骨髄機能を指標とした時もやはり１より少い値を得た． ^{59}Fe 摂取率は低線量で感受性が高く，照射後短期間の骨髄障害が測定出来，線量について比較的正確に摂取率の抑制が見られるのでＲＢＥ測定は好適であり，高エネルギー放射線の骨髄に及ぼす影響を知る上にも興味深い．

1. INTRODUCTION

High energy radiations have been brought into medical and industrial uses in recent years, and many authors have reported the effects of those high energy radiations on living organism[1)2)3)]. The detrimental effect of ionizing radiation on the erythropoietic system of man and animals has been well established by numerous histopathological investigations and extensively reviewed[4,5]. Quantitative studies[6)7)10)18] of the effect of acute ionizing radiation on the erythropoietic system, utilizing ^{59}Fe, have been shown to reflect radiation-induced functional changes. Major interest in the use of radioiron tracer studies to evaluate radiation damage to the erythropoietic system was stimulated by the work of Hennessy and Huff[6].

The present work has been concerned with a study to determine the relative biological effectiveness (RBE) of ^{60}Co gamma rays, 15 Mev X-rays and 200-kvp X-rays using a indicator of ^{59}Fe turn over in red cells.

2. EXPERIMENTAL METHOD

1) Physical methods

a) The physical factors of the X-irradiation were as follows: 200-kvp, 20 mA, filter; 1.5 mm Cu+0.5 mm Al, H.V.L.: 1.8 mmCu, dose rate; 44.7 r per min., 50 cm target-specimen distance.

The animals were given whole-body irradiation with 50–400 rads. The dosage was measured on the bottom of the wooden box with a Siemens Universal Dosemeter.

b) The physical factor of ^{60}Co were: distance to target 50 cm, dose rate 50 r per min. It contained 2,000 Curies.

c) As for 15 Mev X-ray, 15 Mevp Betatron made by the Shimadzu Co. Ltd. was used. A victoreen Chamber was inserted into acrylite phantom of 11.5 cm and measured the dose. The animals was irradiated into the box (7×15×15 cm) which was placed between acrylite phantom (3 cm thick) the upper and lower sides.

d) X-radiation of 200 kvp was used as the baseline radiation. The conversion factor (Rads per roentgen) are 0.95 for 200 kvp X-rays, 0.97 for ^{60}Co-rays, and 0.93 for 15-Mev X-rays.

2) Biological Methods

a) The measurement of ^{59}Fe uptake

Normal white rats (Wistar Strain), weighing 150 to 200 gm, were used, water being allowed ad lib.

Irradiated and control rats were injected with approximately 1 μc of ^{59}Fe citrate at 24 hours after acute whole body irradiation. Injection were made into the left femoral vein while the animsls were under light nembutal anesthesia. Five days after injection, the rats were anesthetized, weighed and 0.2 ml of whole blood was drawn from right femoral vein into a syringe wet with heparin. The whole blood was countered with well-type scintillation counter as it was, because the radioactivity in plasma was already neglected at that time.

The percentage of ^{59}Fe uptake were calculated from the equation:

$$\frac{(\text{cpm/ml of whole blood})\times(\text{whole blood volume in ml})}{\text{Total cpm injected}}\times 100 = \%^{59}\text{Fe uptake}$$

whole blood volumes used here, were 5.5 ml per 100 gr.

The percentage of normal ^{59}Fe uptake was calculated as follows:

$$\frac{\%\ ^{59}\mathrm{Fe\ uptake\ (irradiated\ rats)}}{\%\ ^{59}\mathrm{Fe\ uptake\ (control\ rats)}} \times 100 = \%\ \mathrm{normal}$$

b) ^{59}Fe disappearance time in plasma

One μc of radioiron were injected and the rats were bled while under anesthesia at 30, 60, 120, 150, and 180 min. after injection and its radioactivity was measured. It may safely be said that the radioactivity of whole blood was regarded as that of plasma because in that time injected radioiron were not utilized into circulating red blood cells yet.

Injected radioiron immediately combined with β-globulin of plasma and decreased exponentially from the circulating blood. The half time ($T^1/_2$) was obtained; That was defined by the time requied to reach half of the initial activity.

c) ^{59}Fe concentration in femoral bone marrow

One μc of radioiron was injected 24 hours after irradiation, and later the femoral bone was separated and its radioactivity was measured at daily intervals without being ashes. The experiments were performed in both groups of whole body irradiation and irradiation of right hind leg.

3, EXPERIMENTAL RESULTS

1) ^{59}Fe uptake

a) Radioiron was injected at one day after total body irradiation with 256 rads and hereafter ^{59}Fe uptake was measured at daily intervals as shown Fig. 1.

After about 4 to 6 days in control animal, the uptake of ^{59}Fe in red cells reached its maximum value of around 70–80% of the injected amount. In the irradiated group, however, the uptake was depressed very markedly initially and then rose to a constant maximum value at a level lower than that of a group of control animals and dependent on the dose given. The 5-day uptake level has been used as a standard of comparison.

b) The RBE of ^{60}Co gamma rays

The rats were given whole body irradiation with 50, 100, 200 and 300 rads. The percentage of normal ^{59}Fe uptake (% ^{59}Fe uptake of irradiated rats X 100/% ^{59}Fe uptake of control rats) was measured as shown Table 1.

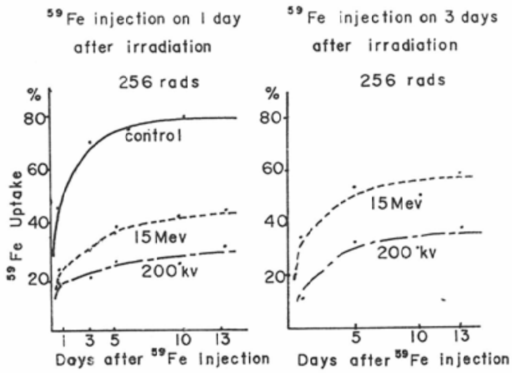

Fig. 1. ^{59}Fe uptake in red cells with time

A linear regression was obtained when percentage of normal ^{59}Fe uptake was plotted as a function of the log of radiation dose. It was found that the decrease of ^{59}Fe uptake was exponentially related to dose over a range of 50 to 300 rads. (Fig. 2) The mean RBE values of ^{60}Co rays to a 200 kvp X ray base line was 0.75.

c) The RBE of 15 Mev Xray

The data of ^{59}Fe uptake of 15 Mev Xray were shown in Table 2. In case of 15 Mev X-ray, also, ^{59}Fe uptake decreased exponentially over a range of 50 to 400 rads. (Fig. 3). By this, the mean RBE values of 15 Mev X-rays was 0.72.

Table. 1. ^{59}Fe uptake (% of control)-200 kVp Xrays, ^{60}Co-

	50 rads		100 rads		200 rads		300 rads	
	200KV	^{60}Co	200KV	^{60}Co	200KV	^{60}Co	200KV	^{60}Co
1	81.0	96.5	51.0	68.5	32.5	50.3	22.7	41.2
2	79.3	93.6	54.2	72.1	46.2	61.2	23.6	38.2
3	73.2	88.5	49.8	61.3	38.6	49.5	15.2	35.8
4	70.0	92.0	53.9	59.1	40.1	57.6	40.2	37.2
5	81.5		50.9	78.9	43.2	62.3	21.5	36.1
M ±S×	78.2 ± 1.6	92.7 ± 1.7	52.0 ±0.88	68.0 ± 3.6	40.1 ± 2.5	56.2 ± 2.7	24.6 ± 4.1	37.8 ±0.98
RBE	0.84		0.76		0.71		0.60	

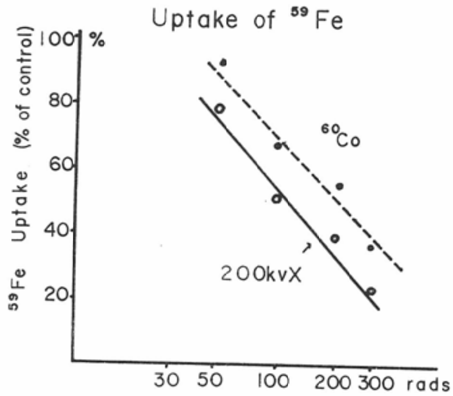

Fig. 2. ^{59}Fe uptake (% of control)

Table. 2. ^{59}Fe uptake (% of control)-200 kVp and 15 Mev Xrays-

	50 rads		148 rads		256 rads		392 rads	
	200KV	15 Mev	200KV	15 Mev	200KV	15 Mev	200KV	15 Mev
1	81.3	96.5	42.5	58.6	38.5	57.3	19.4	22.5
2	70.5	89.3	52.3	65.8	28.6	45.5	25.5	35.5
3	86.3	95.0	48.0	70.5	35.0	48.5	20.3	28.0
4	84.5	93.5	42.5	52.5	34.5	55.5	17.3	38.4
5	79.3	98.5	38.5	65.8	36.1	52.0	26.0	28.2
6	66.1	96.0	46.2	65.0	26.2	42.0	23.5	33.0
M ±S×	78.0 ± 3.3	94.6 ±1.31	45.0 ±1.98	63.0 ±2.62	33.2 ±1.93	50.1 ± 2.4	22.0 ±1.44	30.8 ±2.31
RBE	0.83 (+= 4.6)		0.71 (+=5.48)		0.66 (+=55.5)		0.71 (+= 3.9)	

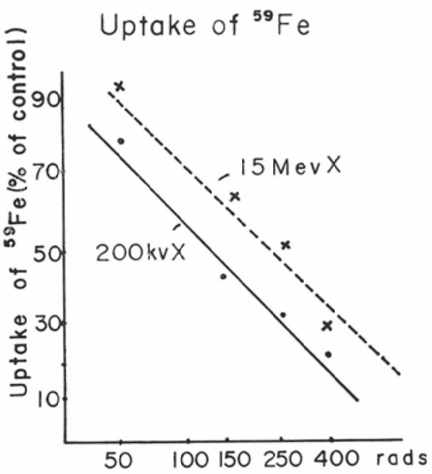

Fig. 3. ^{59}Fe uptake (% of control)

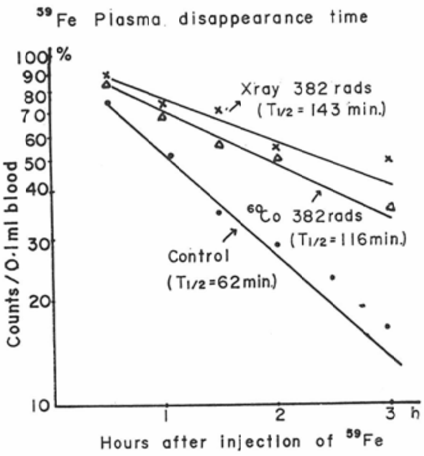

Fig. 4. ^{59}Fe plasma disappearance curve

Table. 3. Plasma disappearance time ($T^1/_2$)

	control	200 kv Xray			^{60}Co γ-ray		15 Mev X ray	
		186 rad	279 r	382 r	186 r	382 r	279 r	382 r
1	45	90	140	138	85	120	104	149
2	55	88	105	138	93	100	98	105
3	72	104	123	137	120	115	109	140
4	67	110	135	164	78	135	115	111
5	68	123	110	141	96	104	125	127
6	64	105	118	145	80	121	118	106
M $\pm S\bar{x}$	62 $\pm$ 4.1	103 $\pm$ 5.3	122 $\pm$ 5.6	14.3 $\pm$ 4.6	92 $\pm$ 6.1	116 $\pm$ 5.2	112 $\pm$ 4.2	125 $\pm$ 7.3
M $\pm 2\sigma m$	62 $\pm$10.5	103 $\pm$13.6	122 $\pm$14.4	143 $\pm$11.8	92 $\pm$15.7	116 $\pm$13.4	112 $\pm$10.3	125 $\pm$13.8
RBE					0.89 (+=1.36)	0.81 (+= 3.9)	0.91 (+=1.44)	0.87 += 2.0

2) ^{59}Fe plasma disappearance time

The rats /20 were exposed to irradiation doses of 382 rads and counts per 0.1 m1 blood was measured 30, 60, 120 and 180 min. following injection of ^{59}Fe citrate. The radioactivity was plotted on semi-logarithmic paper against time and the decrease in activity was exponential (Fig. 4). The rate of decrease was obtained by the time required to reach half of initial activity-half time ($T^1/_2$). The half time were 62 min. in control rats, 143 min. in 200 kvp Xrays and 116 min. ^{60}Co gamma rays (Table 3).

A linear regression curve was also obtained when half time was plotted on semilogarithmic paper as a function of the dose. (Fig. 5). By this, the RBE values were 0.8g for ^{60}Co gamma rays versus 200 kvp X-rays, 0.90 for 15 Mev X-rays versus 200 kvp X-rays.

3) The variation of ^{59}Fe concentration of femoral bone marrow

a) whole body irradiation

The disappearance rate of ^{59}Fe concentrtion in femoral bone marrow of a rat receiving

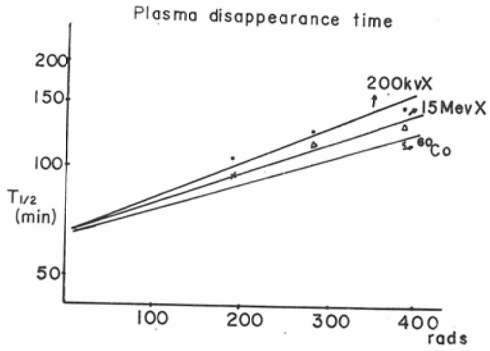

Fig. 5. Plasma disappearance time ($T^1/_2$)

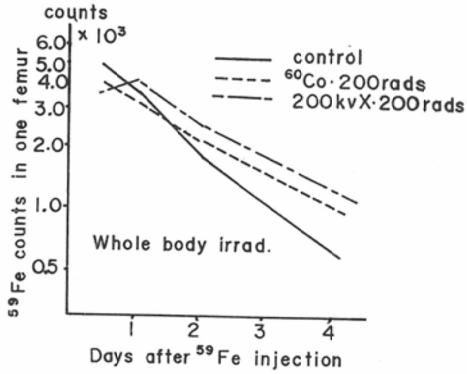

Fig. 6. ^{59}Fe counts curves in femoral bones of control and irradiated rats

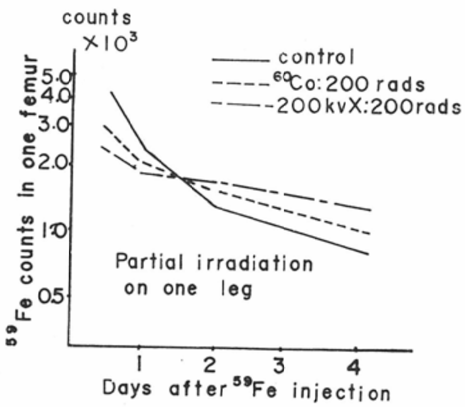

Fig. 7. ^{59}Fe counts curves in control and irradiated femoral bones

200 rads whole body irradiation was markedly smaller than that of a non-irradiated rat as shown Fig. 6. The ^{59}Fe concentration in one femur was exponentially decreased to the days after ^{59}Fe injection. By this, the value of RBE of ^{60}Co was 0.8.

b) local irradiation

The next experiment was carried out, in which only right hind leg of a rat was irradiated with doses of 200 rads, and ^{59}Fe concentration curve ef its femur was compared with that of another femur of the same rat.

The femoral bone marrow of rat has cylindrical shape without complex trabeculae aud general influences are not added in such local irradiation. The same result was obtained as in the case of whole body irradiation. The concentration of irradiated femoral bone marrow was much higher than that of control at one day later after injection of radioiron. The RBE value of ^{60}Co was 0.81. (Fig. 7).

4. DISCUSSION

The high susceptibility to radiation of blood forming organ has drawn the attention of numerous investigators. When animals are exposed to a single dose of ionizing radiation in the range of about LD 50, the degree of destruction of erythroblasts in the bone marrow is much more marked than that of megakaryocytes or leukoblasts and other precursor of white blood cells. This, however, is not reflected in the composition of circulating blood, where the disturbances in white blood cells are very prominent, even after small doses of radiation. Neither the number of erythrocytes, nor haemoglobin values show a marked alteration unless the dose of radiation is at or above LD_{50}. The evaluation of the functional state of the erythrocyte forming tissues is usually carried out by measurement of the incorporation of ^{59}Fe. The rationale of the method is based upon the fact that iron is incorporated by erythrocyte-forming cells, but not by mature red cells.

Depression of ^{59}Fe uptake by red blood cells has been used by a number of investigators as a measure of radiation effect on the bone marrow[7)8)9)]. Hennessy and Huff[6)] have demonstrated a depression of erythropoiesis in the rat after X-irradiation with as little as 5 r, when the ^{59}Fe was injected 24 haurs after irradiation and determinations made on a daily basis thereafter. When rats were injected 48 hours after irradiation and the uptake in cells measured 24 hours after injection, Belcher et al[7)]. found that a minimum observable response was obtained with 30 r. Therefore, it may be said that ^{59}Fe uptake is a very sensitive indicator in small doses and most suitable objects for studies on the RBE and acute radiation damage. Baum et al[10)14)]. reported that the decrease of radioiron uptake was exponential related to dose given: that was confirmed by us.

An extensive series of studies have been carried out by Storer et al[10)]. on iron incorporation by bone marrow of animals irradiated with X-rays 250 kvp, gamma rays (4 Mev, ^{60}Co) and neutrons (14 Mev fission and thermal column), and the RBE values of 4-Mev gamma rays was 0.61, 14 Mev neutrons was 0.84 (250 kvp X-rays was a base line radiation). Sinclaire et al[11)]. reported the RBE values of 0.85 for ^{60}Co gamma rays versus 200 kvp X-rays, and 0.84 for 22 Mev X-rays versus 200 kvp X-rays, based on the absorbed dose to soft tissue. These values fairly coincided with our data.

Girvin[12)] and Hevesy[13)] have shown that plasma radioiron turnover is depressed following irradiation and the iron disappearance time in plasma much prolonged after irradiation. In our experiment, the same results was obtained and half time increased exponentially as the doses augmented. The RBE values of this cases were 0.8-0.9 which comparatively coincided with the RBE of ^{59}Fe uptake.

Delayed appearance of ^{59}Fe tagged erythrocytes in irradiated rats was indicated in Fig. 1. This fact indicate that radiation not only suppresses the mitosis of bone marrow cells but delays the appearance of that tagged cells into circulating blood. As shown in Fig. 6 and 7, the disappearance rate of radioiron concentration in femoral bone marrow of a rat receiving radiation was markedly smaller than that of a non-irradiated rat. These results verify that radiation delays the appearance of tagged bone marrow cells into peripheral blood.

The RBE values which was summarized in Table 4 were obtained from various sides using a indicator of bone marrow function. In that case, we used the absorbed dose to soft tissue as conversion factors for bone marrow(rads per roentgen). The bone is a particular

Table. 4. The values of RBE Values of RBE (Base line radiation: 200 kv X ray)

Test System	Radiation	RBE
^{59}Fe Uptake	^{60}Co 1.5 Mev	0.75 0.72
Plasma disappear. time	^{60}Co 1.5 Mev	0.88 0.90
Fe concent. in femoral bone a) whole body irrad.	^{60}Co	0.87
b) partial irrad.	^{60}Co	0.83

kind of tissue. The flared ends of long bones have internal support which is in the form of a scaffolding of connecting bars and narraw plates of bone termed trabeculae[15]. The spaces between these trabeculae contain the red bone marrow. When an animal is exposed to whole body X-irradiation, the energy absorbed by the bone marrow may be greater than that absorbed by soft tissue parts distant from bone. This is because bone contains elements of higher atomic number than are present in soft tissue, which results in an increased photoelectric absorption coefficient for bone[16]. W.K. Sinclair made autoradiographing sections of rats and mice and found that for both animals the bone marrow dose is grater than the soft tissue dose. This suggests that, if mammalian RBE values are based on bone marrow dose, for bone marrow functions in the rat the RBE for ^{60}Co versus 200 kvp should be multiplied by 1.06, and for 22 Mev versus 200 kvp the RBE should be multiplied by 1.06.

Many authors[1)3)19)25)10] have reported the RBE values of high energy radiation and described the RBE values of ^{60}Co and X-ray of high energy radiation generally were smaller than 1, whatever the test system was used. In our experiment the RBE values ranged from 0.72 to 0.90 (Table 4).

5. SUMMARY

Iron turnover rates of plasma and erythrocytes of the rats were studied 24 hours after whole body irradiation with dosis over a range of 50 to 400 rads. The RBE values of ^{60}Co gamma rays and 15 Mev X-rays compared with 200 kvp X-rays were determined from these bone marrow functions.

1) The decrease of ^{59}Fe uptake was exponentially related to dose.

2) Radioiron disappearance time prolonged after irradiation and the half time also extended exponentially to dose.

3) The ^{59}Fe concentration of irradiated femoral bone was much larger than that of non irradiated femoral bone.

4) The RBE values were summalized in Table 4, ranging from 0.72 to 0.90 which was smaller than 1 on the whole.

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References

1) H.I. Kohn and S.E. Gunter: Rad. Ref. 5, 688, 1956. — 2) S. Lesher and H.H. Vogel: Rad. Res. 9, 560, 1958. — 3) W.J. Meredith, et al.: Brit. J. Radiol. 30, 337, 1957. — 4) W. Bloom and M. Bloom: J. Lab. Clin. Med. 32, 654, 1947. — 5) C.E. Dunlap: Arch. Pathol. 34, 562, 1942. — 6) T.G. Hennessy an dR.L. Huff: Proc.Soc. Exptl. Biol. Med. 73, 436, 1950. — 7) E.H. Belcher et al.: Brit. J. Radiol. 27, 387, 1954. — 8) H.L. Huff et al.: J. Lab. Clin. Med. 36, 40, 1950. — 9) J.E. Funchner and J.B. Storer: Los Alamos Scientific Laboratory Report LA-1544, 1953. — 10) J.B. Storer, et al.: Rad. Res. 6, 188, 1957. — 11) W.K. Sinclair, et al.: Rad. Res. 16, 363, 1962. — 12) E.C. Girvin and J.K. Hampton: Pro. Soc. Exp. Biol. Med. 100, 481, 1959. — 13) G.V. Hevesy: Strahlenther, 102, 341. — 14) S.J. Baum and E.L. Alpen: Rad. Res. 11, 844, 1959. — 15) H.E. Johns: The physics of radiology. — 16) E.R. Epp et al.: Rad. Res. 11, 184, 1959. — 17) W.K. Sinclair: Rad. Res. 16, 369, 1962. — 18) L.G. Lajtha and H.D. Suit: Brit. J.Haematol. 1, 55, 1955. — 19) H. Quastler and R.K. Clark: Amer. J. Roentgenol. 54, 723, 1945. — 20) H. Fitz-Niggli: Experientia, 10, 209, 1959. — 21) H.B. Chase et al.: Amer. J. Roentgenol. 57, 359, 1947. — 22) D.G. Cogan and D.D. Donaldson. 45, 508, 1951. — 23) J.H. Rust et al.: Amer. J. Roentgenol. 72, 135, 1955. — 24) W.S. Moos et al.: 67, 697, 1956. — 25) L.L. Haas, et al.: Amer. J. Roentgenol, 1, 68, 644, 1952.