Studies of Biological Sieve Effects

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生物学的篩効果について

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レ線(h.v.l.: 0.9～ 1.2 mmCu)を篩を通じて空間的に分割照射すれば，同線量を普通照射法により均等に照射した場合に比し，人体皮膚は約5倍の大量の線量に耐える．また全身照射されたマウスの生存率をみるに，同線量が照射されているに拘らず，篩照射法にて照射した場合には，均等照射に比し，生存率が高い．このような篩照射法による特異の効果，すなわち生物学的篩効果は，篩の一つ一つの開放部の大きさが小さいことによるほか，線量分布における開放部に相当する最大値と，被覆部に相当する最小値との比(不均等度)が大きいことによるものと考えられる．言うまでもなく体内にては，吸収と散乱により，この不均等度は浅在部にては大きく，深在部にては小さい．したがつて篩照射法では浅在部と深在部における生物学的篩効果もまた異つてくるであろうことが推測される．この論文は浅在部と深在部における生物学的効果を，レ線では家兎睾丸を用いてその障害度を比較することによりまたマウス全身照射では，その生存率を比較することにより，Co^{60} のγ線では同じく全身照射によるモルモットの生存率を比較することにより検討したものである．何れの実験においても平均線量として同線量が照射されているに拘らず，浅在部では深在部に比し障害が軽度であることを認め得た．また不均等度が2以下では均等照射と生物学的効果の上では差が見られないという興味ある結果を得ることができた．

It seems generally accepted on a clinical and experimental basis that the sieve therapy in which X-ray is irradiated fractionally in space causes less radiation hazards to normal tissue, organs and animals than a conventional irradiation therapy.

The sieve irradiation technique is featured by two points, one, a small size of the opening for adequate irradiation, the other, the stress on the difference between a peak subjected to the opening and a bottom subjected to the covered area in terms of dose distribution curve.

When a sieve is used, difference of dose distribution is remarkable on the surface, but the difference becomes smaller in consequence of absorption and scattering, as irradiation reaches deeper, suggesting a certain biological significance.

In the previous paper, Kaneda and Tanei[1] discussed superficial sieve effects on rabbit's testis by means of a fine sieve with openings 2 mm. in diameter permitting 40% of direct beam to pass. They indicated that the degree of injuries of various germ cells irradiated with the sieve method was always slighter than with a conventional method and that these injuries with both methods were most marked ten weeks after irradiation at a single dose of 600 r.

This paper deals with experimental studies upon biological sieve effects on irradiation at various depths.

For the first experiment, the testis of healthy 10–13 months old rabbits of a similar strain weighing 2.5 Kg on an average were used. Irradiation was performed under the following conditions: Voltage, 200 KV., Current, 25 mA., Filter, 0.7 mm. Cu and 0.5 mm. Al, h.v.l., 1.1 mm. Cu, Distance, 40 cm. and at a dose of 112.4 r per minute. Sieve: 1.5 mm. Pb in thickness, perforations 2 mm. in diameter, arranged in square lattices, open area: covered area =4:6. Through this sieve the rabbit's testis was to be irradiated with about 40 fine X-ray beams.

For the irradiation, the rabbit was fixed on his back and a one percent Urethan solution was injected subcutaneously at a dossage of 10 cc. per 1 Kg. of body weight and a paraffin block of 5 cm. or 10 cm. in thickness was put on the testis. To irradiate a local dose of 600 r on the testis under the paraffin block of 5 cm. in thickness an air dose of 800 r was required if a sieve was not used; to irradiate an average local dose of 600 r with a sieve 2000 r was required as air dose; in case of a 10 cm. paraffin block, 1622 r was required as air dose without sieve and 4050 r with a sieve.

Ten weeks after irradiation the testes were removed and fixed in a 10% formalin solution. Microtomic serial sections five microns thick were prepared, being imbedded in paraffin and stained with haematoxylin-eosin. The sections were made in accordance with the direction of X-ray beams. Out of the specimens, five were taken at random and observations were made of various kinds of the germ cells in 20 circularly sectioned seminiferous tubules (those non-circularly sectioned were rejected) obtaining a similar result of counting for 100 seminiferous tubules.

As no injuries on the Sertoli's cells irradiated with a single dose of 600 r were observed and the numbers of the cells of the specimens were within a 5% difference at any time after irradiation, the number of the germ cells divided by Sertoli's cells was expected to indicate a percentage of Sertoli's cells.

Five experimental and control rabbits each were comparatively observed as to their degrees of injury following three irradiations, surface, 5 cm. deep, and 10 cm. deep, on the basis of the percentage of Sertoli's cells (Table 1,2 and 3).

The various cells with the sieve method are always larger in number than with the conventional method. There was observed a difference within 5% at a 5 cm. depth showing no significant difference at a 10 cm. depth (Fig. 1).

Considering that a ratio of the germ cells for the sieve and non-sieve techniques at a 10 cm. depth is 1.17, it is assumed no specific biological sieve effects resulted at a 10 cm. depth. Since a physical ratio of the maximum to the minimum dose, viz., an inhomogeneity

Table 1. No. of germ cells of rabbit testes and ratios against control 10 weeks after irradiation with a local dose of 600 r on the surface.

		No. of animals	No. of germ cells 1	2	3	4	5	Average	Per cent of Control
without sieve	A	Spertoli	327	388	407	411	460	408	
	B	Spermatogonia	179	146	167	226	174		
		B/A	0.478	0.408	0.411	0.550	0.378	0.439	43.12
	C	Spermatocytes	507	360	637	941	535		
		C/A	1.356	0.900	1.565	2.290	1.141	1.456	38.33
	D	Spermatids	189	235	700	734	532		
		D/A	0.505	0.349	1.720	1.785	1.113	1.146	19.64
	E	Total cells	875	741	1504	1901	1241		
		E/A	2.339	1.909	3.695	4.625	2.697	3.053	28.55
with sieve	A	Sertoli	388	429	401	444	463	425	
	B	Spermatogonia	296	318	264	456	180		
		B'/A'	0.737	0.739	0.658	1.027	0.390	0.710	69.74
	C	Spermatocytes	836	927	682	1339	635		
		C'/A'	1.800	2.156	1.701	3.015	1.156	2.042	53.76
	D	Spermatids	1345	1548	1134	1650	756		
		D'/A'	3.466	3.600	2.828	3.716	1.657	3.053	52.32
	E	Total cells	2477	2793	2080	3445	1571		
		E'/A'	6.384	6.510	5.187	7.759	3.393	5.846	54.68

Table 2. No. of germ cells of rabbit testes and ratios against control 10 weeks after irradiation with a local dose of 600 r in 5 cm depth.

	No. of animals	No. of germ cells 1	2	3	4	5	Average	Per cent of control	Significance
without sieve	(A) Sertoli	464	418	441	426	468	443.4		
	(B) Sp. go.	265	152	150	248	139	190.8		
	B/A	0.571	0.364	0.340	0.582	0.297	0.430	42.24	
	(C) Sp. cy.	815	448	592	450	480	557		
	C/A	1.756	1.072	1.342	1.056	1.026	1.256	33.07	
	(D) Sp. tid.	217	309	406	500	304	347.2		
	D/A	0.468	0.739	0.921	1.174	0.695	0.783	13.42	
	(E) B+C+D	1297	909	1148	1198	923	1095		
	E/A	2.795	2.175	2.603	2.812	1.972	2.470	23.11	
with sieve	(A) Sertoli	534	497	475	472	476	490.2		
	(B) Sp. go.	302	289	296	326	274	297.4		
	B/A	0.566	0.585	0.623	0.691	0.576	0.607	59.63	+
	(C) Sp. cy.	1012	704	864	949	684	842.6		
	C/A	1.895	1.416	1.819	2.011	1.449	1.719	45.26	+
	(D) Sp. tid.	805	654	393	399	450	540.0		
	D/A	1.507	1.324	0.827	0.845	0.945	1.102	18.89	−
	(E) B+C+D	2119	1647	1553	1674	1408	1680.2		
	E/A	3.961	3.314	3.269	3.547	2.958	3.428	32.07	+

Table 3. No. of germ cells of rabbit testes and ratios against control 10 weeks after irradiation with a local dose of 600 r in 10 cm depth.

	No. of animals	No. of germ cells 1	2	3	4	5	Average	Per cent of control	signi-fican-ce
without sieve	(A) Sertoli	473	592	541	547	578	546.2		
	(B) Sp. go.	201	309	245	272	250	255.4		
	B/A	0.425	0.522	0.453	0.497	0.433	0.468	45.97	
	(C) Sd. cy.	728	688	743	521	802	692.4		
	C/A	1.539	1.128	1.373	0.952	1.388	1.268	33.39	
	(D) Sp. tid.	404	222	389	224	426	333		
	D/A	0.854	0.375	0.719	0.410	0.737	0.610	10.45	
	(E) B+C+D	1333	1199	1377	1017	1478	1280.8		
	E/A	2.818	2.025	2.545	1.859	2.557	2.345	21.94	
with sieve	(A) Sertoli	464	441	547	508	600	512.0		
	(B) Sp. go.	256	220	315	254	252	259.4		
	B/A	0.552	0.499	0.576	0.500	0.420	0.507	49.80	(−)
	(C) Sp. cy.	704	699	714	723	686	705.2		
	C/A	1.517	1.585	1.305	1.420	1.143	1.377	36.26	(−)
	(D) Sp. tid.	510	285	476	532	420	444.6		
	D/A	1.099	0.646	0.870	1.045	0.700	0.868	14.88	(−)
	(E)=B+C+D	1470	1204	1505	1509	1358	1409.2		
	E/A	3.168	2.730	2.751	2.970	2.263	2.752	25.74	(−)

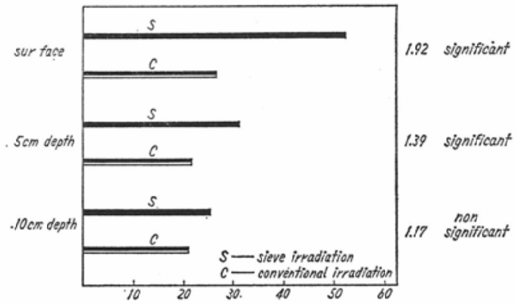


Fig. 1 Different ratios of germ cells observed in rabbit testis irradiated with a local dose of 600 r on the surface, in 5 cm depth and 10 cm depth under the paraffin block.

quotient for the open area to that for the covered area is 6.8 on the surface, 2.2 at a 5 cm. depth and 1.8 at a 10 cm. depth (Table 4), it is considered that specific biological sieve effects do not result in the living body in which the inhomogeneity quotient of dose distribution proves less than 2.

When a formula of Marcus and Menting[2] is employed, viz., the value of S at a 5 cm. depth is 1.5 and 1.2 at a 10 cm. depth.

$$S \text{ (sieve effect)} = \frac{F1}{Fst}\,\frac{D1}{Dst}$$

In the second experiment, where biological sieve effects in terms of depth were pursued,

Table 4. Inhomogeneity Quotient of Dose Distribution Following Sieve Irradiation.

depth (cm)	X-Ray (h.v.l.; 1.2mmCu)	Co-60
0	6.8	(0.5cm) 7.3
1	4.0	7.0
2	3.2	6.6
3	2.7	5.7
4	2.4	5.1
5	2.2	4.6
6	2.1	4.3
7	2.0	4.1
8	1.9	3.9
9	1.8	3.7
10	1.8	3.5
15	1.4	2.8

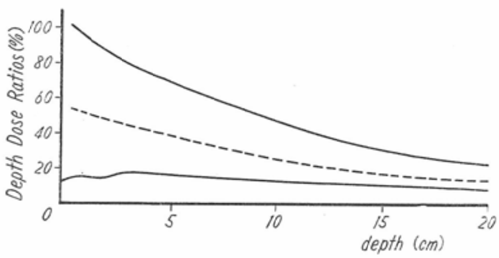
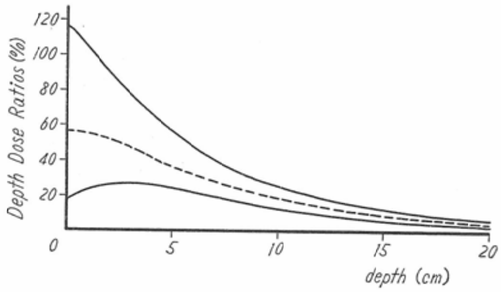


Fig. 2 Depth dose curves of maximum dose under the opening and minimum dose under the covered area following Cobalt sieve irradiation.



Depth dose curves of maximum dose under the opening and minimum dose under the covered area following X-ray sieve irradiation.

male mice of d.d.N. strain were used. The survival rate of mice irradiated with the same local dose in different depths under a paraffin block was observed.

The physical conditions for irradiation were the same as the first experiment in which a fine sieve was employed. The height of a mouse being two cm. on an average, the standard of depth to estimate a local depth dose was fixed at the center of a mouse, i.e. 1 cm. from the dorsum. Depth dose were measured with the Toshiba dosemeter.

In order to keep animals still during irradiation, five mice were packed together close in a square space (100 cm^2) of the paraffin block of 2 cm. in thickness ; no anesthetics were used. Each group consisted of 15 mice. Mice placed in a 2 cm. depth (under the paraffin block of 1 cm. in thickness) and a 7 cm. depth (under the paraffin block of 6 cm. in thickness) were subjected to whole body irradiation with local doses of 600 r, 800 r and 1000 r.

Their survival rates during one month were observed. The survival rate of A group which was irradiated at a 2 cm. depth was 26.6% and that of B group at a 7 cm. depth was 13.3%, revealing that the same local dose resulted in a higher survival rate for sur-

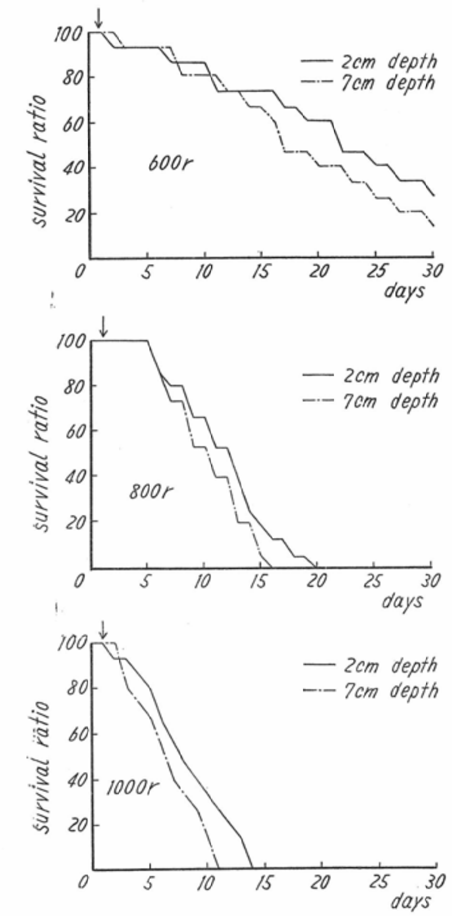


Fig 3. Survival ratio of mice irradiated with the same local dose on whole body placed in 2 cm and 7 cm depths under the paraffin block.

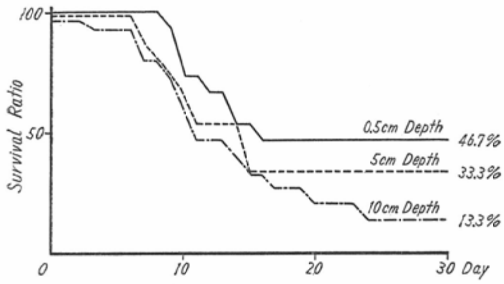


Fig 4. Survival of guineapigs irradiated whole body with a local dose of 400 r with Cobalt sieve method.

face irradiation than deep irradiation. Hence, the larger the irradiated local dose, the shorter the survival period. But in all cases the survival of mice irradiated with a sieve was higher than that without a sieve. Namely in all cases the survival of mice irradiated with a sieve was higher than that without a sieve, and the larger the irradiated dose, the shorter the survival period.

Likeweise, the third experiment was performed with the telecobalt sieve method with male guineapigs, weighing 300 g, which were employed in consideration of size of the opening of a sieve for telecobalt therapy.

The sieve was made of lead 4.6 cm. in thickness, perforated with cylindrical holes of 1 cm. in diameter in a radiated direction from the source. Three adjacent holes make an equilateral triangle, its open area being 55% on the source side, 45% on the patient's side and 50% in the center. The dose distribution of this sieve was measured with a fluoroglas dosemeter (Toshiba Co. in Japan) by Maeda and Yamada.[3)]

As shown by an average depth dose and inhomogeneity quotient (Table 4), inhomogeneity was higher in cobalt at the depth than in X-ray, the former reaching 3.5 even at the depth of 10 cm.. Thus, it is considered that in the case of telecobalt sieve irradiation biological sieve effects developed at the depth of 10 cm.

Whole body irradiation was given to guineapigs at a local dose of 400 r which was placed on the surface, 5 cm. and 10 cm. deep under the paraffin block. Each experimental group consisted of 15 guineapigs. During irradiation, each animal was fixed in an akrylite box under Thyopentathol anesthesia 25 mg. per Kg.. The survival rate a month after irradiation on the surface was 46.7%, at a 5 cm. depath 33.3% and at a 10 cm. depth 13.3% (Fig. 4).

A so far accepted theory that the higher the inhomogeneity the more marked the biological sieve effect has been confirmed by the present experimental results which verified that the shallower the irradiation the higher the survival rate in the sieve irradiation.

Discussion

The sieve therapy enables the human skin to tolerate more dose of X-ray which is fractionally irradiated in space. In this way, Marks[4)] gave a total dose of 24000 r in 28 days, while Freid, Lipman and Jacobson[5)], Gros, Wolf and Burg[6)] and Botstein and Harris[7)] iradiated at the same dose. In the authors' department, a total dose of 24000 r was given to the thoracic skin of a patient in 4 weeks resulting in the development of leucoderma on the irradiated area followed by teleangiectasis about a year later. But the skin trouble was free from serious symptoms such as skin ulcer. A dose of 24000 r is assumed as a maximum permissible dose in sieve irradiation of the human skin. It has been accepted that the human skin, if irradiated fractionally in space, can endure a dose five times heavier than that given with a conventional homogeneous irradiation.

Marks, Cohen and Palazzo[8)], Pfeifer and Seidel[9)] and Kaneda[10)] who is one of the present authors, have reported a smaller rate of radiation sickness occurring in the patient irradiated through a sieve. Kaneda has also referred to the haemographic data following a sieve irradiation, mentioning a very little decrease in erythrocytes, and leucocytes as well as in haemoglobin.

The same results were obtained by Becker, Stodtmeister, Fliedner and Kuttig[11)] on animal experiments. Morozumi[12)], a previous worker at the present authors' department, obtained a similar result concerning a decrease in the number of platelets and reticulocytes, which was found very slight in rabbits irradiated through a sieve.

The above data indicate that the living body irradiated fractionally in space can tolerate a large dose in both local and whole body irradiations. It is assumed that biological sieve effects were influenced by the following two factors, one, the smallness of the size of each opening, and the other, the difference of dose distribution between a maximum dose subjected to the opening and a minimum dose subjected to the covered area.

As to the former, it is considered that the effect of the so called field size factors works upon the living body, and that the field size factor has significantly something to do with the ratio of the length of circumference against the size of the opening. About this problem many papers have been published including those of Mayer[13], Erskine[14], McKee et al[15]., Goldberg[16], Belisario[17], Joyet ank Hohl[18], Kaneda[19] and Tanikawa[20].

As to the latter, the influence of the difference in dose distribution on the living body does not seem to have been satisfactorily pursued, except for the articles of Marcus and Menting and Kröker[21].

As a matter of course, the difference in dose distribution between the opening and the covered area becomes smaller as irradiation is deeper because of absorption and scattering.

A possible difference suggested in biological sieve effects between the surface and certain depths has led the present authors to perform their experimentation.

As a result it is demonstrated that radiation injuries of animals on the surface were slighter than those at certain depths in spite of the same dose irradiated through a sieve.

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