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ORIGIN OF RADIATION RELEASED SEROTONIN IN RABBITS AND MICE

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It is generally agreed at present that the early rise of plasma and urinary serotonin (5-HT) levels caused by moderate doses of ionizing irradiation are due to a release of 5-HT from the body stores (ALTMAN et coll. 1970, STREFFER 1969).

For practical purposes, i.e. for the localization of injury after radiation accidents more or less specific biochemical indicators are of meaningful value (Biochem. Indicators of Radiation Injury in Man); 5-HT has been mentioned as such. It therefore appeared worthwhile to investigate the origin of the 5-HT present in body fluids after exposure to several types of ionizing irradiation. 5-HT is bound to subcellular structures in various tissues throughout the body; significant quantities occur in the brain, lungs, liver, intestinal tract, spleen, skin and blood platelets (GARATTINI & VALZELLI 1965). Partial body irradiation has enabled a number of these organs to be examined as being the possible potential sources of the 5-HT released into the blood plasma; the experiments were mainly executed with moderate doses of roentgen irradiation; a few were carried out with 14 MeV neutron irradiation. All indicated the intestinal tract as the principal site of origin for the enhanced amount of free 5-HT in the circulation.

Methods. Random bred Chinchilla rabbits of about 3 kg and institute inbred grey mice weighing between 20 and 30 g were used. The rabbits were individually

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fixed to an operating table after being anaesthetized with 25 mg/kg sodium pentobarbital. Both animals species were kept without food for 24 hours prior to the experiments.

Partial body irradiation was performed by covering the non-irradiated parts of the body with 4 mm lead, and employing a Philips Müller MG 300 apparatus at 200 kV, 15 mA, and 0.5 mm Cu and 0.5 mm Al filtration. The dose rate varied with the focus to object distance. In rabbits the average dose rate as measured in cadavers by a Philips Universal dosimeter was 45 rad/min and in mice amounted to 74 rad/min for all regions irradiated. To obtain platelet irradiation both carotid arteries of the rabbits were bared for 3 cm; a piece of 4 mm lead measuring 5 cm $\times$ 3 cm was then placed underneath and the whole animal covered by 4 mm lead with a 3 cm $\times$ 3 cm hole above the carotids. The dose rate measured was 700 rad/min, a value which was obtained with the 0.5 mm Al filter only.

The diameter of the carotid arteries was estimated to find the dose received by the circulating blood and the blood volume exposed to the roentgen beam was calculated. Assuming the blood content of the rabbits to be 52 ml/kg body weight (SCHILDT & SCHILDT 1963), and recognizing that the exposure time in the experiment was 2 1/2 h, the total roentgen dose received by the whole blood was calculated according to the equation: $D_t = \frac{V_e}{V} \times t \times D_s$, where D_t = total dose

V_e = volume of the exposed carotid part in ml

V = total blood volume in ml

t = exposure time in s

D_s = radiation dose per s

Venous blood in 4.5 ml samples from the ear for the first three samples and from one of the carotid arteries for the next three samples were collected in plastic tubes containing 0.5 ml EDTA 5 %. The first two samples were taken prior to irradiation. Blood sampling from the mouse was executed by orbita extraction, 0.8 ml blood being collected into 0.2 ml EDTA 2.5 % in physiologic saline. All samples were kept at 4° C and spun at 1 250 g in a cooled centrifuge for 10 min. Plasma was withdrawn by aspiration and respun at 1 250 g for 30 min. Two samples of the platelet-free murine plasma were pooled. All samples were analysed for their 5-HT content by the method of ASHCROFT et coll. (1963). Hemolytic samples were discarded. To prevent breakdown of the blood 5-HT the animals were intraperitoneally injected 30 min before the start of the experiment with either 10 mg/kg tranylcypromine or 10 mg/kg iproniazide; both are well known inhibitors of the enzyme mono-amine oxidase.

In vitro irradiation of the rabbit platelets was realized by centrifuging 4.5 ml

Table 1

The effect of 250 rad roentgen to various regions on the blood plasma level of 5-HT in the rabbit. n represents the number of plasma samples

Irradiated region	5-HT ng/ml plasma (Mean±SE)				
	Control		5 min		
		n		n	p
Total body	100±00	16	140±10	8	<0.001
Head	160±10	22	160±10	11	n.s.
Thoraco-gastric region	210±10	27	220±20	11	n.s.
Abdomen	160±10	21	230±10	8	< 0.001
Abdomen sham-irradiated	120±10	8	140±20	4	n.s.
Abdomen splenectomized	60±10	18	110±10	9	< 0.0025

samples of whole blood replenished with 0.5 ml EDTA 5 % at 600 g cold for 15 min. Platelet rich plasma was obtained by suction and dispersed in a 5 cm plastic Petri dish. Non-irradiated and sham-irradiated samples of the same animal served as controls. The samples were centrifuged at 1 250 g at various intervals after irradiation and treated as described above for the 5-HT estimation.

The areas of the rabbit irradiated were the whole body, the head, the thoraco-gastric region, including the liver, and the abdomen intact and 72 hours after splenectomy. Abdominal sham-irradiation was also performed. The irradiation pattern for mice was virtually similar, but the irradiation of blood platelets and of the thoraco-gastric region was omitted. The number of animals in each group varied; it can be deduced from the n value in the tables.

For the neutron exposure the mice were placed in 2.5 cm perspex tubes with a wall thickness of 0.2 cm, five of the tubes being grouped vertically around the 14 MeV neutron source at an ax to ax distance of 4.5 cm to the ion tube of the generator. A dose of 600 rad was delivered to the central part of the abdomen which was perpendicularly positioned in relation to the neutrons emitted. The dose was calculated from flux measurements with a value of 6.7×10^{-9} rad/n $\times$ cm^{-2}.

Results

Rabbits and mice bore a remarkable resemblance in plasma 5-HT response after irradiation of the various body regions. Only after irradiation of the abdomen was a significant increase in the plasma 5-HT observed (Tables 1, 2, 3,

Table 1 (*cont.*)

15 min			30 min			60 min		
	n	p		n	p		n	p
110±10	7	n.s.	110±10	8	n.s.	100±00	8	n.s.
180±20	10	n.s.	180±10	11	n.s.	180±10	11	n.s.
240±20	13	n.s.	220±20	13	n.s.	200±20	13	n.s.
210±20	8	0.01	200±10	10	< 0.05	190±20	10	n.s.
130±20	4	n.s.	130±20	4	n.s.			
80±10	9	n.s.	80±20	9	n.s.	100±20	8	n.s.

4). This increase was already present immediately after the irradiation treatment. The response in rabbits levelled off in the course of 1 hour (Figure) and reached normal values at 4 hours. Exposure of mice to 14 MeV neutrons also led to an increase in the plasma 5-HT (Table 5), comparable to that after roentgen irradiation of whole mice (Tables 3, 4).

Splenectomy failed to prevent the response to irradiation of the abdomen. The control values for the plasma 5-HT lay at the level of approximately a factor 2 1/2 times lower than normal in the rabbits. Abdominal irradiation of normal mice caused 5-HT values significantly less than those appearing after total body exposure as well as those in abdominally irradiated splenectomized mice (Tables 3, 4).

Irradiation of rabbit platelets in vitro with roentgen doses up to 10 000 R was ineffective (Table 6).

Discussion

Early radiation induced changes in the 5-HT content of internal organs generally correlate with the changes observed in the excretion pattern of the amine after irradiation. Early observed decreases in the intestinal tract thus correspond with increases in the blood and urine of 5-HT as well as of its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) (Table 7). Organs other than the intestinal tract in general do not react. Irradiation of only the abdomen results in a significant increase in the plasma 5-HT level in rabbits as well as in mice. Since splenectomy fails to influence this effect there appears to be justification for indicating the intestinal tract as the source of the released 5-HT.

Table 2

5-HT concentrations in rabbit blood plasma after in vivo roentgen irradiation of thrombocytes during MAO inhibition

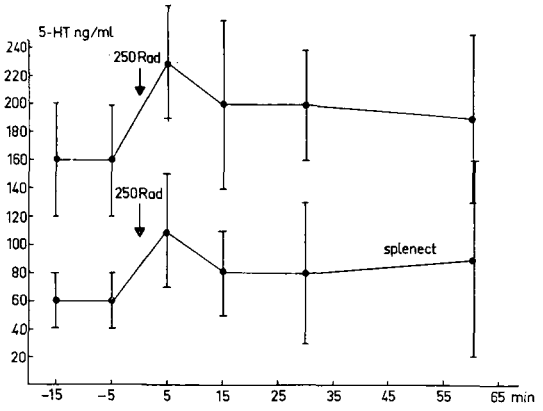
Roentgen dose (rad)	5-HT ng/ml plasma			
	Control		Experimental	
			5 min	30 min
60	190	200	120	110
60	—	—	230	220
90	210	210	170	150
90	190	250	250	180
90	170	140	130	190
90	180	170	130	130
170	130	120	120	120
200	—	—	110	110
250	180	180	170	170
Average ± SE	180 ± 9		160 ± 17	150 ± 13

The intestinal mucosa contains the bulk of body 5-HT in its enterochromaffin cells (GARATTINI & VALZELLI 1965). The amine is stored in granules known to be sensitive to roentgen doses of 500 to 1 200 R as observed during the first hours after treatment (UHER 1958, ANSARI et coll. 1962). At a later stage, i.e. 12 hours post irradiation, the granules present a normal quantitative and qualitative appearance (ANSARI et coll. 1962, PENTTILLÄ & KORMANO 1971). The present observations are in accordance with these findings as well as with those that demonstrated a drop in the intestinal 5-HT content early after irradiation (WILLOUGHBY 1960, PENTTILLÄ & KORMANO 1971).

Table 3

The effect of 575 rad roentgen to various regions on the blood plasma level of 5-HT in the mouse. C = control; E = experimental. The experimental samples were collected immediately after irradiation

Irradiated region	5-HT ng/ml plasma Mean ± SE		Number of samples		Significance (p)
	C	E	C	E	
Total body	50 ± 2.8	80 ± 4.2	13	25	< 0.001
Head	50 ± 2.8	50 ± 2.9	13	12	n.s.
Head + Thorax	60 ± 2.9	50 ± 2.7	12	14	n.s.
Abdomen	50 ± 2.0	60 ± 2.2	25	55	0.005
Abdomen splenectomized	50 ± 3.3	70 ± 3.4	9	19	< 0.002



5-HT concentrations of rabbit blood plasma at different intervals before and after irradiation with 250 rad roentgen to the abdominal region. The upper curve gives the data of normal animals; the lower curve is constituted of data obtained after splenectomy.

It is reasonable to assume that the 5-HT released will enter the blood stream. A part will become oxydized by the enzyme mono-amine oxidase (if this is not inhibited) to 5-HIAA. This process will lead to an increase in the urinary 5-HIAA (Table 7); another part of 5-HT, released by the irradiation, can be excreted as such. An increase in urinary 5-HT has been observed in some animal species (BRINKMAN & VENINGA 1962).

Splenectomy in the rabbits leads to an apparent decrease in the original 5-HT level in blood plasma. This means that in this animal species, in contrast to the mouse, the spleen significantly contributes to the normal level of the plasma 5-HT. The spleen in the mouse has been demonstrated to be involved in a 5-HT retentive process (RITZÉN et coll. 1965). Hence, free circulating labelled 5-HT was rapidly taken up by the spleen, and a number of other organs such as the lungs, bone marrow, adrenal medulla and the thyroid. This uptake might explain the difference in the rise of plasma 5-HT after abdominal irradiation of normal

Table 4

Comparison of some regions of mice irradiated with either roentgen or 14 MeV neutrons with regard to the release of 5-HT into the blood plasma.

Regions compared	Respective 5-HT values Mean ± SE		Significance (p)
Total body/Abdomen	80 ± 4.2	60 ± 2.2	< 0.001
Abdomen/Abdomen splenectomized	60 ± 2.2	70 ± 3.4	= 0.001
Total body roentgen/Total body neutrons	80 ± 4.2	95 ± 6.0	n.s.

Table 5

The effect of 14 MeV neutron irradiation directed to the abdomen of mice upon the blood plasma level of 5-HT

	5-HT ng/ml plasma	
	Controls	5 min post−600 rad 14 MeV neutrons
	50	120 120
	90	110 80
	50	40 90
	50	80 90
	80	90 90
	60	70 140
	60	40 140
	90	130 110
	60	70 120
		110 110
		50 110
		80
Av. ±SE	65±5.3 (n = 9)	95±6.0 (n = 23)
	Difference 30±1.4 p = 0.002	

Table 6

5-HT in rabbit plasma after in vitro irradiation of whole blood

Roentgen dose	5-HT ng/ml plasma			
(R)	Control		Experimental	
			5 min	15−30 min
1 000	90	90	—	110
2 000	100	100	100	110
5 000	100	100	110	110
10 000	60	90	—	60
10 000	140	150	140	170
		180		
Average ±SE	110±10		120±15	110±18

Table 7

The effect of roentgen irradiation on the 5-HT content of different organs as well as on the concentration of 5-HIAA in urine as reported by several authors. The upper part of the table indicates values obtained after shorter intervals after roentgen exposure, whereas the middle part presents longer term values.

Organ	Animal	Dose (R)	Effect	Interval (hours, days)	Authors
5-Hydroxytryptamine					
Liver	Mouse	810	none	0—2h	MELCHING et coll. (1960)
Spleen	Mouse	810	none	0—2h	MELCHING et coll. (1960)
Stomach	Rat	1 025	drop	3h—15d	EISEN et coll. (1956)
Intestine	Rabbit	500—2 000	drop*	0—3h	MATSUOKA et coll. (1966)
Intestine	Rat	1 500	drop	24h	WILLOUGHBY (1960)
Intestine	Mouse	1 000—3 000	drop	12—24h	PENTTILLÄ & KORMANO (1971)
Brain	Rat/guinea pig	500—1 000	rise	0h	CHERNOV & RAUSCHENBACH (1960)
Brain	Rat/mouse	810	none	0—2h	MELCHING et coll. (1960)
Brain	Rat	900—4 000	none	0—24h	RANDIĆ et coll. (1961)
Hypothalmus	Rat	1 000	drop	2h	RENSON & FISCHER (1959)
Blood	Rat	800	rise	6h	FRANZEN et coll. (1963)
Blood	Mouse	250—600	none	0—5d	MOMMA et coll. (1967)
Urine	Rat	400—800	rise	3h—21d	FRANZEN et coll. (1963)
Skin	Rat	600—1 000	drop	5—10d	EISEN et coll. (1956)
Skin	Rat	600	drop	6d	LEITCH et coll. (1957)
Spleen	Rat	450—900	drop	1—6d	ERSHOF et coll. (1962)
Spleen	Rat	600	rise→drop	2—4d, 9d	LEITCH et coll. (1957)
Intestine	Rat	450—900	drop	1—6d	ERSHOF et coll. (1962)
Intestine	Rat	600	rise→drop	6d, 6—16d	LEITCH et coll. (1957)
Intestine	Mouse	1 000—3 000	none, drop	2—4d	PENTILLÄ & KORMANO (1971)
Brain	Rat	900	none	6d	ERSHOF & GAL (1961)
Blood	Rat	900	drop	6d	ERSHOF & GAL (1961)
Blood	Rat	600	drop	4—9d	LEITCH et coll. (1957)
5-Hydroxyindoleacetic acid					
Urine	Rabbit	1 000	rise	?h	FISCHER & RENSON (1959)
Urine	Rat	810	rise	0—2h	MELCHING et coll. (1960)
Urine	Rat	800	rise	6—24h	RANDIĆ & SUPEK (1962)
Urine	Rat	200—800	rise	6—12h	DEANOVIĆ et coll. (1963)
Urine	Frog	400	rise	4h	BRINKMAN & VENINGA (1962)
Urine	Man	200—300	none	24h	BETTENDORF et coll. (1959)
Urine	Man	150 rad	rise	24h	SMITH & LANGLANDS (1966)

* As reflected in depression of intestinal motility

mice as compared to splenectomized animals (Tables 3, 4). The spleen in normal mice predominantly remains out of the irradiation field and may thus participate in the retentive process. The steeper increase in the plasma 5-HT after total body irradiation as compared to abdominal exposure of normal mice may be interpreted by assuming a disturbance of the process due to damage of these, for the greater part, radiation sensitive organs.

The response of 5-HT observed was not restricted to roentgen irradiation; 14 MeV neutron irradiation also proved to be effective if mainly directed to the abdomen of mice. The increase in plasma 5-HT observed was even somewhat more marked although the difference was not significant if compared to the total body roentgen irradiated mice.

The reaction on different types of irradiation might represent an early biochemical indicator of radiation injury to the abdominal region. It might then be preferable to class the reaction as dose dependent. Although a linear relationship between radiation dose and 5-HIAA excretion in the urine of rats has been established (DEANOVIĆ et coll. 1963), a tentative trial in rabbits failed to indicate a dose relationship for the roentgen induced 5-HT increase in the blood plasma (VENINGA, unpublished).

Another uncertainty concerns the 5-HT response in man. SMITH & LANGLANDS (1966) observed a rise in the urinary 5-HIAA content of patients receiving partial body irradiation with 150 rad. However, BETTENDORF et coll. (1959) were unable to demonstrate significant differences in 5-HIAA excretion in patients treated with roentgen irradiation. A complication is produced by the alimentary 5-HT intake in human subjects giving rise to fluctuations in the normal excretion pattern of 5-HIAA. This may turn out to be a significantly counteracting factor in the development of 5-HT as a biochemical indicator of local radiation injury. For the present however it nevertheless seems worthwhile to extend investigations to other mammalian species so as to obtain an impression of the generality of the phenomenon.

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SUMMARY

Blood plasma levels of free 5-hydroxytryptamine (serotonin, 5-HT) have been determined in rabbits and mice following zonal roentgen irradiation of the animals to identify the source of the 5-HT released. The results indicated the intestinal tract as the most prob-

able site from whence the 5-HT was originating. Further investigation of the liberation as a potential indicator of radiation injury in the abdominal region would appear to be required.

ZUSAMMENFASSUNG

Der Blutplasmaspiegel von freiem 5-Hydroxytryptamin (Serotin, 5-HT) wurde bei Kaninchen und Mäusen im Anschluss an Partialkörper-Röntgenbestrahlung, um die Quelle der 5-HT-Freisetzung festzustellen, bestimmt. Die Ergebnisse deuten darauf hin, dass der Intestinaltrakt die Stelle ist, von der am wahrscheinlichsten 5-HT herstammt. Weitere Untersuchungen erscheinen notwendig, um die Freisetzung als potentiellen Indikator eines Strahlenschadens des abdominalen Gebietes verwenden zu können.

RÉSUMÉ

Les auteurs ont déterminé le taux dans le plasma sanguin de la 5-hydroxytryptamine libre (sérotonine, 5-HT) sur des lapins et des souris après une irradiation régionale par les rayons roentgen pour identifier la source de la 5-HT libérée. Les résultats indiquent que l'intestin est la source la plus probable de la 5-HT. Il est nécessaire de continuer les recherches sur la libération de la 5-HT comme indicateur possible de radio-lésion dans la région abdominale.

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