

PERIPHERAL RETICULOCYTE COUNT AS BIOLOGIC DOSIMETRY OF IONIZING RADIATION

Experiments in the mouse

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During the past 25 years many efforts have been made to find suitable biologic indicators to detect effects of ionizing radiation in man, particularly at sublethal doses. Among the various methods the chromosome aberration test is considered to be the most promising one for evaluating persons subjected to exposure occupationally or otherwise (BENDER & GOOCH 1962, BUCKTON et coll. 1969, BREWEN & GENGOZIAN 1971, KOMAROV 1973, LLOYD et coll. 1976). However, this test demands considerable time and manpower. Attempts were therefore also made to obtain more simple tests, i.e. estimating lymphocytes in the peripheral blood (BARNES et coll. 1971), which are known to be very sensitive to radiation. However, these tests were found to be unsuitable because the lymphocytes are rapidly eliminated from circulation after irradiation (EDGREN & WEBER 1976).

The reticulocytes are much more numerous in the peripheral blood than the lymphocytes. They are also known to be highly sensitive (ELTGEN et coll. 1961). Moreover the amount of reticulocytes in blood reflects the bone marrow activity. BOND et coll. (1965) suggested that a reticulocyte analysis could be used for evaluating radiation injury. Therefore, an analysis of the reticulocytes in the peripheral blood was made, using an apparatus capable of microfluorometric quantification of the

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reticulocytes over an analogue computer. In spite of the various weaknesses of this prototype equipment clear indications were obtained regarding the usefulness of such an analysis as a biologic dosimetry of ionizing radiation, which has also been indicated by OTTO (1973).

Material and Method

Male NMRI mice of 20 g average body weight were used. Ten μ l peripheral blood was stained in suspension with acridine orange and fixed partially with glutaraldehyde according to the method described by EDER & THAER (1969).

A known volume of this cell suspension was spread homogeneously in a narrow trail on a non-fluorescent electromagnetic tape using a fine mechanical device, and dried. The tapes carrying the blood cells were then scanned under an incident light fluorescent microscope mounted with two photo-multipliers to monitor the red and green fluorescent impulses emitted from the intracellularly bound acridine orange. These photo-impulses were received and by an analogue computer displaying the data on the amount of reticulocytes and their various stages of maturity.

Two experiments were designed to test the method.

In the first experiment the mice were divided into six groups, which received different doses of 250 kV roentgen irradiation ranging over 12.9 to 232.2 mC/kg (50 to 900 R). Blood samples were obtained by puncturing the retro-orbital plexus at various intervals to count the reticulocytes.

In the second experiment the animals were exposed to a lower range of radiation dose, 3.2 to 103.2 mC/kg (12 to 400 R), to explore the sensitivity of the reticulocyte system. Both experiments were repeated twice.

Results

In both experiments a dose dependent reticulocytopenia was observed (Figs 1, 2). The time of onset of the reticulocytopenia appeared to be more or less independent of the dose, being recorded already 24 hours following exposure. Subsequently, through compensatory activity of the bone marrow a period of reticulocytosis was observed in all groups. Though the maximum of reticulocytosis in animals exposed to higher radiation doses was comparatively higher, the onset of reticulocytosis was delayed corresponding to the radiation dose.

Discussion

The prospect of developing a biologic dosimetry of exposure to irradiation by means of the radiation induced reticulocytopenia seems to be encouraging. The present technique of microfluorometry is superior to the conventional technique by virtue of its quicker operation and unbiased analysis. The reticulocytes were found

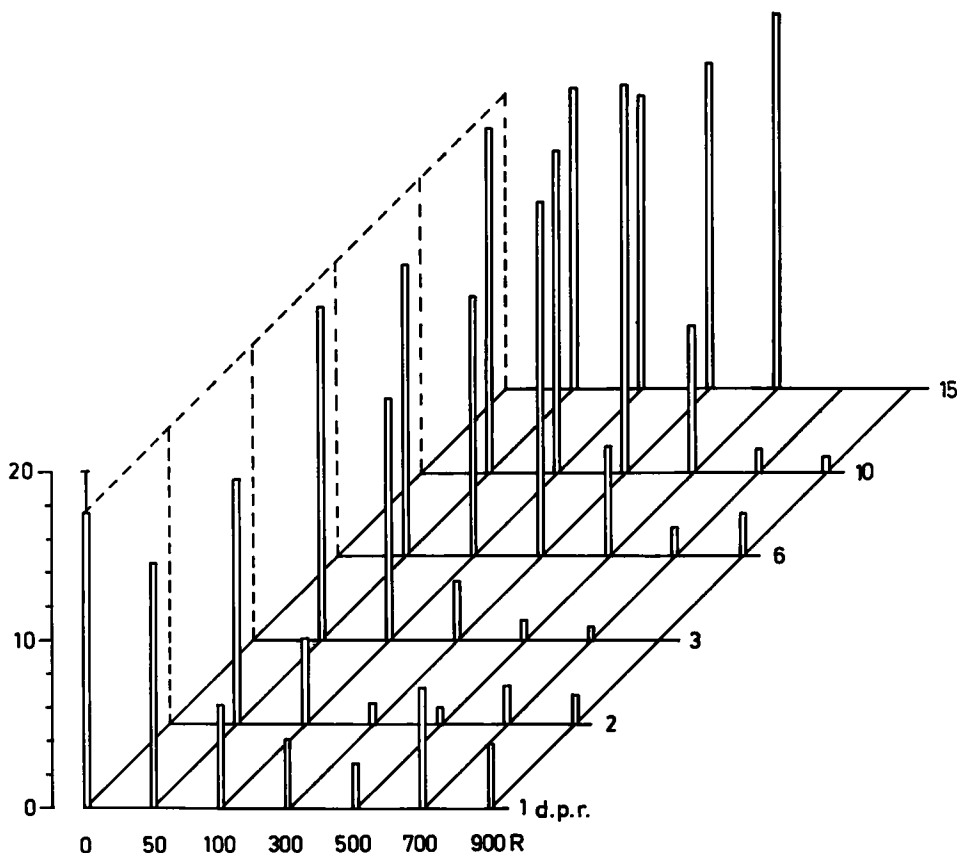


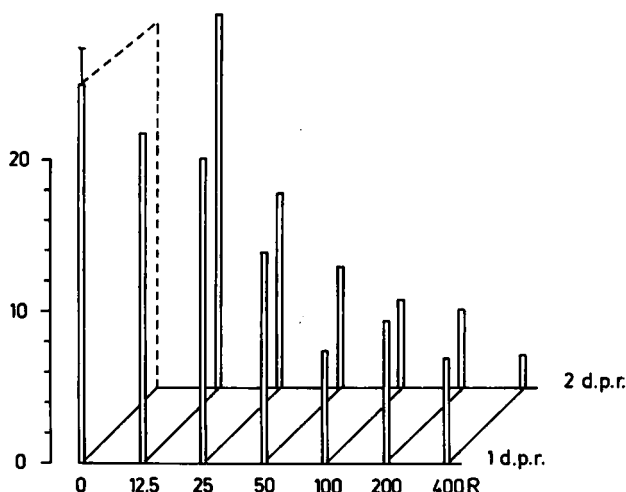
Fig. 1. Dose dependent reticulocytopenia following whole body irradiation. Ordinate: Number of reticulocytes in 10 000 per μl blood. 1 R = 258 $\mu\text{C/kg}$.

to react even at as low a dose as 3.2 mC/kg (12 R), and already at 24 h post-irradiation.

Therefore, reticulocyte analysis in the context of biologic radiation dosimetry appears to be superior to the chromosome aberration test, particularly when the time and manpower needed for the lymphocyte culture and for the preparation and analysis of chromosomes are taken into consideration. Moreover, the quantity of blood necessary for a reticulocyte analysis is only 10 μl , which can be obtained from a finger tip or ear lobe, whereas for a chromosome aberration test at least 500 μl blood is necessary, a quantity requiring venipuncture.

When the degree of sensitivity of these two tests is compared, it is found that the reticulocyte test is in no way inferior to the chromosome test, because as low a dose as 3.2 mC/kg (12 R) can be monitored using reticulocytes. Though the lowest dose causing lymphocyte chromosome aberrations after *in vitro* irradiation has been found

Fig. 2. Dose dependent reticulocytopenia following sublethal whole body irradiation. Ordinate: Number of reticulocytes in 10 000 per μ l blood. 1 R = 258 μ C/kg.



to be around 1.3 mC/kg (5 R) (LLOYD et coll. 1975), a dose of about 6.5 mC/kg (25 R) is necessary in practice for an accurate evaluation as the background frequency of human chromosome aberrations must be taken into consideration. The sensitivity of a reticulocyte test may be increased if the maturity stages of the reticulocytes are also determined.

In case of a radiation catastrophe, be it military or civil, or even in case of minor radiation accidents with physical injuries like burns, etc., in addition to the radiation damage, a knowledge of the radiation dose to the individual victim is an imperative for the planning of the medical management. In such cases a dependable technique of dosimetry is necessary, which should not only be rapid, but also be universally applicable and suitable to screen a population. These requirements may be fulfilled by developing computerized dosimetry through microfluorometric quantification of peripheral blood reticulocytes.

Such a method may be easily adaptable to screen human populations by improving the sensitivity of the present equipment, taking into consideration that human blood has a lower concentration of reticulocytes and that they are poorer in RNA content than the mouse reticulocytes.

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SUMMARY

The flow of reticulocytes in the peripheral blood reflects the bone marrow activity and can be used for evaluation of radiation injury. Using an apparatus capable of microfluorometric quantification of reticulocytes in suspension, the peripheral blood reticulocytes of

mice exposed to various doses of ionizing radiation were examined. A dose dependent reticulocytopenia was observed. The advantages of this method in biologic dosimetry over that of evaluating chromosomal aberrations, and its applicability for screening a human population, are discussed.

ZUSAMMENFASSUNG

Der Einstrom der Reticulozyten in das periphere Blut spiegelt die Knochenmarksaktivität wieder und kann verwendet werden, um Strahlenschäden festzustellen. Unter Anwendung eines Apparates zu microfluorometrischen quantitativen Bestimmungen von Reticulozyten in Suspension wurden die peripheren Blutreticulozyten von Mäusen, die verschiedenen Dosen von ionisierender Strahlung ausgesetzt waren, untersucht. Eine dosisberuhende Reticulozytopenie wurde beobachtet. Die Vorteile dieser Methode bei der biologischen Dosimetrie gegenüber der Feststellung von Chromosomaberrationen und für die Anwendbarkeit als Screeninguntersuchung bei menschlichen Populationen werden diskutiert.

RÉSUMÉ

La circulation des réticulocytes dans le sang périphérique reflète l'activité de la moelle osseuse et peut servir à évaluer l'effet nocif des radiations. En utilisant un appareil permettant la quantification microfluorométrique des réticulocytes en suspension, les auteurs ont examiné les réticulocytes du sang périphérique de souris exposées à différentes doses de radiations ionisantes. Ils ont observé une réticulocytopenie dépendant de la dose. Ils examinent les avantages de cette méthode en dosimétrie biologique par rapport à la méthode d'évaluation des aberrations chromosomiques, ainsi que la possibilité de l'appliquer au dépistage dans une population humaine.

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