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INVESTIGATIONS INTO THE MECHANISM OF ACTION OF IONIZING RADIATION*

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Model tests were performed to gain further information on the mechanism for inducing malignancies by ionizing radiation. Precise variation of the physical parameters of grid irradiations made a separate study of particular test conditions feasible. Barley seeds were sown in dry and in wet soils and they were exposed to grid and to open-field radiations of varying dose; the different effects were measured at different times. Macroscopic migration is supposed. Such migration might be appropriate to explain the differences found between the biological effects of open-field and grid irradiations in man.

Knowledge of the mechanism for inducing malignancies by ionizing radiation is, for the time being, rather scanty. The number of phenomena that cannot be explained even today, after over eighty years of routine applications of ionizing radiations in therapy, is surprisingly high. On the other hand, it was precisely investigations into such manifestations that appeared to be the most suitable to lead to the discovery of further general tendencies of the mechanism of radiations effects.

One such manifestation observed by several researchers is that of grid irradiation. We do not wish to assess the by no means unequivocal results of therapeutic grid irradiations, but only intend to lay emphasis upon the empirical finding generally admitted, according to which the sensitivity of the skin to radiation substantially decreases with grid irradiation. The extent of the decrease in sensitivity is a function of numerous factors, furthermore the conditions for obtaining the results were not the same.

Some authors observed the appearance of the standard erythema of the skin in the case of grid irradiation after the application of doses three, four, or five times higher than in the course of irradiation without a grid, that is to say, at 600 cGy skin tolerance dose generally adopted.

The essentials of the phenomenon can be summarized thus: a skin surface of say, 1 cm^2, will show the same extent of skin erithema only by a significantly larger dose when it is surrounded by other, non irradiated skin portions, than when the same skin surface of 1 cm^2 makes part of a larger, uniformly irradiated skin surface, that is to say, encircled all around by irradiated skin.

* Dedicated to Prof. I. Tarján on his 70th birthday.

This fact is usually explained by stating that in case of a grid irradiation the portion of the irradiated skin can regenerate from the neighbouring, non irradiated skin surfaces.

The process cannot be studied directly on living humans, and there is no information available in the literature on animal experiments. It seems that, for the moment the difficulties of test procedures prevent the carrying out of such experiments.

In order to attempt to make a step towards the discovery of the mechanism of radiation effects, we have tried to perform conclusive model experiments by applying exact biophysical methods:

A *model* was constructed in which the biological matter itself and its environment could be varied independently. Thus the effects of ionizing radiation could be studied under variable conditions, and the correctness or incorrectness of certain theoretical assumptions ascertained.

The biological matter in the model consisted of various seeds, mainly barley, the intercellular matter of sand, with a bed of pebble under it, through which the sand layer of 5 cm thickness could be saturated promptly and uniformly with water. Barley was obtained from the Tápiószentkereszt Research Institute as a material of 100 per cent viability. On every occasion, 900 seeds were sown in sand, previously dried into cubes of 1 cm^2 of a square grid of 30 by 30 cm, with the germinating end of the seeds pointing upwards. The test box was placed into another box. (Fig. 1). As water was let flow into the second box, the liquid level rising in the pebble bed wetted the sand simultaneously and uniformly, without displacing the seeds, in a perfectly reproducible way.

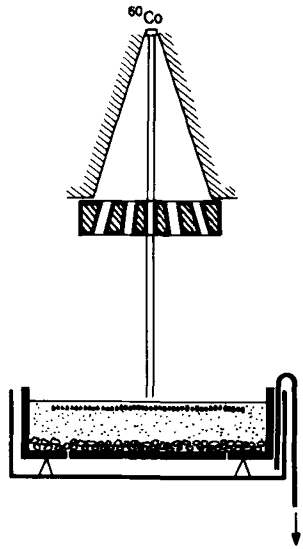

Fig. 1. The 50 per cent grid-filter made of lead, with openings of 25 mm diameter

A feature of great importance described above is that in every phase of the experiment the model accurately simulates the natural conditions.

The model was irradiated by a double-tank system Rotacert cobalt unit developed in our Institute. The charge of the unit was ^{60}Co of 130 TBq activity. After the irradiation treatment the models were transferred to our (constant temperature) laboratory where observations and measurements were performed and photos taken.

The task

Our aim was to check whether during irradiation a macroscopic migration takes place in the biological system and if so, what is its mechanism. We wished to study the problem by a model test with grid irradiation.

Test methods

The Rotacert cobalt unit [1], [2] was, first of all, completed by a projection grid [3] that could be rigidly fixed to the irradiation head at a distance of 50 cm from the source, and could project the gamma field of the grid assembly upon a larger surface. The grid filter and model are illustrated in Fig. 1. The openings, directed towards the 20 by 20 mm cobalt source, are 25 mm in diameter. Half of the total area is screened. The thickness of the lead plate is 45 mm, the screened parts letting 10 per cent of the ^{60}Co radiation through. Thus, grid irradiation gave strongly irradiated areas (100 per cent at the grid points) and slightly irradiated ones (10 per cent through the screened areas) (Fig. 2).

Open-field irradiations were performed in flower pots, grid irradiations in the wooden boxes described above. The irradiations were directed vertically downwards, perpendicularly to the sown areas. In order to limit the risk of errors. irradiations in dry and in wet soil were performed for comparison at the same time and by a single exposure.

The barley seeds previously germinated, sprouted on the fourth day. Observations and measurements were continued for a fortnight.

Aspects of evaluation

We considered the following:

— The number of seeds sprouted in a given area, e.g. in a grid opening area.
— The summed heights of the plants sprouting in a given surface area.
— The colour of the sprouting plants.

An issue of importance was the comparison of the radiation effects brought about in dry and in wet soil. In view of this the wet germinated seeds had to be sown

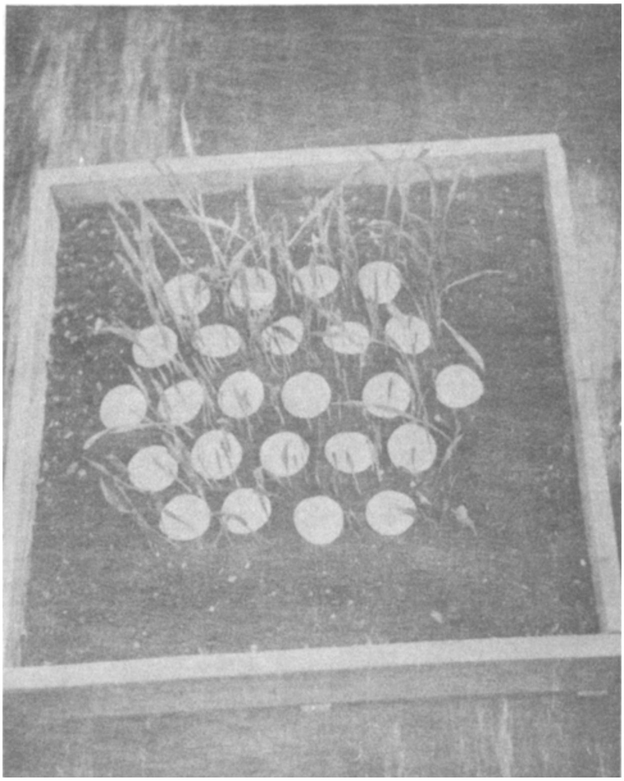

Fig. 2. Plants that sprouted on the tenth day after grid irradiation. The seeds failed to sprout at the grid points, where the white paper circles lie smoothly upon the soil

partly in dry, partly in wet soil. Thus, the conditions of development were variable even without irradiation, as the dry soil gradually absorbed the moisture of the seeds. The latter by and by became desiccated, thereby causing the growth rate to slacken, as compared with the rates of growth of the seeds in wet sand.

Assuming the time taken by the sowing of 900 barley seeds under the conditions outlined above to be 2 to 3 hours, and the irradiation taking a half to two hours, we had to reckon with a significant percentage drying. Therefore we additionally examined the influence of the drying process as a function of time, also looking for practicable ways to reduce the time.

A significant shortening of time was possible by sowing barley seeds soaked in water (germinated), instead of dry seeds. The fact that quickly cleaving cells in quick cleavage are as a rule more sensitive to radiation than cell populations multiplying by bipartition at normal rates is well known. The delivery of doses several times smaller took significantly shorter irradiation times. For our purposes the optimum soaking time for barley was found to be 20 hours so this was the time we used.

The barley seeds soaked in water for 20 hours were sown in groups of 20 in dry sand, and the groups were watered after 1/4, 1/2, 1, 2, 3 and 6 hours. The effects of drying were ascertained by measuring the heights of the plants on the sixth day. Our measurements showed that the slackening of the growth rates of the plants, watered within the first 2 hours still keeps below 5 per cent. This means that the added times of sowing and irradiation had to be brought down to below 2 hours. Even though this raised rather thorny problems, yet the discussion of such details will not be entered into here.

Test results

The results can be summed up as follows:

The 900 barley seeds were sown in dry sand corresponding to a square network of 1 cm mesh. The sown area of 30 by 30 cm was split into two equal parts by a vertical aluminium plate. In the first part of the box the sand was wetted. Immediately after that the box was placed under the Rotacert cobalt unit and the two parts were irradiated at the same time with 6,500 cGy gamma dose measured at the grid points.

Dosimetry was performed with an instrument of our own construction containing a small-sized ionization chamber [4]. After exposure the second part of the

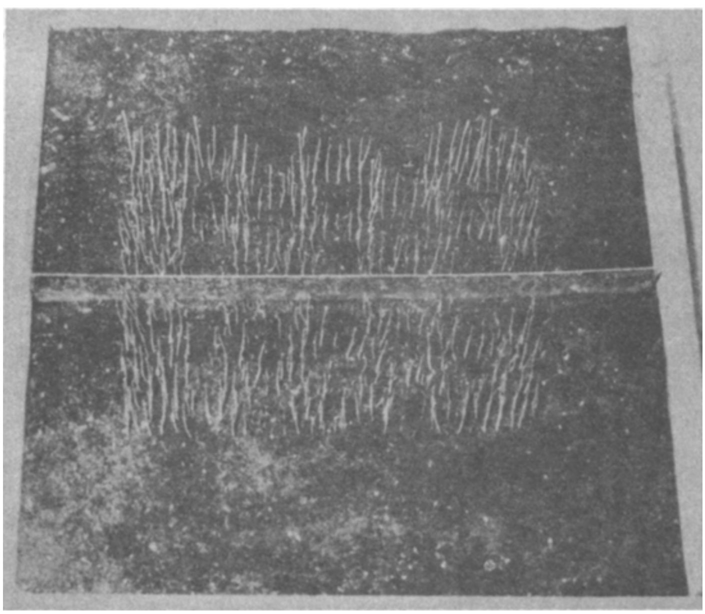

Fig. 3. Plants that sprouted after irradiation with a dose of 8000 cGy dose, on the eight day. At the time of irradiation the soil in the upper half of the box was wet, that in the lower part dry

Table 1

Data on barley sown in dry and in wet soil. The data were taken on the seventh day after a grid irradiation at five grid points for each soil.

Experimental conditions	Number of plants sprouting at five grid points	Summed heights of plants	Dose at grid point	Ratio of heights
Irradiation in wet soil	42	605 mm	5500 cGy	$\frac{605}{172} = 3.5$
Irradiation in dry soil	27	172 mm		
Irradiation in wet soil	17	92 mm	8000 cGy	$\frac{92}{30} = 3.1$
Irradiation in dry soil	14	30 mm		

box was also wetted and the boxes transferred to the laboratory for further observations and for the performance of measurements.

The plants that came up on the fifth day at the grid points were counted every third day and their summed heights obtained. In the screened regions all barley seeds were sprouted (doses of 700 cGy), and the plants were grown similarly to the controls (Fig. 3). The test was then repeated with doses of 8000 and 20000 cGy (Fig. 3).

The results obtained are given in Table I. According to the findings in case of irradiation in wet soil both the numbers of the plants sprouting at the grid point and their summed heights were found to come three times greater than those of the plants sprouting from seeds irradiated in dry soil.

Thus, between the effects of radiation in dry and in wet soils quite significant differences were found with grid irradiation. It is manifest that the cause of this must be the presence of water. The phenomenon can be explained by a sort of "poisonous matter", unknown for the time being, produced in the barley seeds by the irradiation. This matter cannot migrate in dry soil, whereas it can easily do so in water, by diffusion. Thus the poisonous matter produced in the barley grains exposed to large doses can diffuse from the grid points towards the screened areas receiving ten times lower doses only and so also the originally produced concentration of the poisonous matter may be surmised to be of ten times lower concentrations there. This causes the damaging of the seeds at the grid point to be less severe than that suffered by the seeds subjected to radiation in dry soil where migration is impossible. The tests have indicated a factor of three.

It is evident, on the other hand, that the damaging of the seeds under the screened areas, on account of the migration will be more severe than that of the seeds irradiated by the same doses but in dry soil.

It shoud be expected that, in the case of exposures to larger doses of grid irradiation performed in wet soil, the picture of the grid pattern simply vanishes from

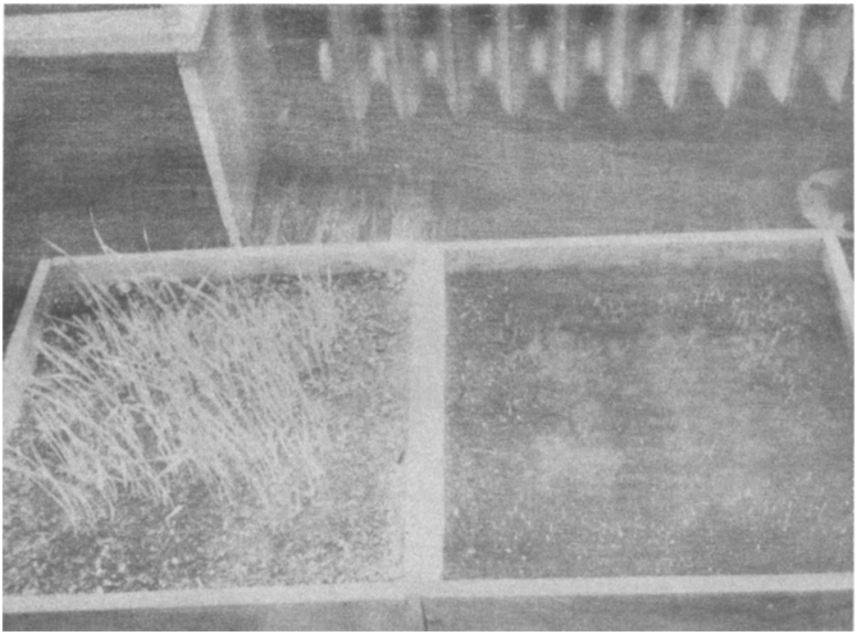

Fig. 4. Plants on the tenth day after irradiation with a dose of 80 000 cGy in a dry soil (left-hand side box) and in wet soil (right-hand side box)

the sown area and homogeneous damaging appears, because the grid points suffer lower, the screened areas suffer more severe damage on account of migration than those that would be motivated only by the dose of irradiation in these regions without migration, as in the case of dry soil. This is also substantiated by the photos of Fig. 4.

This inference was fully verified by further experiments. After exposure to irradiation of 40 000 cGy dose the grid pattern is still clearly visible in both dry and wet sands, like in Fig. 3, with a dose of 100 000 cGy, however, though in dry sand the holes corresponding to the grid formation still do appear; with wet sand the picture of the grid formation completely vanishes, severe uniform damage by radiation is found, and the few dwarfish plants sprouting here and there, will not grow higher than 2—3 mm, unhealthy looking greyish, distorted formations appear, and even the soil displays conspicuous discolouration.

For final conclusions we examined the effects of open-field irradiations without a grid, under various conditions.

In order to secure identical test conditions barley was sown in flower pots in which the soil was separated into three parts by aluminium plates, and the pots were simultaneously irradiated with doses of 10 000 cGy of gamma rays.

In the pots (Fig. 5) each upper third part contains the plants sprouting of 20 dry barley seeds sown in dry sand; the left-hand side sections of the pots those sprouting from 20 seeds sown in dry sand after a previous germinating for 4.5 h and for 20 h,

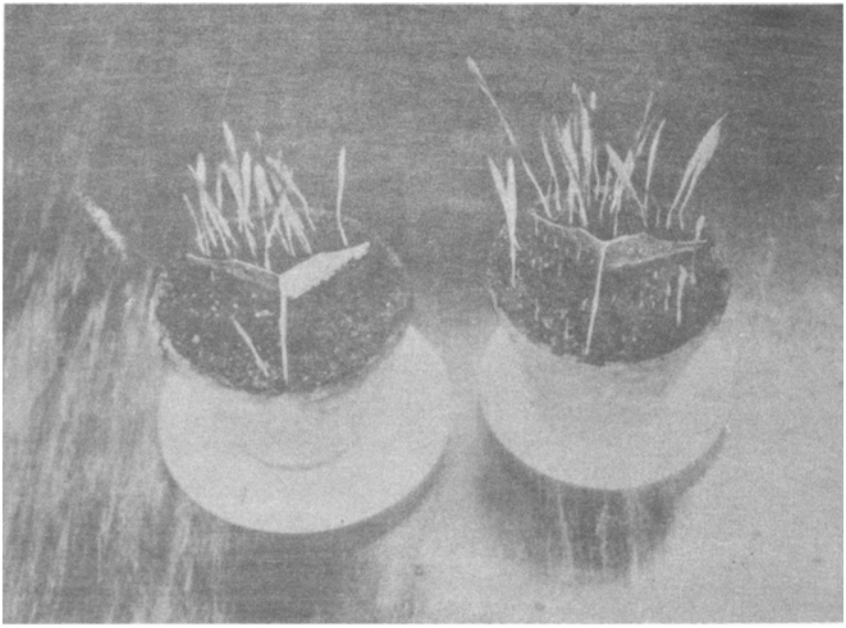

Fig. 5. Plants on the seventh day after irradiation with a dose of 10 000 cGy. The seeds were preliminarily germinated for 4.5 h (left-hand side spot) and for 20 h (right-hand side spot), respectively. In the two top sectors dry seeds were irradiated in dry sand, and in the two left-hand side sectors preliminarily germinated seeds in dry sand, in the two right-hand side sectors germinated seeds in wet sand were irradiated

respectively. The right-hand side sections of the pots contain the plants sprouting from 20 seeds sown in wet sand, after germination for 4.5 h and 20 h, respectively, on the seventh day. It is clearly seen that:

a) between the dry seeds and those preliminarily germinated, differences of 20—50 times are found. The dry seeds sown in dry sand all sprouted, the plants are of a nice green colour. Also they later displayed the same rates of growth as the controls.

b) The plants from germinated seeds sown in dry and in wet sand respectively, and irradiated (the two bottom sectors in Fig. 5) are practically the same, in contrast with the differences up to 300 per cent found after grid irradiation. The seeds germinated for 4.5 hours (right-hand side pot) are perhaps less damaged than those subjected to 20-hour germination — and so obtaining somewhat greater sensitivity (left-hand side pot) where the seeds irradiated in wet sand died off completely. The differences are rather small.

This observation gives substantial support to our hypothesis, namely that, in the case of larger irradiated areas, the damaging of the application of germinated seeds with identical doses is the same, irrespectively of the application of irradiation in a dry or a wet soil, simply for the reason that the surmised "poisonous matter" produced in the barley seeds keeps stationary in both cases. In dry sand the dryness of the soil itself, in a wet soil the "poisonous matter" present all around in uniform concentration, will prevent diffusion.

Similar tests were performed and the same results obtained with alfalfa and lettuce seeds.

Conclusions

1. The model tests of some phenomena of radiobiology seem to give a properly reproducible, exact basis for the development of a hypothesis suitable for bringing us nearer by a step to the knowledge of the mechanism of action of radiation. From our well-proved model tests showing that lesions produced at grid points are much less severe when radiation is applied in wet sand than those exposed in dry sand, the assumption can be made that with radiation in the presence of water a migration takes place, e.g. through diffusion, that cannot occur in dry sand. This migration directed from the grid opening toward the screened areas, takes away a part of the "poisonous matter" surmised to be produced by the re-unification of the molecule particles breaking down as a consequence of the ionizing radiation. Because of this, the remainder of the "poisonous matter" causes damage corresponding to smaller doses than that which would be produced in dry sand, where the displacement of the poisonous matter toward the surroundings and so the drop of concentration involved in this cannot follow.

2. As can be seen, the results of the model tests performed without a grid fully agree with this hypothesis. The reason why, for an open field irradiation with wet sand, no differences are found compared with those obtained in dry sand is that the poisonous matter does not move from the inner points towards the surroundings, not even in the case of wet sand, because in consequence of irradiation with the same dose, the poisonous matter is already present in uniform concentration all around.

3. We may state that the fact experienced with grid irradiations, namely that doses 3 to 4 times higher are required to produce the same skin reaction as in an open-field irradiation is essentially in agreement with the results of our model tests. Thus, it is not excluded from the very first that our hypotheses surmising a macroscopic migration of a poisonous matter, which was proved by our model tests, could be attempted to be applied also to the mechanism of radiation effects produced in humans and in other animals, to try to prove the existence of the said poisonous matter at a place and time that seems propitious for the performance of such work.

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