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EFFECTS OF IRRADIATION ON THE INTESTINAL CELL POPULATION¹

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I. CLINICAL AND PATHOLOGY PICTURE

If a C57 mouse is given a whole body X-irradiation with a dose of 1200 rad or more, then it develops a characteristic syndrome which terminates in death $3\frac{1}{2}$ ($\pm \frac{1}{2}$) days after irradiation. On close study, the mouse shows various transient functional disturbances shortly after irradiation, and serious disturbances of gastrointestinal motility and absorption throughout; however, on casual observation, the mouse looks normal until, about 3 days after irradiation, it develops somnolence, hunched back, fluffed-up fur, and severe diarrhoea. This picture remains very much the same if the dosage is raised up to ten-fold and, if the head is protected, even up to twenty-fold. The syndrome is about the same for mice of both sexes and all ages; it occurs, with appropriately adjusted doses, with all kinds of ionizing radiations. With minor differences in dosage range and survival time, it has been found in many mammals and in some other vertebrates.

By anatomical fractionation, testing irradiations first of entire body regions, then of smaller areas, and finally of surgically exposed individual organs, it was found that the syndrome which kills a mouse in $3\frac{1}{2}$ days is mainly due to irradiation of the small bowel: irradiation with 1200 rad or more, of any region which includes the entire small intestine results in death at precisely the same time as irradiation of the whole body, irradiation of any major fraction of the small bowel alone results in death under the same circumstances but at a slightly later time, and irradiation of the entire body minus the protected surgically exposed small intestine (plus such small portions of other organs as are necessarily covered together with the intestine) does not cause a comparable syndrome (1).

This particular mode of radiation death (there are several others) is thus initiated by irradiation of the small intestine, hence the name, acute intestinal radiation death (2). Presence of the irradiated intestine is necessary for the evolution of the syndrome: if a large portion of the small intestine is exteriorized and irradiated with a dose which would cause intestinal death, but then resected within two days after irradiation then death is prevented; even if the irradiated part is only isolated and periodically washed, some animals can be saved (3, 4).

Pathological studies of animals given high doses of irradiation reveal many changes occurring between irradiation and death; but the finding even of severe changes in any organ does not prove that it is critically involved in the mechanism of death. However, there is a sequence of changes in the intestinal mucosa closely correlated with the clinical picture of the intestinal syndrome; its occurrence under various conditions parallels the occurrence of intestinal death. Therefore, it may be identified as the critical sequence of events. It evolves as follows: soon after irradiation, entrance of cells into mitosis ceases (5), and uptake of tracer into DNA is greatly reduced (6). After 1-2 hr, pyknosis develops in many crypt cells. At the end of a day, the crypt cell population is greatly reduced. During this time, the villus cells show little if any change in the light microscope; electron-microscopic study reveals changes particularly of the membranes (7). During the 2nd and 3rd day, there is some evidence of proliferative activity in the crypts, such as DNA synthesis and mitoses (if the dose was not too high); however, mitosis when it occurs is abortive, not resulting in the production of viable daughter cells. The crypt cell

population keeps declining and vanishes on the third day; the numerical reduction is accompanied by qualitative changes of the cells: cells remaining after the first day are larger than normal and show some abnormality of structure; cells remaining after the second day are very large and grossly misshapen (2, 8). The decline in crypt population is succeeded by a reduction in villus cell population, accompanied by a reduction in villus size; also, qualitative changes in villus cells succeed the same changes in crypt cells, and the final depletion of the crypts is succeeded by depletion of the villi. After all cells are lost, the villi collapse into small mounds; the submucosal connective tissue becomes very edematous. The sequence of crypt changes preceding villus changes, together with the knowledge that the cells lining the intestinal lumen are produced in the crypts and decay off the villi, suggests a simple model of the mechanism of intestinal radiation death: irradiation destroys the proliferative cells, while cells which were mature at the time of irradiation decay at roughly normal rates. If production of new cells is blocked for long enough time to allow denudation of the intestinal wall, then the lethal syndrome develops; if the dose was small enough to allow resumption of proliferation before completion of denudation, then the tissue and the whole organism may recover.

Denudation of the intestine means removal of the barrier which separates the intestinal lumen from the intestinal wall. If this loss involves large portions of the gut, then there must result severe disturbances which, if not corrected, are bound to be lethal. Indeed the problem of precisely establishing the mechanism of intestinal radiation death is not

one of finding a plausible mechanism but of deciding which of various disturbances is lethal fastest. It seems that the immediate cause of death is loss of water and electrolytes; parenteral replacement results in immediate improvement of the clinical picture, and sustained administration of water and electrolytes can prevent death (9). The diarrhoea may contribute to the loss but more important seems to be the very severe edema of the intestinal wall which in turn is probably due to irritation by intestinal content. This fits with the observation that washing of the irradiated intestine can be life-saving (4) and that diisopropylfluorophosphate which reduces edema also reduces the damage due to intestinal irradiation (10). It should be mentioned that parenteral administration of water and electrolytes in these animals greatly increases the diarrhoea and thus results in a washing out of the intestinal content, in addition to replacement of water and electrolytes lost.

II. NORMAL CELL POPULATION KINETICS

The sequence of histological changes in the intestinal epithelium after irradiation indicates the close association between evolution of pathology and kinetics of the cell population. Accordingly, a searching analysis of the syndrome must contain a detailed investigation of the cell population kinetics. It has been known for a long time that the cells lining the gut form not a static but a dynamic population; however, a fairly precise and comprehensive knowledge of the normal kinetics of cell proliferation, maturation, movement and decay has been acquired only within recent years (11-15). This progress was largely based on the development

of isotopic tracers and autoradiography. The tracer used most frequently is tritiated thymidine (H^3T) (16). Ideally, this tracer labels instantly and permanently all cells which replicate their DNA at the time of tracer administration. Actually, things are not that perfect, and every one of the six italicized words needs to be qualified, but for short-range studies with the epithelium of the small intestine, the approximation to the ideal characteristics is not bad.

It has been found (17) that in higher organisms, replication of DNA is restricted to a fairly well-defined portion of the interphase separated on both sides from mitosis by definite intervals of time. This divides the proliferative cycle into four phases, designated mitosis, post-mitotic or pre-synthetic "gap," synthesis phase, post-synthetic or pre-mitotic "gap," or shorter, M , G_1 , S and G_2 phase. Labeling with H^3T identifies a cohort of cells which at the time of labeling were replicating their DNA, and study of the population of these cells at various intervals after labeling is the principal method of studying the kinetics of a cell system. Thus, observing the leading edge of labeled cells entering into mitosis yields an estimate of the duration of G_2 ; observing the trailing edge leaving mitosis yields an estimate of the duration of S ; the entrance of the cohort of labeled cells into mitosis takes less time than their leaving mitosis, and the difference between these two times is related to the variance of times spent by individual cells in replicating DNA (13). Observing the rate of increase of labeled cells, and the concomitant decrease in the amount of label per cell, allows estimation of the rate of proliferation, or the time taken for the entire proliferative

cycle. The duration of the transition phase can be determined from the interval which elapses between labeling and the appearance of labeled cells in the surface epithelium; and the life time of mature cells, by the rate of progress of the leading edge of labeled cells along the sides of the villi (12, 13, 15). Information on the total number of proliferating cells in a crypt can be obtained by continuous or repeated labeling. Data obtained by single or repeated labeling can be checked by using two pulses of labeling with thymidine tagged with different isotopes, i.e., H^3 and C^{14} ; this method is also very sensitive for revealing possible effects of internal irradiation caused by the label (18, 19). The application of these methods and the evaluation of the results obtained has become moderately complicated (20, 21). In the intestinal epithelia of mice, rats and men, the duration of the generative cycle is $\frac{1}{2}$ to 1 day, depending on species, strain, age, and location in the gut; the S-phase lasts about 8 hr and thus occupies $\frac{1}{3}$ to $\frac{2}{3}$ of the proliferative cycle; the G_2 -phase (lumped together with more or less of the prophase) lasts from $1\frac{1}{2}$ -3 hr; the G_1 -phase accounts for the rest of the cycle time, and it is largely responsible for differences between cycle times of different populations of cells, and also between those of individual cells within a given population. The normal kinetics of the intestinal epithelia in certain strains of mice and rats is known in considerable detail, and the kinetics in a few radiation-induced abnormal situations is fairly well known--sufficiently well to be quite certain that the simple model "aplasia due to destruction of the proliferative cells with normal decay of mature cells" is not adequate to describe the details of the intestinal radiation syndrome.

III. CELL POPULATION KINETICS IN THE IRRADIATED ANIMAL

In the lower ileum of the C57 Brown mouse, the life time of a mature cell plus the duration of the transition phase is about 2 days, but depletion of the mucosa occurs in 3 days. The difference is much greater than the error in the determination of kinetic parameters; hence, the simple model is inadequate. Indeed one finds by labeling just before irradiation and sacrificing at various times after irradiation that labeled cells--which were proliferating at the time of irradiation--continue to reach the villi, and that they move along the villi at approximately normal rates. A detailed study has revealed the following mechanisms operating between irradiation and final depletion (22):

(i) post irradiation freeze: following irradiation, transitions from one phase to another are blocked for varying length of time. The post-irradiation delay of mitoses is the best known instance of the post-irradiation freeze; others are the transition from the pre-synthetic phase G_1 into the synthesis phase S , and, probably, a transient block of the movement of mature cells along the villus (23);

(ii) early necrobiosis resulting in the death of roughly one-half the proliferative cells, taking place largely between 2 and 12 hr after irradiation;

(iii) precocious maturation, with labeled cells (i.e., in S -phase at the time of irradiation) maturing without having divided;

(iv) abortive resumption of proliferative activity as revealed by resumption of DNA synthesis and mitosis, beginning about one cycle time

after irradiation, and leading to:

- (a) death in mitosis, or
- (b) maturation without mitosis (precocious maturation in the 2nd post-irradiation cycle) with formation of cells which are larger than normal and deviate moderately from the normal structure, or
- (c) cells having presumably gone through one S-phase remaining proliferative without, however, dividing, and going through a second post-irradiation S-phase resulting in very large and very misshapen cells which leave the crypts and constitute the last covering of the villi; these cells have been called ω -cells since they represent the last effort of the proliferative tissue (22).

Depletion of the villus cell population with denudation of the intestinal wall occurs when the ω -cells are sloughed off; hence the total time elapsed between irradiation and depletion can be partitioned into two parts, the time from irradiation to the time when all remaining proliferative cells are ω -cells, which lasts about $1\frac{1}{2}$ days (and is probably composed of time spent in post-irradiation freeze plus two generative cycles), plus the life time of a ω -cell which often is the normal span of about $1\frac{1}{2}$ days. This accounts roughly for the time elapsing between irradiation and depletion; however, the precise kinetics of the formation of ω -cells remains to be determined.

When a major portion of the small intestine is protected, and

sometimes for unknown reasons, the w-cells are not sloughed off. In this case, no lethal loss of water and electrolytes occurs but the barrier between lumen and submucosa is leaky and easily passed by bacteria; death occurs due to bacterial invasion, as a rule some 2 days later than in the typical intestinal syndrome. This syndrome has been called "intestinal radiation syndrome II" (24) and is one of the variants of the intestinal syndrome. Another, characterized by a longer lag between epithelial depletion and death, occurs in the rat if the bile duct is ligated prior to irradiation (25); in the mouse, bile duct ligation has no effect on survival time (Quastler, unpublished data). Still another variant is characterized by death about $\frac{1}{2}$ days earlier than usual; the mechanism of this modification is quite unknown (1).

The discrepancy between the simple model (death of proliferative cells, normal decay of mature cells) and the actual findings brings out an important fact: there occur, in the irradiated organism, phase transitions and states in cell life which are well-defined and different from those observed in normal organisms; the w-cell is an extreme example of a well-defined abnormal state. Irradiation thus reveals potentialities of a cell which under normal conditions are not or but rarely apparent (26).

The occurrence of abnormal phases which together make up a steady state is brought out strikingly by continuous irradiation. Under this condition, the intestinal epithelium reveals an extraordinary power of adaptation. When rats are subjected to whole body irradiation by a Caesium source, at a rate of 415 rad/day, there is great reduction in mitoses and a moderate amount of necrobiosis in the intestinal epithelium which lasts for 1-2 days;

during this time, tracer uptake into DNA is not or only slightly reduced. After these transient changes, cell production resumes and within 3-4 days a steady (or near steady) state is reached and is maintained until the rat dies due to hemopoetic failure (27). When the life of the rat is saved by marrow transplantation, the intestinal epithelium recovers after having received a total dose ten times as high as the dose that would wipe out the whole cell population if delivered in one short session (28).

In the steady state established under 415 rad/day irradiation, the intestinal epithelium is not normal. The crypt population is reduced, the villus population possibly more so; the crypts are slightly shortened and much thinner than normal, the villi are considerably shorter than normal. The kinetic parameters are not much affected; the rate of proliferation shows little if any change, and the life time of mature cells is approximately normal although a larger fraction of this span is spent inside the crypt (27, 29, 30). The crypt cells appear grossly normal, but the villus cells are larger than normal and the polarization of their nuclei is less strict than normal; they are comparable to the first generation of abnormal cells which reach the villi after acute irradiation. Since this situation develops during a time when DNA synthesis appears to be little affected and mitoses are greatly reduced, one might suspect that the cells have double the normal amount of DNA. This, however, is not the case as has been established by cytophotometry (31); therefore the tracer uptake during the transition period not followed by division yet not resulting in doubled DNA must represent yet another abnormal phase, this one a variant of the normal S phase.

The group of syndromes described does by no means exhaust all the radiation responses of the intestine, and the description given does by no means exhaust all the aspects of the syndrome. On the other hand, the interest in the mechanism described transcends the immediate concern with the object discussed. The sequence of changes found in the intestinal epithelium occurs, with some variations, in all cell renewal systems; thus the pattern described is characteristic of a considerable fraction of the known radiation responses (22, 26). Still more important, the condition described is not specific for ionizing radiation but can be produced with some variations, by a number of agents. Thus the effects of aminopterin on the intestinal mucosa, shown by Zamcheck in the lecture which initiated this series of symposia (32) are very similar to the effects of ionizing radiations, and so are the effects of other cytotoxic drugs such as azaserine, nitrogen mustard, actinomycin D (33); drugs acting through different biochemical mechanisms. The appearance of the intestinal epithelium under continuous irradiation is rather similar to the histopathology of sprue. The radiation effects are thus just part of the repertory of responses with which the intestinal epithelium and other cell renewal systems react to abnormal situations.

The analysis of the intestinal radiation syndrome has led into studies of cell population kinetics, and these in turn have opened up a number of interesting perspective in two directions: proceeding toward lower levels of organization, to cell biology, there is the demonstration of a variety of well-defined and stable states of cells which are rarely if ever found under normal circumstances; proceeding toward higher levels,

to organ pathology, cell population kinetics is a powerful tool in elucidating disease mechanisms.

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