Certain Effects of Irradiation and Chemotherapy on Cellular Division and Differentiation*

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Just as the individual chemical constituents of a cell continually change while the cell itself remains a distinct entity, so is a dynamic equilibrium maintained in tissues and organs in which the over-all structure is sustained but the cellular composition is constantly changed. For example, from the epidermal surface of the skin, cells are regularly lost and are replaced by new elements formed in the deeper layers. The epithelium of the intestinal mucosa is another such tissue; the shedding of elements at the tips of the villi is compensated for by the formation of new cells in the crypts. The rapidity of formation of new cells is indicated by the large number of mitotic figures in the epithelium of the small intestine. It has been calculated that mitosis in the jejunum of the rat takes about 30 minutes (14). This calculation is based on the observation that mitotic activity persists for this period after the animal has been irradiated. Irradiation prevents cells from entering into mitosis, but those cells which had already begun to divide at the time of treatment are able to complete mitosis. Irradiation usually prevents cells from entering into mitosis, but those cells which had already begun to divide at the time of treatment are able to complete mitosis.

RESULTS

Young adult (3-6 months) albino rats of the Wistar strain were employed. Groups of animals were given intraperitoneal injections of nitrogen mustard (1 mg/kg), triethylene melamine (1 mg/kg), aminopterin (1.5 mg/kg), urethan (1000 mg/kg), and colchicine (2 mg/kg). Other groups were irradiated (500 r); some received treatment over the entire body, and in others the field of irradiation was restricted to a transverse mid-abdominal portal. (The size of the field did not influence the results of the mitotic counts.) Animals were killed every 1-2 hours during the first 24 hours after treatment and at 12-hour intervals thereafter for 5 days. The duodenojejunal loop was fixed in Bouin's solution, embedded in paraffin, and multiple cross sections, 6 μ thick, were stained with hematoxylin and eosin and by the periodic acid-Schiff method. Mitotic figures were counted at a magnification of 400X. The points on which the curves are based represent the averages of several determinations on different animals. A broken nuclear membrane had to be observed in order for an early figure to be counted; paired daughter nuclei with reestablished nuclear membranes were not counted. Paired chromosomal clumps in anaphase or telophase were counted as one, and two doubtful figures were counted as one. The diurnal variation in the number of mitotic figures is not of sufficient magnitude to significantly affect the alterations produced by the agents employed.

METHODS

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RESULTS

There are approximately 200 mitotic figures, almost entirely in the crypts, in a cross section, 6 μ thick, of the jejunum of a rat. A single injection of nitrogen mustard or triethylene melamine (1

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mg/kg) results in a reduction of the number of mitotic figures to about 20 per cent of the normal value. Subsequently, there is a transitory increase in the number of mitoses, a so-called "compensatory" hypermitosis, followed by a return to the normal level (Chart 1). The effects of a single dose of aminopterin (1.5 mg/kg) are similar (Charts 1 and 2).

To maintain the normal level of mitotic activity, constant numbers of cells must continuously pass from the intermitotic stage into mitosis. The fall in the number of mitoses after treatment probably results from interference with the progress of elements preparing to go into mitosis; the alternative explanation, that the duration of mitosis is shortened, is unlikely. The teleologically designated "compensatory" hypermitosis after the period of depression is actually caused either by an increase in the number of dividing elements or by a lengthening of the time spent in mitosis. A prolongation of the intermitotic phase (15) without inhibition of the later division of the cells would cause a transitory increase in mitotic figures, because those elements which had been delayed in entering mitosis would be added to the normal complement of dividing cells. Shortening of the intermitotic phase would have the same effect as prolonging the duration of mitosis.

Chart 3 attempts to illustrate these points diagrammatically. The alterations in the level of mitotic activity are the end results of events which took place in cells during their interphase. The early changes in the mitotic curve are produced by effects on cells which were in late interphase, nearly ready for mitosis. The later changes are the consequences of cellular reactions in elements which were in early interphase. Reversal of the curve, therefore, makes it possible to correlate altered mitotic activity directly with the changes in the procession of interphasic cells advancing toward mitosis, as drawn at the bottom of the figure. The standard curve in the upper right-hand corner of the diagram has been drawn backward so as to coincide with the column of intermitotic elements drawn below. If the magnitude and duration of the compensatory hypermitotic phase exceeds that of the period of depression, as in the mustard curve (Chart 1, #2), it is necessary to conclude that delay of mitosis is the major inhibitory effect. Suppression of mitosis greater than the extent of compensatory hypermitosis, as in the case of triethylenemelamine (Chart 1, #1), indicates that mitosis is to a large extent completely blocked.

A single dose of 500 r given over a short period results in an immediate depression of mitotic ac-
tivity which lasts about 24 hours and is followed by hypermitosis during recovery. However, about 6 hours after treatment there is a rise in mitotic activity (9) which continues for about 4 hours, and at its peak, reached about 9 hours after irradiation, the number of mitotic figures approaches normal levels (Charts 1 and 4). This period of mitotic activity suggests that cells during a certain portion of the intermitotic phase are not radiosensitive and can still divide subsequent to irradiation (Chart 5). The absence of such a secondary rise when the mustards are employed suggests that the action of the chemical agents is so prolonged that those cells in the hypothetically unreactive phase can progress into the sensitive premitotic phase while the chemical is still effective and can thus be prevented from going into mitosis.

It seems reasonable to postulate that the insensitive phase is a resting stage between two radiosensitive phases of cellular activity. The sensitive premitotic period, interference with which causes the immediate post-treatment fall in mitotic activity, may be a period of formation or rearrangement of nuclear components, such as deoxyribonucleic acid. The earlier radiosensitive phase is probably a period of synthesis of cytoplasmic substances, such as pentosenucleic acid, and should be designated "active" interphase to distinguish it from the resting stage (Chart 6).

The mitotic curves following the administration of urethan (1,000 mg/kg), are consistent with such speculations (Charts 1, 2, and 7). Although there is a slight early depression of mitotic activity after the use of urethan, the delayed major depression probably results from inhibition of the radiosensitive active interphase. The absence of both compensatory hypermitosis and nuclear debris (Fig. 1) after the administration of urethan indicates that this drug abolishes active interphase by inhibiting cytoplasmic metabolic activity (7). Nuclear debris can be observed in the crypts not only following irradiation (Fig. 2) and the injection of colchicine but after treatment with the chemical agents (4) which interfere with normal mitosis by acting during premitotic interphase.

The curves produced by the administration of colchicine (2 mg/kg) (Charts 1 and 8) are unique, because this drug permits entry into but prohibits completion of mitosis. Twelve hours after treatment, the mitotic count is excessively high (Fig. 3), but by 24 hours after injection it falls, and thenceforth the curve is similar to that produced by the administration of the other antimitotic agents. One might expect less compensatory hypermitosis following colchicine than is actually encountered.

Urethan and colchicine, unlike irradiation, the mustards, and aminopterin, interfere to a certain extent with cellular differentiation, an effect which probably results from inhibition of synthetic cytoplasmic activity in postmitotic cells. Differentiation can be followed in the jejunum by tracing the development of mature goblets as the cells move up the villi from the proliferating zone in the crypts (13). At the time of maximal suppression of mitotic activity, after irradiation or treatment with mustard or aminopterin, goblet cells distended with mucus appear in the crypts. Such ele-
Clements are normally encountered only along the villi; their appearance in the crypts coincident with mitotic depression indicates that maturation of undifferentiated cells, namely, the formation of goblet cells from reserve cells, has not been inhibited. Since the new elements which must be formed in the crypts to push cells along the sides of the villi are lacking, the postmitotic cells mature in situ, and the crypts become loaded with goblet cells. When mitosis is reestablished the zone of arrested goblet cells moves distally, as does the wave of damaged epithelial cells.

The appearance of mucous elements in the crypts, once incorrectly interpreted as "mucous degeneration," is a manifestation of mitotic suppression unassociated with inhibition of differentiation. This phenomenon is encountered following irradiation or treatment with nitrogen mustard, triethylene melamine, or aminopterin (Fig. 4). It does not take place after the use of colchicine (Fig. 5) or urethan (Fig. 6), which interfere with differentiation as well as inhibit mitosis.

DISCUSSION
The dissociation of the effects on differentiation and division produced by irradiation, mustard, and aminopterin is not limited to normal intestinal
mucosa. It can be observed in other tissues, such as the testis (8), and in tumors (6).

There are two genera of cells, one of which has the capacity to reproduce but not to differentiate, the other the ability to differentiate but not to divide (Charts 6 and 9). The undifferentiated cells of self-replacing tissues must by their division produce not only new elements for the maintenance of the tissue but must replace themselves (11). Therefore, after each division one daughter cell persists as a reserve element while the other differentiates. The postmitotic differentiating somatic cell synthesizes and accumulates such material as keratohyalin, mucoprotein, enzyme and hormone precursors or whatever is needed for its specialized functions; the postmitotic reserve cell accumulates the nuclear materials necessary for its next division (12). One cannot ignore nuclear-cytoplasmic interrelations, but the postmitotic element differentiates chiefly through cytoplasmic synthetic activity; the reserve cell differentiates largely through nuclear synthetic activity which probably follows a cytoplasmic synthetic stage early in interphase. Preparation of the reserve cell for division and preparation of the somatic cell for differentiation are homologous.

Tumors probably originate from the reserve cells which have the capacity to form dividing and differentiating elements rather than from adult postmitotic cells which have “de-differentiated.” Since a tumor cell may have the capacity both to divide and to differentiate, the resultant new growth may be homogeneous with the parent cell through division, completely heterogeneous through differentiation, or reflect both potentials of the originating element.

Curves of mitotic activity following irradiation or administration of chemotherapeutic agents in neoplastic tissue (2) are comparable to those in similarly treated normal tissue (5). One reason advanced for the effectiveness of radiotherapy is the short intermitotic time (14) of neoplastic cells and the consequent increased opportunity for inhibition of division. Another is the induction of differentiation (6).

Many of the cells in neoplasms do not die as a result of chemical treatment or irradiation but instead enlarge, exhibit nuclear hyperchromatism, and form giant and multinucleate elements. The bizarre changes in such irradiated cells indicate, not increased malignancy, but inhibition of cellular division without interference with cellular synthesis and growth. Similarly, the cells of the treated tumor may exhibit increased differentiation while division is inhibited. Serial specimens taken during the course of treatment of an undifferentiated transitional carcinoma in man may reveal transformation to a well differentiated and cornifying epidermoid growth (8). It is possible to demonstrate similar transformations in experimental tumors. Since transitional growths are basically undifferentiated epidermoid tumors, the potentiality to form squamous cells becomes evident when cellular division is inhibited by treatment with agents which do not prevent differentiation. The ability of the cells to differentiate is manifested, not despite the antimitotic effect of treatment, but because of it.

SUMMARY

Analysis of the reactions produced in tissue by irradiation or the administration of chemotherapeutic agents reveals that such factors do more than merely interfere with cellular division. Some modalities inhibit the completion of mitosis in cells with the capacity of multiplication, some interfere with premitotic nuclear metabolism, and others inhibit an earlier cytoplasmic phase of synthesis. A comparable interference with cytoplasmic synthesis and an inhibition of differentiation are exercised by some of the drugs on the somatic cells which lack the ability to divide, while irradiation in therapeutic doses and other chemical agents do not interfere with differentiation. Although tumors in general are presumed to arise from reserve cells, spontaneous differentiation occurs not uncommonly. The possibility of enhancement of the tendency to differentiate is a relatively neglected but important effect of treating neoplasms by irradiation and selective chemotherapy.
REFERENCES


Fig. 1 (F 1882).—Intestinal crypt 18 hours after injection of urethan (1,000 mg/kg). Note absence of mitoses, lack of nuclear debris, and absence of goblet cells. (Compare with Fig. 2.) X750.
Fig. 2 (F 1860).—Intestinal crypt 6 hours after treatment with 500 r. Note absence of mitoses and presence of nuclear debris. (Compare with Fig. 1.) X750.
Fig. 3 (F 1807).—Intestinal crypt 3 hours after injection of colchicine (2 mg/kg). Note numerous mitotic figures. X360.
FIG. 4 (F 1607).—Intestinal crypt $24$ hours after injection of aminopterin (1.5 mg/kg). Note absence of mitotic figures and presence of numerous goblets. (Compare with Figs. 5 and 6.) $\times 360$.

FIG. 5 (F 1715).—Intestinal crypt $24$ hours after injection of colchicine (2 mg/kg). Note few mitotic figures and absence of goblet cells. (Compare with Figs. 4 and 6.) $\times 360$.

FIG. 6 (F 1886).—Intestinal crypts 18 hours after injection of urethan (1,000 mg/kg). Note absence of mitotic figures and lack of goblet cells. (Compare with Figs. 4 and 5.) $\times 360$. 
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