Latent Injury and Repair in Rat Liver Induced To Regenerate at Intervals after X-Radiation*

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SUMMARY

When young, adult, male rats were exposed to a single dose of x-radiation of 365 or 600 r to the whole body and were partially hepatectomized immediately afterward, the regenerating livers exhibited a lag in initiating both DNA synthesis and mitosis. The lag was most pronounced in livers that had been regenerating for ~7 hours, a time when controls exhibited near maximal levels of activity. When days elapsed between irradiation and the induction of hepatic regeneration the lag gradually diminished so that after 8 weeks the response of the irradiated animals was similar to that of the controls. When a second partial hepatectomy was performed 4 or 5 days after the first, recovery from the above effects of irradiation was expedited. The number of chromosome bridges and other signs of structural damage resulting from irradiation did not diminish with time under any of the above conditions, however. Thus it appears that certain functions, although presumably inactive at the time, are impaired by x-radiation administered when cells are in the nondividing state, but the recovery is accelerated by induction of cell proliferation. Other functions, involving the distribution of genetic material, appear to be more permanently damaged.

The synthesis of deoxyribonucleic acid (DNA) as measured by the incorporation of P^32, glycine-N^15, adenine-C^14, orotic acid-C^14, and formate-C^14 has been extensively studied in both intact and regenerating livers of rats and mice (2, 10, 15–18, 26, 31, 36, 37, 41). The results, irrespective of precursor, consistently indicate that the amount of DNA synthesized in intact adult liver is very low. In regenerating liver of the rat a sharp rise begins approximately 15–18 hours after partial hepatectomy, reaching a peak at 24–30 hours and exhibiting elevated levels for several additional days. Mitotic figures are very rare in normal adult liver; they first appear in appreciable numbers 22–24 hours after partial hepatectomy with a maximum at 27–34 hours, followed by a fluctuating decline during the next few days. The pattern of regeneration in mouse liver is similar, but there is a longer delay before the initial rise in DNA synthesis (8, 13, 18, 29, 31, 45).

As regards the effects of x-radiation upon regenerating liver, there has been general agreement upon the following points. The incorporation of labeled precursors into DNA can be either lowered or completely inhibited depending upon the size of the dose and the stage of regeneration at which it is applied; smaller doses of irradiation are required to reduce incorporation into DNA if applied during the relatively sensitive presynthetic interval than during the relatively resistant period of active DNA synthesis. Mitotic activity is also inhibited by x-radiation; it is even more sensitive and can be completely blocked by doses that do not depress DNA labeling (6, 13, 30). A few experiments have shown that a dose of irradiation to the intact animal as long as 10 hours before partial heptectomy is equally as effective as treatment during the sensitive early period of regeneration (6, 23).

The present study is concerned with the dura-
tion of the effects of x-radiation upon intact liver. At the dose levels employed there were no signs of injury until the liver was induced to regenerate. Latent damage thus brought to light was evaluated by measuring (a) the incorporation of C14-labeled orotic acid into DNA, (b) the amount of mitotic activity, and (c) the occurrence of mitotic abnormalities.

MATERIALS AND METHODS

Animals.—Young adult male rats of the Harvard-Wistar strain, weighing approximately 200 gm., were anesthetized with ether and partially hepatectomized, usually during the morning hours by the method of Higgins and Anderson (21). They were given Rockland Farms rat diet and water ad libitum.

In a few experiments, in which two partial hepatectomies were performed upon the same rat, the average initial weight of the animals was 116 gm. The second hepatectomy, which was carried out after the liver had been regenerating for 4 or 5 days, consisted in removal of the ventral portion of the right lateral lobe, or approximately 30–40 per cent of the liver then present. Postoperatively these animals received penicillin, streptomycin, and 15 ml. of 5 per cent glucose subcutaneously. In addition, they were offered 10 per cent sucrose solution ad libitum.

Irradiation.—A Picker-Waite Therapy machine operating at 220 kvP, 20 ma., and 45 r per minute with a filter of ½ mm. Cu and 1 mm. Al, yielding a half-value layer of 1 mm. Cu, was used. The animals, in groups of four, free to move laterally but not vertically, were housed in a circular plastic cage 50 cm. from the source of irradiation. The dose was determined with a Victoreen roentgen meter in the “liver region” of a phantom made to simulate an average size rat.

Analyses.—Rats were given injections intraperitoneally of 1.2 amoles of neutralized orotic acid-6-C14 in saline. The specific activity was 1.36 μc/μmole. In all cases the orotate was injected 4 hours before the animals were killed.

Animals were anesthetized with ether and the livers rapidly excised. Samples of the tip of the left lateral lobe were fixed for histological studies. The remaining liver was quickly frozen and stored at –10° C. for future biochemical analysis.

The method of Huribert and Potter was followed for extraction of nucleic acids and isolation of DNA (26). To insure freedom from contamination with ribonucleic acid (RNA), the DNA was reprecipitated 4 times from alkaline solution by acidification. The final DNA precipitate was dissolved in 30 per cent NH4OH, plated on glass planchets, and assayed for radioactivity in a gas flow counter fitted with an ultra-thin window (density less than 150 μg/sq cm). The counting efficiency was approximately 35 per cent. The samples, still on planchets, were dropped into digestion tubes and analyzed for phosphorus by the method of King (38).

For determination of mitotic activity, tissue fixed with Stieve’s fluid was sectioned and stained by the Feulgen method with fast green counterstain. Counting was carried out at a magnification of 200X under a microscope fitted with an ocular field stop, and was continued until at least 30 mitotic figures were recorded or until 20–30,000 nuclei had been examined. The results were expressed as percentage of hepatic parenchymal nuclei undergoing mitosis (11).

To estimate the proportion of abnormal mitotic figures, tissue was fixed in Bouin’s fluid, sectioned at 5 μ and stained with hematoxylin and eosin. Although various kinds of abnormalities occurred, only chromosomal bridges in anaphase and telophase were recorded (9) because objective criteria for scoring these are more readily established. Thus the results, expressed as per cent of all anaphases and telophases having bridges, convey the relative degree of injury to the mitotic mechanism in different livers, but not the total extent of damage, which is greater but which cannot be scored as dependably in material of this kind (46).

RESULTS

X-radiation followed immediately by partial hepatectomy.—Groups of animals were partially hepatectomized within 1, or at most 3, hours after receiving 600 r. They were killed at 27, 38, and 48 hours postoperatively, C14-labeled orotic acid having been injected 4 hours previously.

Following the injection of labeled orotic acid the specific activity of DNA in normal liver is very low, averaging less than 25 counts/min/mg DNA. As noted above, DNA labeling does not begin to rise to significant levels until 15–18 hours after partial hepatectomy. The control curve in Chart 1 shows the expected high levels of orotic acid incorporation in the 27- and 38-hour groups, followed by a sharp decline at 48 hours. Because the points were far apart the exact time at which the peak level of activity was reached is not known. In the irradiated livers there was a delay in initiating DNA synthesis, and hence incorporation was still low at 27 hours. By 38 hours the lag had been at least partially overcome, and the activity was up to 50 per cent of the control...
level. The decline in the experimental curve appeared to be more gradual than in the control.

Mitotic activity was suppressed to a far greater degree than DNA synthesis by x-radiation; although the controls approached a peak at 27 hours, the irradiated livers showed no detectable rise for at least an additional 11 hours (Chart 2). By 48 hours, however, they exhibited at least partial recovery. The small rise in the control curve at 48 hours probably reflects the diurnal rhythm previously noted by other investigators (4, 27).

The above results demonstrated maximum difference between control and irradiated groups at 27 hours after partial hepatectomy, and, accordingly, in all subsequent experiments, animals were killed at this time.

No obvious morphological changes characterized the irradiated livers that had been regenerating for 27 or 38 hours other than the failure of mitoses to develop. In the 48-hour group, which did exhibit an increase in mitotic activity, abnormal figures were detectable similar to those described below. Also at this time giant interphase nuclei were first seen in appreciable numbers (see below).

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**X-radiation followed by partial hepatectomy at varying time intervals.**—In these experiments the interval between irradiation and partial hepatectomy was varied, whereas the survival time after hepatectomy (i.e., the duration of regeneration) was kept constant at 27 hours. As demonstrated in Table 1, irradiation with 600 r 6 days prior to partial hepatectomy resulted in a highly significant depression of DNA synthesis, although it was somewhat less pronounced than in animals irradiated and hepatectomized immediately. Mitotic activity exhibited a similar pattern; when 6 days elapsed between irradiation and operation, the mitotic index was still extremely low, but showed a definite trend toward recovery (Chart 3, 600 r group).

Animals begin to manifest deleterious effects from irradiation with 600 r shortly after treatment. It is therefore possible that the persisting depression in DNA synthesis noted above might be due to general debility and starvation (31). Accordingly, a smaller dose (365 r) was administered to rats that were studied for longer postirradiation intervals. Rats receiving this dose continued to grow and gain weight (although at a slower rate than the controls). Since the results in this group (see below) agreed with the previous ones, inanition appears to be ruled out as the basic source of the depression.
In the 365-r group mitotic activity and incorporation of orotic acid into DNA were both dose-dependent; the initial depression with 365 r was significantly less than with 600 r (Chart 3 and Table 1, 365 r, 0 day group). As before, DNA labeling was less severely depressed than mitotic activity.

As in the 600-r group, the livers of animals given 365 r showed signs of gradual recovery. By 28 days after irradiation the difference between 59 days after irradiation (Table 2). The various kinds of abnormal figures seen were the same as those previously described in rat (9) and mouse liver (1). Their morphological characteristics (width of bridges, disruption of metaphases) did not change with time—an indication of persistence of the initial degree of damage.

**X-radiation (600 r) followed by one or two partial hepatectomies.**—Rats were irradiated with 600 r and partially hepatectomized within several hours.

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**TABLE 1**

<table>
<thead>
<tr>
<th>TIME IRRADIATION TO HEPATECTOMY (days)</th>
<th>SPECIFIC ACTIVITY OF DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Irradiated</td>
</tr>
<tr>
<td></td>
<td>Dose (r)</td>
</tr>
<tr>
<td>0</td>
<td>600</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
</tr>
<tr>
<td>0</td>
<td>365</td>
</tr>
<tr>
<td>28</td>
<td>&quot;</td>
</tr>
<tr>
<td>48</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

* All rats were sacrificed 27 hours after partial hepatectomy, 4 hours after injection of labeled orotate. Specific activity is expressed as counts/min/mg of DNA ± standard error of the mean.† This value for P was obtained when the 28- and 48-day groups were combined.

Specific activities of DNA in irradiated and control livers was of doubtful significance (Table 1). There was considerable variation among groups, but when the 24- and 48-day groups were combined to provide a larger sample for analysis, the difference was significant at less than the 5 per cent level, showing that recovery is a long drawn out process (Table 1). The mitotic data presented in Chart 3 point even more clearly to the same conclusion. By 48 and 59 days after irradiation, the 27-hour regenerating livers of irradiated rats exhibited levels of mitotic activity similar to those of the controls. The decline in the control curve in Chart 3 and also the decrease in DNA specific activity with time in the controls in Table 1 are presumably attributable to the increasing age of the rats (35).

There were no visible changes in the livers of intact irradiated rats during the 59-day period of observation. However, in 27-hour regenerating livers at all intervals up to 59 days after irradiation, a high incidence of mitotic abnormalities appeared. The number of anaphase and telophase bridges did not decrease significantly even by 59 days after irradiation (Table 2). The various kinds of abnormal figures seen were the same as those previously described in rat (9) and mouse liver (1). Their morphological characteristics (width of bridges, disruption of metaphases) did not change with time—an indication of persistence of the initial degree of damage.

**CHART 3.**—Effect upon mitotic activity of 600 r and 365 r total-body x-radiation, followed at varying intervals by partial hepatectomy. All animals were killed when livers had been regenerating for 27 hours.

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A second partial hepatectomy was performed on one group 4 days and on another group 5 days later. In both groups animals were killed 27 hours after the second hepatectomy, C¹⁴-labeled orotic acid having been injected 4 hours previously. As no significant differences were observed in the results of the two groups, the data were combined. Controls were treated in the same manner, but received no irradiation. The results are shown in Table 3, in which irradiated and control animals treated similarly but hepatectomized only once are included for comparison. In the once-hepatectomized rats, as noted above, 600 r resulted in a pronounced depression of both DNA synthesis and mitotic index. In contrast, in the twice-hepatectomized animals the depression attribut-

### Table 2

**Occurrence of Chromosome Bridges in Livers Induced To Regenerate at Varying Intervals After X-Radiation**

<table>
<thead>
<tr>
<th>Time Irradiation to Hepatectomy (Days)</th>
<th>Chromosome Bridges†</th>
<th>Controls‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (r)</td>
<td>No. rats</td>
<td>Av. per cent</td>
</tr>
<tr>
<td>0 §</td>
<td>600</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>20 (15-30)‡</td>
</tr>
<tr>
<td>0</td>
<td>365</td>
<td>5</td>
</tr>
<tr>
<td>28</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>48</td>
<td>5</td>
<td>10 (6-16)</td>
</tr>
<tr>
<td>59</td>
<td>8</td>
<td>10 (6-14)</td>
</tr>
</tbody>
</table>

*See Table 1, footnote *. †Expressed as per cent of all anaphases and telophases showing bridges. ‡The same rats served as controls for all groups. The incidence of chromosome bridges is very low in nonirradiated livers. §No value is given for the 0 day 600-r group because the mitotic index was so low that reliable evaluation was not feasible. ‡Range in parentheses.

### Table 3

**Effect of X-Radiation Followed by One or Two Partial Hepatectomies**

<table>
<thead>
<tr>
<th>No. of Partial Hepatectomies</th>
<th>Specific activity of DNA</th>
<th>Chromosome Bridges†</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. rats Av. sp. act.</td>
<td>No. rats Av. sp. act.</td>
<td>No. rats Av. per cent</td>
</tr>
<tr>
<td>Irradiated</td>
<td>Control</td>
<td>P</td>
</tr>
<tr>
<td>Irradiated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>7</td>
<td>1395 ± 335</td>
</tr>
<tr>
<td>Two</td>
<td>5</td>
<td>1395 ± 335</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mitotic Index</th>
<th>No. rats Av. per cent</th>
<th>No. rats Av. per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>7</td>
<td>1.4 ± .53</td>
</tr>
<tr>
<td>Two</td>
<td>6</td>
<td>1.7 ± .53</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of Partial Hepatectomies</th>
<th>No. rats Av. per cent</th>
<th>No. rats Av. per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>7</td>
<td>1.4 ± .53</td>
</tr>
<tr>
<td>Two</td>
<td>6</td>
<td>1.7 ± .53</td>
</tr>
</tbody>
</table>

*See Table 1, footnote *. The first partial hepatectomy was performed immediately after exposure to 600 r, the second 4 or 5 days later. †See Table 2, footnote †. ‡This group of animals also appears in Tables 1 and 2. §Range in parentheses.
able to x-radiation was eliminated; the values for DNA labeling and mitotic activities in the irradiated and control rats were not significantly different. (The low response of the controls to the second hepatectomy as compared to the first was attributable to the weaker regenerative stimulus—i.e., the smaller percentage of total liver excised.) It should be noted that at the time of the second operation—i.e., in the livers that were regenerating for 4 or 5 days—the average mitotic index in the irradiated animals was 0.16 per cent. Hence the tenfold increase resulting from the second hepatectomy is indicative of a real rise and not persistence of an already elevated rate.

The development of morphological changes in these livers can best be described by reviewing the findings in the previous groups. X-radiation produced definite changes in microscopic appearance of livers only after they were induced to regenerate. These changes became more obvious as regeneration progressed. Although livers regenerating for 27 or 38 hours after irradiation with 600 r showed little except the absence of mitoses, by 48 hours they contained occasional giant interphase nuclei. Compared with the normal (Fig. 1), after 4 days of regeneration, the disparity in nuclear sizes was pronounced (Figs. 2 and 4); giant nuclei were more frequent, and abnormally small nuclei were also in evidence. Binucleate cells were far more numerous than in the controls (Figs. 3 and 4); random scoring of ten microscopic fields in 4- to 5-day regenerating livers yielded 9 per cent (range 6–11) binucleate cells in six irradiated livers compared with 1 per cent (range 0–2) in four control livers. Since the true population of binucleate cells in liver is approximately 3 times higher than the values determined by counting tissue sections (42), the percentages obtained above should be corrected to 27 per cent and 3 per cent, respectively, emphasizing the extent of the radiation effect.

The control livers after the second hepatectomy resembled the controls regenerating for the first time, except that the lobular patterns seemed sometimes more irregular (Fig. 5). In the irradiated animals the second operation served to exaggerate many of the effects already noted. Interphase nuclei showed even more variation in size and bizarreness in shapes than seen at 4 days after the first hepatectomy (Figs. 6, 7, and 8). Although the number of anaphase–telophase bridges was not significantly higher than after a single hepatectomy, other kinds of mitotic aberrations and polyplaid figures were far more prominent (Fig. 6). The percentage of binucleate cells remained approximately the same as after a single hepatectomy: 9 per cent (6–10) in the irradiated and 1 per cent (0–1) in the controls, uncorrected for the geometry of sectioning. Thus the recovery of capacity to initiate both mitosis and DNA synthesis induced by the second hepatectomy was not paralleled by the morphological findings which, as in the long-term groups, again provided ample evidence of persisting damage.

**DISCUSSION**

Although the majority of studies have dealt with the effects of x-radiation administered while the liver was regenerating, ancillary observations of Holmes (22) and Beltz et al. (6) have shown that treatment within a few hours before partial hepatectomy is as effective as within a few hours afterwards. Our data are in substantial agreement with these findings.

The signals for activating DNA synthesis and mitosis appear to be separable (23, 25, 30). In our experiments, the over-all patterns of response of these two processes to x-radiation were similar, however. When irradiation was administered to intact animals in which nearly 100 per cent of hepatic cells could be assumed to be in interphase, and livers were induced to regenerate immediately afterwards, the onset of both DNA synthesis and mitosis was delayed. The delaying effect on DNA formation, although definite, was less obvious (Chart 1) than in the experiments carried out at earlier time intervals by Holmes et al. (22). The effect on mitosis, which follows DNA synthesis and is more radiosensitive, was pronounced (Chart 2). In a matter of hours some degree of recovery took place, and both processes eventually showed increased activation (Charts 1 and 2). The maximal deficiency induced by irradiation was found in the group of animals examined at 27 hours after partial hepatectomy. In livers not stimulated by partial hepatectomy the impaired capacity to initiate DNA synthesis and mitosis persisted, so that even when several weeks elapsed between irradiation and operation, livers regenerating for 27 hours still exhibited a residual depression of these two functions. The mitotic lag gradually lessened over approximately the same period of time as the DNA lag. In the former case recovery appeared complete by 48 days, whereas in the latter the final degree of recovery was somewhat equivocal; the methods of measurement were not sufficiently sensitive to determine whether recovery of the ability to initiate DNA synthesis was partial or complete at that time; but it is clear that a considerable degree of repair had occurred.

In animals subjected to two partial hepatectomies 4–5 days apart, restoration of competence...
to activate DNA synthesis and mitosis was accelerated; i.e., in rats irradiated but hepatectomized only once these two functions remained depressed, while in the doubly hepatectomized animals they approximated the levels in the doubly hepatectomized controls. Possibly selective multiplication of less damaged cells might result in an altered cell population that could account for this apparent recovery, but more probably the recovery is attributable to an overcoming of the lag during the response to the first regenerative stimulus (see below).

Examples of similar forms of recovery may be cited. Henshaw found that when arbusca eggs are irradiated prior to fertilization there is a delay in the first cleavage, whereas if an interval elapses before fertilization there is no delay (19). Baldwin, studying x-ray burns in cells of insect epidermis, reported that the delaying effects of x-radiation on mitosis gradually disappeared if the cells were maintained in a resting phase for some weeks before they were induced to divide (3). Holmes has mentioned that irradiation to the liver region at 12 hours prior to partial hepatectomy caused less of a delay in mitosis and DNA labeling than irradiation immediately before the operation (28), an observation suggesting a degree of recovery in accord with our findings. On the other hand, Leong et al. (34) have very recently reported performing repetitive partial hepatectomies at 8, 11, and 14 weeks after two doses of 400 r each to the whole body. These authors found the mitotic index to be consistently 50-60 per cent below nonirradiated controls, although the mass of restored liver was not significantly less. The failure of the mitotic function to evidence recovery in these experiments is puzzling but might be attributable to the larger doses of radiation employed and the longer intervals between operation and killing the animal.

Histological examination of our material indicated that recovery was somewhat less complete than implied by determinations of DNA synthesis and mitotic activity. During the time the two latter processes were returning toward normal the number of anaphase-telophase bridges did not decline significantly, still averaging 10 per cent by 2 months after irradiation with 365 r. Because of losses due to the planes of the section and its thinness (5 μ) the actual number would approximate several times this value. Thus a kind of damage to the cellular reproductive mechanism was revealed that was extensive and far more enduring than the ability to enter mitosis. The tendency to form bridges has been reported to persist undiminished for as long as 8-9 months following irradiation in mouse liver stimulated to proliferate at the end of that time by injection of carbon tetrachloride (1). In our experiments the number of bridges was not significantly altered by the second partial hepatectomy, and in the series of Leong et al. (34) 75-85 per cent of all mitoses were found to be aberrant and to persist at the same high level even after three partial hepatectomies. Brues has reported that a second partial hepatectomy in itself leads to no change in mitotic abnormalities in either control or irradiated livers (9). The microscopic appearance of irradiated livers regenerating for the second time showed persistence of a severe degree of damage reflected not only in the high number of abnormal mitotic figures, but also in the distorted appearance of interphase nuclei, the frequent occurrence of wide-angle spindles and giant nuclei indicative of polyploidy, and the high incidence of binucleate cells which were 9 times more abundant in irradiated livers than in controls.

Although many investigators have found that the incorporation of labeled precursors into DNA is depressed by x-radiation, there is not universal agreement on this point. The diversity of biological materials examined, the variety of dose ranges, time intervals, and other experimental conditions employed have led to seemingly conflicting interpretations. A change in cell population (24, 30), an arrest of mitosis with no effect on DNA synthesis per se, a direct effect on biosynthetic mechanisms, or a combination of factors, have all been implicated. In regenerating liver, during the early stages at least, changes in cell population through either cell death or mitotic arrest appear to be ruled out (24): microscopic evidence of a significant degree of cellular degeneration is lacking, RNA synthesis is unaffected, and DNA synthesis which precedes mitosis in a synchronized population of cells of this kind is measured in the first generations of dividing cells. Further, the persistence of chromosome bridges in undiminished numbers argues against a change in cell population as the basis for recovery of ability to initiate mitosis and DNA synthesis in the twice-hepatectomized irradiated animals.

As regards a direct effect on biosynthetic mechanisms in the liver, recent studies (5, 7, 28, 38, 44) have shown that, when rats are irradiated during the early stages of regeneration, their livers fail to show the increase in activity of certain enzymes mediating the incorporation of labeled thymidine into DNA that normally occurs during the first 14 hours after partial hepatectomy. As regeneration proceeds and these enzymes have reached
high levels of activity, but still before DNA synthesis has begun, x-radiation can nevertheless depress subsequent DNA labeling. Presumably it then acts on the initiating mechanism in some other way, since the enzymes themselves are not inhibited. In addition, it has been suggested that damage to the DNA template may occur (7, 38, 39).

The increasing body of evidence that x-radiation in moderate doses has no effect upon DNA synthesis per se (12, 14, 30, 40) has largely been obtained from tissues in a phase of active growth. In such cells it is probable that the organizing of a DNA-synthesizing system—whether through enzyme formation, activation or stabilization (20), or some ancillary process—has already taken place. On the other hand, in adult liver, in which cell division is normally rare, prior development of mitotic machinery seems a likely prerequisite for regeneration. Beltz et al. (6) found that, although DNA synthesis was totally blocked by irradiation during the early sensitive period of regeneration, 100 per cent inhibition could not be achieved if irradiation were administered at the time when the number of cells engaged in synthesis was maximal. This result was interpreted to mean that there is a radiation-insensitive period during the time that DNA synthesis is actually in progress.

There is general agreement that the inception of mitosis is delayed by x-radiation but that, once under way, as in the case of DNA synthesis, its further progress is not arrested by such treatment. In regenerating liver, as in other tissues, mitosis is initiated separately from DNA synthesis; Cater et al. have shown that cell division is delayed by x-radiation even when administered after the synthesis of DNA for the ensuing mitosis has already been completed (13). There is little information about the biochemical nature of this phenomenon (43).

Thus, it appears likely that, although moderate doses of x-radiation damage multiple facets of the hepatic cellular reproductive function, DNA synthesis per se and the carrying through of mitosis to completion are relatively unaffected, and the injuries lie, rather, within the mechanisms that initiate these processes. These are subject to gradual repair, whereas other functions, as evidenced by persisting chromosome bridges and certain structural anomalies, seem to be more permanently damaged. Further investigations may disclose whether or not the transient forms of injury may reflect temporary interference in the formation or activation of certain enzymes, and whether the more enduring effects may stem from alterations in the DNA template.

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The purpose of this paper was to study the primary growth of leukemic cells. The relatively slight difference between these two series for mice with AK4 leukemia early massive blood-borne invasion of organs. For the transition between various strains of leukemias compared to those bearing scantier local growths suggests that metastatic infiltrative growth of all leukemic strains the difference in the amount of tissue either at the site of inoculation or from peritoneal exudate. It appears from the results on leukemias reported above and those on other tumors with regard to their two tendencies to spread extensively into organs is paralleled by their trend to grow as free cells in body fluids, to be separated from the tissue, and to infiltrate into organs. Thus, primary localization of malignant cells is based on their interdependence, while the ability to grow as free cells in the blood, and to infiltrate into organs is a specific characteristic of malignant cells due to their reduced adhesiveness. The mortality assay of leukemic strains showed the shortest survival periods in the intraperitoneal series and the longest in the flank series. It was found with the help of organ assay their primary growth in the peritoneal exudate. This was attributed to their autonomy and, perhaps, more precisely to their reduced adhesiveness. The role of implanted cells into abdominal organs from subcutaneous growth. This was attributed to the role of primary growth as the direct lethal factor and--indirectly and directly--in the death of the host. This factor is least significant for leukemias with a high degree of cell autonomy and nonleukemic tumors. However, shorter survival suggests that for this strain the role of implantation from peritoneal exudate was overshadowed by the role of primary growth as free cells in the blood, and to infiltrate into organs by blood route at the expense of the primary growth and--indirectly and directly--in the death of the host. This factor is least significant for leukemias with a high degree of cell autonomy and nonleukemic tumors. However, shorter survival of animals with large tumor growths in the scalp as opposed to those bearing scantier local growths. It may be presumed that tissue growth 'from benign and malignant cells is based on their interdependence, while the ability to grow as free cells in body fluids, to be separated from the tissue, and to infiltrate into organs is a specific characteristic of malignant cells due to their reduced adhesiveness.

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Fig. 5.—Control. 27-hour regenerating liver following a second hepatectomy. Many normal mitotic figures and interphase nuclei. X450.

Fig. 6.—Irradiated. 27-hour regenerating liver following a second hepatectomy. Giant interphase nuclei and polyploid mitotic figures. X450.

Fig. 7.—Irradiated. 27-hour regenerating liver following a second hepatectomy. Elongated, imperfectly reconstituted nuclei. X450.

Fig. 8.—Irradiated. 27-hour regenerating liver following a second hepatectomy. Giant bizarre-shaped interphase nuclei and abnormal telophase with chromosome bridge. X450.
The purpose of this paper was to study the primary growth of leukemic cells. The relatively slight difference between these two series for mice with AK4 leukemia early massive blood-borne invasion of organs. For peritoneal exudate was overshadowed by comparison from peritoneal exudate. It appears from the results on opposite tendencies to spread extensively into and nonleukemic tumors with regard to their two primary growth and to be implanted into connective organs by blood route at the expense of the primary growth earlier and to a greater extent than grafts that organs were invaded from intraperitoneal growth. This was attributed to permeable growth and to be implanted into connective organs by blood route at the expense of the primary growth earlier and to a greater extent than grafts that organs were invaded from intraperitoneal growth earlier and to a greater extent than grafts that organs were invaded from intraperitoneal growth. The purpose of this paper was to study the primary growth of leukemic cells.
Latent Injury and Repair in Rat Liver Induced To Regenerate at Intervals after X-Radiation

Mary D. Albert and Nancy L. R. Bucher


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