The factors controlling the initiation, maintenance, and cessation of hepatic cell mitosis in response to partial hepatectomy are not completely understood. Evidence offered by parabiont studies favors considering the presence of a humoral factor (7, 10, 16).

The present study demonstrates the effect of the postulated humoral factor upon tumor cells present in the liver during the 12-day period following partial hepatectomy, as well as the effect of the tumor cell presence upon the hepatic cell proliferation following partial hepatectomy.

MATERIALS AND METHODS

Strong A mice weighing from 20 to 28 gm were employed. The animals were housed in wooden cages, in a controlled temperature and environment (21-24°C), and were given Purina Lab Chow and water ad libitum. No attempt was made to regulate light and dark hours.

Ten animal groups were selected and submitted to the following procedures: 1, no treatment; 2, partial hepatectomy; 3, intrasplenic saline injection and sham hepatectomy; 4, intrasplenic saline injection and partial hepatectomy; 5, intrasplenic liver homogenate injection and sham hepatectomy; 6, intrasplenic liver homogenate injection and partial hepatectomy; 7, intrasplenic reticulum cell sarcoma injection and sham hepatectomy; 8, intrasplenic reticulum cell sarcoma injection and partial hepatectomy; 9, intrasplenic Ehrlich ascites tumor injection and sham hepatectomy; 10, intrasplenic Ehrlich ascites tumor injection and partial hepatectomy.

All groups, except the nontreated controls, were further subdivided into 12 subgroups of 8-10 members each. These subgroups were sacrificed on the 12 consecutive days following sham or partial hepatectomy.

Intrasplenic Injections

The method of intrasplenic injection offered a means of delivering the tumor cells and control inoculi to the liver sinusoids. It was found that the portal vein of the mouse did not offer a suitable entry route due to its size and anatomic position. Cells of the inoculum were observed in the hepatic sinusoids seconds after the intrasplenic injection was performed.

Four types of intrasplenic inoculations were employed, 2 different tumor cell suspensions, macerated liver, and sterile
Surgical Procedure

Entry into the peritoneal cavity was accomplished by a longitudinal incision through skin and body wall, ventromedial to the thoracoepigastic vessels. The spleen and the attached portion of the greater omentum were delivered and directed in a cephalolateral position with the medial surface of the spleen uppermost and lying on a small square of sterile gauze moistened with sterile physiologic saline and maintained in an ice bath until inoculation. The resulting suspension consisted of cell debris, individual cells, and cell clusters containing as many as 20 cells per group. Cell counts were difficult to make, but quantitative attempts were made to approximate the population size of the two tumor inoculi.

The fourth inoculum was sterile physiologic saline.

RESULTS

1. No Treatment

Mitotic figures were not common among hepatic parenchymal cells of nontreated control animals (0.4 cells/1000 ± 0.28).

2. Partial Hepatectomy

An elevation of mitotic rate occurred on the second day following partial hepatectomy. A peak of hepatic parenchymal cell mitosis was reached at the third day, followed by a return to near control levels by Day 4 (Chart 1).

3. Intrasplenic Saline Injection

Significant differences did not exist between the hepatocyte mitotic indices of partially hepatectomized and saline-injected partially hepatectomized animals (Chart 1). Comparable agreement existed between nontreated controls and saline-injected, sham-hepatectomized animals.
4. Intrasplenic Liver Homogenate Injection

Injection of liver homogenate did not constitute a graft of liver tissue. One day after injection, remains of the inoculum were observed in smaller divisions of the portal vein and in the hepatic sinusoids. Cytoplasmic material was strongly eosinophilic. Nuclear components were distinguishable as vague outlines which were not discernible by the third day. Leukocytes were present in these depositions; their number increased with time. By the sixth day no trace of the injected material existed, although leukocyte aggregations still were present.

Liver homogenate injection did not alter the mitotic response to partial hepatectomy during the first 6 days (Chart 2). Sham-hepatectomized mice, receiving this inoculum, demonstrated mitotic activity comparable to nontreated controls during this same period (1.0 cells/1000). However, elevations of mitotic activity occurred in each of the liver homogenate groups during the second half of the experimental period. A peak mitotic index was noted 5 days after hepatectomy (Chart 2). A lesser mitotic peak occurred at this time in animals subjected to sham hepatectomy (Chart 2).

5. Hepatic Parenchymal Cell Mitotic Frequency in Tumor-injected Animals

Difficulty was not encountered in distinguishing tumor cells from hepatic parenchymal cells. The higher nuclear to cytoplasmic ratio and cytoplasmic basophilia of the tumor cells made this classification possible.

Peak hepatic cell mitosis did not occur at Day 3 in partially hepatectomized, tumor-injected animals (Charts 3, 4). Instead, a peak in hepatic parenchymal mitosis was found in the sixth postoperative day in both tumor-injected groups. Following attainment of these peaks, the mitotic activity remained elevated and did not return towards control level, as was observed in animals subjected to partial hepatectomy alone.

Slightly elevated hepatic parenchymal cell mitotic activity occurred in sham hepatectomized tumor-injected animals, from Days 7–12 in groups bearing reticulum cell sarcoma and from Days 6–12 in groups bearing Ehrlich ascites tumor (Charts 3, 4).

6. Tumor Cell Mitotic Frequency

Some difficulty was encountered in distinguishing between the reticulum cell sarcoma population and Kupfer cells. This problem was met most frequently in the Day 1 and 2 groups (i.e., second and third days post-tumor injection), when the cells were found either singly or in small groups throughout the hepatic sinusoids. A distinction was made on the basis of cell size, nucleolar size (in the case of intermitotic nuclei), and relation of the cell to the sinusoid epithelium. The distinction was found to be facilitated as the tumor cell population size increased (Fig. 1). No problem was met in distinguishing Ehrlich tumor cells from host cells (Fig. 2).

Reticulum cell sarcoma and Ehrlich ascites tumor mitotic rates were consistently higher in partially hepatectomized than in sham-hepatectomized animals (Charts 5, 6). In each case, the differences were more pronounced during the first half of the experimental period.

![Chart 1](chart1.png)

Chart 1. Hepatic cell mitotic indices following saline injection and sham or partial hepatectomy. Vertical bars indicate the 95% confidence limits of the mean.
Chart 2. Hepatic cell mitotic indices following liver homogenate injection and sham or partial hepatectomy. Vertical bars indicate the 95% confidence limits of the mean.

Chart 3. Hepatic cell mitotic indices following reticulum cell sarcoma injection and sham or partial hepatectomy. Vertical bars indicate the 95% confidence limits of the mean.
Chart 4. Hepatic cell mitotic indices following Ehrlich tumor injection and sham or partial hepatectomy. Vertical bars indicate the 95% confidence limits of the mean.

Chart 5. Mitotic indices of reticulum cell sarcoma cells residing in the liver following sham or partial hepatectomy. Vertical bars indicate the 95% confidence limits of the mean.
No Treatment, Sham, and Partial Hepatectomy

Hepatic parenchymal cell mitotic activity is rare in normal adult rodent liver. Mitosis was not common in the present study (0.4 ± 0.3 cells/1000). Similar rates were reported (5) in rats. In the present study mitotic rates within the control range were found in sham-hepatectomized, saline-injected animals. On the other hand, the extirpation of a portion of the liver stimulated a marked proliferation among the remaining parenchymal cells. A peak mitotic index of 29.6 cells/1000 was observed in our work 3 days after removal of 30% of the liver (Chart 1). Proportionately higher peak indices were reported (31) when 70% hepatectomy was performed upon mice of the same strain. Similar observations have been made employing rats. Bucher and Swaffield (8) postulated the existence of a threshold phenomenon. A minimal mitotic response occurred when less than 30% of the liver was removed, but when 30% or more was removed, a marked increase was observed proportionate to the amounts of liver removed. If a similar situation exists in mice, the degree of hepatic insufficiency evoked in the present study may be closed to threshold levels.

Intrasplenic Liver Homogenate

Mitosis has been observed in adult liver parenchyma following procedures other than partial hepatectomy. It is known that injection of certain organ homogenates will be associated with hyperplasia in the homologous organ of the recipient (1, 24-26, 28). These studies employed epidermis, outer orbital gland, gut mucosa, mesonephros, and thymus. Injection of liver homogenate in the present study and in earlier reports (4, 18, 20, 27, 30) has been accompanied by similar increases in mitotic frequency. In the experiment described in this paper, the intrasplenic injection of liver homogenate into sham-hepatectomized animals was followed 8-9 days later by elevated parenchymal cell mitosis (Chart 2). Evidence that this hyperplasia was not brought about through mechanical stimulation is offered from the data obtained from sham-hepatectomized animals receiving intrasplenic injections of saline (Chart 1). It is significant that a more elevated, but similarly timed peak in hepatic cell mitosis occurred during the same time period among partially hepatectomized animals receiving liver homogenate (Chart 2). This peak was preceded by another, occurring at Day 3, which was undoubtedly related to hepatic insufficiency (Chart 3). The second, 8-9-day peak was in some way related to the injection of liver homogenate. It therefore appears as if a two-stimulus sequence existed, i.e., liver extirpation and liver homogenate injection.

Intrasplenic Tumor Injection

Presence of tumor is still another stimulus to hepatic cell mitosis. Hyperplasia was noted from Day 7-12 in sham-hepatectomized animals injected with reticulum cell sarcoma, and from Day 6-12 in similarly treated animals previously injected with Ehrlich ascites tumor. These findings are in agreement with earlier reports of hepatic cell mitotic activity in tumor-injected animals (2, 3, 17, 19). Baserga and Kisielieski (8) noted greater mitotic index elevations in the littoral cell

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**DISCUSSION**

No Treatment, Sham, and Partial Hepatectomy

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**Chart 6. Mitotic indices of Ehrlich ascites tumor cells residing in the liver following sham or partial hepatectomy. Vertical bars indicate the 95% confidence limits of the mean.**
population than among hepatic parenchymal cells. There were no indications of this nature in the present study.

Three observations were made in regard to mitotic rates in tumor-injected, partially hepatectomized mice: (a) a delay in the appearance of hepatic parenchymal mitosis, (b) an elevation in tumor cell mitotic rate, (c) a prolongation of hepatic parenchymal cell hyperplasia.

The results associated with the first observation were undoubtedly related to the presence of the tumor in the liver (Charts 3, 4). Liver homogenate injection prior to hepatectomy did not evoke a similar response (Chart 2). The delay of hepatic parenchymal mitosis may have been due to a general homeostatic mechanism involving the liver parenchyma to such an extent that mitosis was precluded for a short period following tumor administration. Further work is necessary to establish whether or not a situation peculiar to neoplastic tissue is responsible for the delay in onset of hepatocyte mitosis.

The second observation suggests the presence of a mitotic stimulatory factor. Reports exist in the literature which indicate the presence of such a substance following partial hepatectomy (6, 7, 10, 16). Neither the molecular species nor its mechanism of action has been determined. Several authors have doubted its existence (6). In the present study the tumor cell mitotic peak in each of the two groups of partially hepatectomized animals was at Day 3 (Charts 5, 6). It should be noted that this is the day that peak hepatic parenchymal cell mitosis should be expected (Chart 1). If a mitotic stimulatory factor is responsible for the compensatory hyperplasia following hepatectomy, it is logical to assume that it would be present at peak levels during this period. Increased tumor take (11, 13), tumor growth (21, 29), and tumor induction (14) have also been reported following partial hepatectomy. Paschkis et al. (22) found hyperplasia and hypertrophy of non-hepatic normal tissue following partial hepatectomy. These findings and the findings of the present study support the mitotic stimulatory factor theory, as well as suggest that its action is not limited to hepatic parenchymal cells.

The third observation presents an interesting problem. In systems such as regenerating liver, which demonstrate a systematic, regulated sequence, a negative feedback type of control is indicated. If this is true, production of the growth-promoting factor should cease when hepatic cell number is restored. This does not occur in mice bearing one of the 2 tumors. Instead, a sustained period of elevated hepatocyte mitosis follows the attainment of the 6-day mitotic peak. Somehow, the presence of tumor has disrupted the usual, well-controlled mitotic response. A possible explanation of the prolongation is offered by the mitotic index data obtained from the 2 groups of sham-hepatectomized tumor-injected animals. Mitosis occurred among hepatic parenchymal cells from Day 7 to 12 in each of these groups, thereby suggesting a tumor-evoked stimulus to mitosis. If such a stimulus were exerted upon a parenchyma capable of intense proliferation, a more vigorous mitotic response might occur. This, if true, would explain the prolonged elevation of the mitotic rate. Similar etiology may explain the second peak of hepatocyte mitosis observed in partially hepatectomized animals pretreated with liver homogenate. In some way, injection of the liver homogenate causes an elevation of the hepatocyte mitotic rate. When this stimulus is exerted upon a parenchyma not previously geared for proliferation (i.e., following sham hepatectomy), only moderate hyperplasia is noted. However, if the metabolic pathways have been preconditioned for anabolism, enhancement of the mitotic rate occurs.

The elevation of tumor cell mitotic rates following partial hepatectomy offers indirect evidence supporting the existence of a nonspecific, mitosis-promoting factor. Similar evidence has been reported by other workers in regards to both normal and neoplastic tissues. A delay in the production of such a factor is also indicated. Additional experiments are under consideration to further study these hypotheses.

REFERENCES

Fig. 1. Mouse liver with reticulum cell sarcoma deposited in the liver after intrasplenic injection. The animal was subjected to partial hepatectomy one day after tumor injection and was sacrificed six days following hepatectomy. H & E, × 1000.

Fig. 2. Mouse liver with Ehrlich tumor cells deposited in the liver after intrasplenic injection. The animal was subjected to partial hepatectomy one day after tumor injection and was sacrificed six days following hepatectomy. Arrows indicate mitoses of hepatic parenchymal cells. H & E, × 400.
Alteration of Tumor Cell and Hepatic Parenchymal Cell Mitotic Rates in Tumor-injected Partially Hepatectomized Mice

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