Partial hepatectomy initiates a complex pattern of cellular changes in the parenchymal cells of the residual liver. The sudden shift of the cell population from normal functional biosyntheses toward syntheses in preparation for cell division provides an ideal system for the study of various aspects of cell metabolism. The morphological and biochemical changes associated with liver restoration have been described and reviewed by many investigators (1, 14, 22, 26, 37). The earliest changes noted are the conspicuous nucleoli and a cytoplasm rich in ribonucleic acid, a pattern typical of cells engaged in increased or altered protein synthesis. It is now generally accepted that nucleic acids are intimately involved in cellular growth and biosyntheses. Ribonucleic acid (RNA) changes are correlated with the variations in the rate of protein synthesis, while deoxyribonucleic acid (DNA) is associated with mitotic activity (reviews, 5, 43, 47).

Previous studies have shown that thioacetamide (CH₃CSNH₂) induces cellular changes in rat liver similar in some respects to those occurring after partial hepatectomy (29). The most striking effect is the increase in nuclear ribonucleoprotein (29, 31). The nucleoli increase to gigantic proportions, and nuclear RNA synthesis is greatly accelerated. Drug treatment by injection (31) or feeding for 10 weeks (20) does not impair regeneration after partial hepatectomy. It was felt that thioacetamide (TA)-treated animals would provide a useful system for studying the effect of increased nuclear RNA on growing and dividing cells. TA-treated rats were subjected to partial hepatectomy and some of the general aspects of restorative growth analyzed, i.e., rate of regeneration, volume changes, DNA synthesis, mitotic rates, cytoplasmic basophilia, and polyploidy. The following questions were considered during the course of this study: Will the TA-induced nucleoprotein be utilized, or will there be a period of reorganization comparable to that of the controls before restoration proceeds? Will the presence of large amounts of nucleolar RNA interfere with DNA synthesis or mitosis? Will the distribution of cytoplasmic ribonucleoprotein be altered? The results indicate that the TA-induced ribonucleoproteins do not interfere with the restorative process. The cellular reorganization characteristic of the early period of regeneration occurs concomitantly with the TA-induced alterations.

MATERIALS AND METHODS

Male Wistar rats weighing about 200-220 gm. were given daily subcutaneous injections of thioacetamide as a 1 per cent solution in 0.85 per cent NaCl, at a dose level of 5 mg/100 gm body weight for 7 days. Partial hepatectomy was performed whereby 70 per cent of the liver was removed (26). In twelve normal rats sacrificed immediately after partial hepatectomy (9). The necrotic lobes weighed 69.6 ± S.D. 2.3 as per cent of the total liver. In two subsequent experiments animals were sacrificed in pairs 9, 18, 20, 23, 26, 31, 48, 96 hours, and 10 days following surgery. The liver remnants removed at autopsy were weighed, and the rate of regeneration was plotted as a percentage of the estimated weight of the original whole liver (7). Daily TA injections were continued during regeneration in one group (Group C). In a second group TA injections were stopped at the time of partial hepatectomy (Group B). Saline-injected, partially hepatectomized rats (Group A) and sham-operated, TA-injected rats served as controls.

Tissues were fixed in 10 per cent neutral formalin and 3:1 alcohol:acetic acid, processed through the isopropyl alcohol-paraffin schedule (11), and sectioned at appropriate thicknesses. Samples of liver were taken at the time of partial hepatectomy and at sacrifice. Animals were fed Animal Foundation Laboratory Diet (Standard Brands, Inc., N.Y.) ad libitum throughout the experiment.

Deoxyribonucleic acid (DNA) determinations.—The relative amount of DNA per nucleus was determined by photometric determinations of Feulgen-stained preparations as previously described (29). Sections to be measured were mounted in oil matched to the refractive index of the cytoplasm (1.564). DNA was estimated by measurement of individual nuclei according to the "two-wavelength" method (34, 35). The photometric apparatus used was that described by Pollister (36).
Volume determinations.—Nuclear and nucleolar volumes were determined by direct measurement of whole nuclei in 20-μ sections of Feulgen-fast green preparations and in 10-μ sections stained with Azure B. Fifty tetraploid nuclei containing a single nucleolus each were measured from each liver sample.

Mitotic rates.—The percentage of cells in mitosis was determined by counting 1,000–4,000 nuclei for each liver sample. Feulgen-stained preparations were used. Random fields of at least three different pieces of tissue were scanned.

Polyploid distribution.—The classification of nuclei into polyploid classes (Class I, diploid; Class II, tetraploid; Class III, octaploid; Class IV, 16-ploid; Class V, 32-ploid) was made by scoring whole Feulgen-stained nuclei within random fields.

RESULTS

Rate of regeneration.—The rate of regeneration in saline-injected, partially hepatectomized control animals (Group A) was similar to that recorded by previous workers (7, 26). The liver remnant increased in weight from 30 per cent of the total liver mass immediately following partial hepatectomy, to 50 per cent at 31 hours, 58 per cent at 48 hours, 73 per cent at 96 hours, and 89 per cent at 10 days (Chart 1).

Animals treated with TA prior to partial hepatectomy, then allowed to recover from the drug during the regeneration period (Group B), showed a slightly faster rate of restoration after the first 48 hours. The liver remnant increased to 80 per cent at 96 hours and 95 per cent at 10 days (Chart 1).

Animals treated with TA 7 days prior to partial hepatectomy and with daily injections continued during the regeneration period (Group C) showed the widest variation in the rate of regeneration. The mean rate was slightly depressed (Chart 1) after the first 96 hours when compared with the controls.

Volume changes.—Changes in nuclear and nucleolar volumes following partial hepatectomy are summarized in Table 1. Nine hours after surgery the mean nuclear volume was increased 36 per cent; at 18 hours, 46 per cent; and at 23 hours, 63 per cent. The initial increase observed at 9 hours was not associated with DNA synthesis. The mean tetraploid nuclear volume increase in 28-hour samples varied from 51 per cent in one animal to 86 per cent in a second animal. Since mitosis started at 24–26 hours, the nuclear population at 28 hours consisted of nuclei preparing for division and nuclei which had just completed the mitotic cycle. Tetraploid nuclei ready to undergo division, i.e., nuclei which had synthesized DNA in the process of chromosomal duplication, could not be distinguished from true interphase octaploid nuclei. Those nuclei which may have increased in volume from 350 cu μ to 600 cu μ (see Chart 3) were not included in the measurements of Table 1. On the other hand, nuclei which divided prior to fixation were included in the measurements. One would expect such nuclei to have smaller volumes temporarily, until the uncoiling of the chromosomes during the reconstruction period was completed. The wide variation in mean nuclear volumes in the two 28-hour samples indirectly reflected the mitotic and synthetic activities of the nuclear population.

Nucleoli showed more striking increases in volume during this early period of restoration. At 9 hours there was a twofold increase. As more nuclei responded the mean increase rose to three- to five-
fold at 23–28 hours. The increase in nucleolar volume appears to be associated at least in part with increased amounts of nucleoprotein (37, 45). The irregularly shaped nucleoli characteristic of regenerating liver and their affinity for making contact with the nuclear membrane (Figs. 1 and 2) may be correlated to changes in the position and physical state of the chromosomes with which they are associated. This may involve a loosening of the netlike organization of the nucleolus as reportedly occurs in liver cells of fasting rats (13). Measurements of the irregular nucleoli in 23- and 28-hour samples were obviously less accurate, being higher than actual.

Dally treatment with TA for 7 days resulted in an 80 per cent increase in the nuclear volume of the tetraploid parenchymal cells and a ninefold increase in nucleolar volume. This was calculated

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Time after partial hepatectomy (hr.)</th>
<th>Nuclear vol.* (cu μ)</th>
<th>Increase (per cent)</th>
<th>Nucleolar vol.* (cu μ)</th>
<th>Increase (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>0</td>
<td>257 ± 5.7</td>
<td>37</td>
<td>6.7 ± 0.296</td>
<td>80</td>
</tr>
<tr>
<td>A2</td>
<td>9</td>
<td>338 ± 8.4</td>
<td>12</td>
<td>12.1 ± 0.514</td>
<td>130</td>
</tr>
<tr>
<td>A3</td>
<td>0</td>
<td>264 ± 5.5</td>
<td>35</td>
<td>6.0 ± 0.350</td>
<td>130</td>
</tr>
<tr>
<td>A4</td>
<td>9</td>
<td>307 ± 8.5</td>
<td>13.8 ± 0.668</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A5</td>
<td>0</td>
<td>244 ± 5.5</td>
<td>6.8 ± 0.301</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td>A6</td>
<td>18</td>
<td>354 ± 8.1</td>
<td>23 ± 0.845</td>
<td>208</td>
<td></td>
</tr>
<tr>
<td>A7</td>
<td>0</td>
<td>246 ± 5.3</td>
<td>7.1 ± 0.298</td>
<td>208</td>
<td></td>
</tr>
<tr>
<td>A8</td>
<td>18</td>
<td>374 ± 9.2</td>
<td>21.8 ± 0.892</td>
<td>208</td>
<td></td>
</tr>
<tr>
<td>TA-treated:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>0</td>
<td>463 ± 15.4</td>
<td>15.0 ± 23</td>
<td>6.8 ± 0.306</td>
<td>280</td>
</tr>
<tr>
<td>B2</td>
<td>9</td>
<td>430 ± 8.5</td>
<td>6.9 ± 0.350</td>
<td>350</td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td>9</td>
<td>427 ± 9.4</td>
<td>29.7 ± 2.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B4</td>
<td>0</td>
<td>235 ± 5.1</td>
<td>6.8 ± 0.546</td>
<td>307</td>
<td></td>
</tr>
<tr>
<td>B5</td>
<td>23</td>
<td>438 ± 11.9</td>
<td>33.8 ± 2.37</td>
<td>307</td>
<td></td>
</tr>
<tr>
<td>B6</td>
<td>0</td>
<td>362 ± 11.0</td>
<td>7.3 ± 0.380</td>
<td>307</td>
<td></td>
</tr>
</tbody>
</table>

* Values represent the mean ± standard error of 50 tetraploid nuclei (Class II) containing a single nucleolus.

† Per cent corrected increase in nuclear volume was calculated as: volume increase/control mean nuclear volume (249 cu μ), thereby subtracting the increase in volume due to thioacetamide.

‡ Per cent corrected increase in nucleolar volume was calculated as volume increase/control mean nucleolar volume (6.8 cu μ).
from the mean 0-hour values tabulated in Table 1. Following partial hepatectomy the nuclear and nucleolar volumes showed additional increases (Table 1, Figs. 3 and 4). The magnitude of these increases was far smaller than that observed for corresponding controls. If, however, the increases in nuclear and nucleolar volumes following partial hepatectomy are calculated as the per cent of the normal mean nuclear (249 cu μ) and nucleolar values of normal adult liver samples (0 hour) fell into distinct classes corresponding to diploid, tetraploid, and octaploid DNA amounts. Intermediate DNA values (values falling between diploid and tetraploid, or tetraploid and octaploid, amounts) represent nuclei in the process of synthesizing DNA. Such intermediate nuclei were found most numerously in 20-, 23-, and 28-hour regenerating livers.

**NUCLEAR DNA**

![Chart 2](chart2.png)

**Chart 2.**—Amounts of DNA (Feulgen) in nuclei of regenerating rat liver from saline-injected control animals, and from animals treated with thioacetamide (TA) prior to partial hepatectomy.

TA-treated rat livers generally tend to have an increased number of nuclei with intermediate DNA values (8, 25, 29). This was reflected in the measurements of sham-operated TA-treated rats as graphed in Chart 2. A sharp decrease in the number of intermediate nuclei was noted in 9-hour regenerating liver samples. Repeat measurements of 6-, 9-, and 12-hour samples confirmed this finding. During the initial 12-hour period following partial hepatectomy there was essentially no DNA synthesis. This was true for both saline control and TA-treated animals.
Intermediate DNA values in the TA-treated rats were most numerous in 20-, 23-, 28-, and 96-hour samples. The percentage of intermediate nuclei was higher in these livers than in corresponding controls. In measuring TA-affected nuclei, selection of the more normal nuclei in the peripheral regions of the lobule was favored. These nuclei were smaller than those lying proximal to the central vein and therefore a higher number of uncut nuclei were encountered in this region in random scanning of sections. To check whether a greater number of nuclei were synthesizing DNA relating to the following questions: (a) Is DNA synthesis initiated only after a particular volume is reached? (b) What proportion of the observed increase in volume is related to the duplication of DNA?

As can be seen in Chart 3, DNA synthesis did not begin until a 40–50 per cent increase in nuclear volume had occurred. This may reflect qualitative chromosomal changes (4) and the uptake of nucleic acid precursors (51) that were found to occur in dividing cells. Some nuclei doubled in volume before synthesis started.

Chart 3.—The relation between amounts of DNA (Feulgen) and nuclear volume in a normal rat from liver samples in the periportal regions as compared with nuclei proximal to the central vein, 25 nuclei were measured in each of two circumscribed areas: (a) immediately surrounding the central vein, and (b) in the periportal region of the lobule. Intermediate nuclei were found in both groups but were more numerous in the periportal regions. There were five intermediate nuclei in the periportal zone in contrast to one in the area immediately proximal to the central vein. Identical regional differences were found in 20-hour samples of control livers.

Nuclear volume and DNA synthesis.—The volume and DNA content of individual nuclei in 0- and 18-hour samples of a saline control rat are shown graphically in Chart 3. It was hoped that such a representation would provide information taken at the time of, and 18 hours following, partial hepatectomy.

The proportion of the nuclear volume change directly associated with DNA duplication could not be determined. Whether a direct increase in volume occurs once DNA synthesis starts, or whether a nucleus already doubled in volume prior to DNA synthesis maintains its volume unchanged during the synthetic process by a shift in chemical components, still remains unanswered.

Mitotic rates.—Immediately after partial hepatectomy the mitotic activity of the remaining liver drops sharply. No mitotic figures were found in 18- or 20-hour samples (Table 2), even when these were taken in the early morning when mitotic activity is at a peak owing to the diurnal periodicity exhibited by the rat (28). Mitoses were first seen in 23-hour samples and culminated in an outburst be-
between 25 and 30 hours (Table 2). Experiments with colchicine to block mitosis at metaphase revealed that 25–40 per cent of the nuclei divided within this 5-hour period (unpublished). The spatial distribution of cells in mitosis was predominantly in the perportal and midlobular region, with very few immediately around the central veins, confirming previous reports (21). A comparison showed that the accumulated mitotic figures amounted to 5 per cent in the region immediately around the central vein versus 47 per cent in the perportal region. This corresponds directly to the regional differences in DNA synthesis observed in the photometric measurements of 20-hour samples. In terms of the structural liver unit of Rappaport et al. (38), the cells first to be supplied with blood rich in oxygen and nutrients are the first to divide. The mitotic activity gradually decreased and after 10 days was only slightly higher than normal (Table 2).

Treatment with TA prior to partial hepatectomy did not affect the mitotic activity in the regenerating remnant. Rats recovering from the drug (Group B) had a higher division rate at 96 hours than corresponding controls (Table 2). This in itself is not significant, since individual variation and the cyclic nature of the mitotic activity in the rat liver result in a wide range of values for any group. However, the higher percentage of nuclei synthesizing DNA at 96 hours (Chart 2) and the greater mean weight of the regenerated liver (Chart 1) suggest that pretreatment with TA may slightly enhance the restorative process. The possibility that pretreatment results in a slower rate of DNA synthesis and in larger cells would also explain these data. The mitotic activity in the livers of rats that continued to receive TA during the regenerative period (Group C) was generally lower than that of the controls (Table 2). However, there was greater individual variation, especially after the first burst of division. Proliferation of nonparenchymal elements was more prominent than in the controls.

Polyploidy.—The classification of nuclei into polyploid classes from liver taken prior to partial hepatectomy and from samples taken 96 hours and 10 days later is summarized in Table 3. In the control animals (Group A) the percentage of diploid nuclei decreased rapidly during the first 96 hours, while the higher polyploids increased. The frequencies remained stabilized after this period, showing no additional change at 10 days. Similar polyploid changes have been described by Himes et al. (27) in regenerating rat liver following carbon tetrachloride poisoning.

Animals treated with TA for 7 days showed a shift toward higher polyploid frequencies and a decrease in the percentage of diploid nuclei (compare 0-hour values of Group A and Group B, Table 3). Three mechanisms have been postulated to account for the increase in polyploidy in these livers (25, 29): (a) fusion of interphase nuclei in binucleate cells frequently seen in these livers, (b) fusion of metaphase figures during mitosis in binucleate cells (2) since mitosis is also increased, and (c) chromosomal duplication without mitosis.

The diploid nuclear population of the TA-treated rat livers (Groups B and C) decreased only slightly during the first 96 hours of regeneration, while the frequencies of the higher polyploid nuclei increased (Table 3). Animals recovering from the drug (Group B) showed no further change at 10 days. In contrast, rats receiving TA during restoration (Group C) showed significant increases in the percentage of diploid nuclei after 10 days (Table 3). This was correlated with the presence of nonparenchymal elements which are more prominent than in the controls.
of many enlarged cuboidal cells extending in strands or nests from bile ducts. These cells had ovoid nuclei, prominent nucleoli, and densely basophilic cytoplasm. Groups of such "transition-al" cells, often seen in ductlike formation, resembled diploid parenchyma to varying degrees. Although most of these cells did not contain gly-

TABLE 3

POLYPLOID CLASS FREQUENCIES IN REGENERATING RAT LIVER*

<table>
<thead>
<tr>
<th>Animals</th>
<th>Time after partial hepatectomy</th>
<th>Per cent Class I</th>
<th>Per cent Class II</th>
<th>Per cent Class III</th>
<th>Per cent Class IV</th>
<th>Per cent Class V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group A)†</td>
<td>0</td>
<td>20.6</td>
<td>77.0</td>
<td>2.4</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96 hours</td>
<td>4.3</td>
<td>74.1</td>
<td>19.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>0</td>
<td>13.1</td>
<td>84.1</td>
<td>2.8</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96 hours</td>
<td>2.6</td>
<td>75.0</td>
<td>19.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>0</td>
<td>10.4</td>
<td>86.6</td>
<td>3.0</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 days</td>
<td>2.3</td>
<td>75.0</td>
<td>19.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>0</td>
<td>12.5</td>
<td>85.0</td>
<td>2.5</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 days</td>
<td>3.2</td>
<td>75.3</td>
<td>18.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA-treated (Group B)‡</td>
<td>0</td>
<td>7.9</td>
<td>85.1</td>
<td>6.7</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>TA1</td>
<td>96 hours</td>
<td>5.8</td>
<td>68.0</td>
<td>22.6</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>TA2</td>
<td>96 hours</td>
<td>9.4</td>
<td>82.2</td>
<td>7.8</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>TA3</td>
<td>10 days</td>
<td>7.7</td>
<td>82.3</td>
<td>9.8</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>TA4</td>
<td>10 days</td>
<td>8.3</td>
<td>84.2</td>
<td>7.3</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>TA-treated (Group C)§</td>
<td>0</td>
<td>7.6</td>
<td>84.8</td>
<td>7.6</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>TR1</td>
<td>96 hours</td>
<td>5.2</td>
<td>63.6</td>
<td>25.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TR2</td>
<td>96 hours</td>
<td>8.5</td>
<td>63.0</td>
<td>25.4</td>
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<tr>
<td>TR3</td>
<td>10 days</td>
<td>7.2</td>
<td>79.8</td>
<td>12.6</td>
<td>0.4</td>
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<tr>
<td>TR4</td>
<td>10 days</td>
<td>9.2</td>
<td>82.0</td>
<td>8.2</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

* Percentages are based on counts of 1,000 nuclei for each sample.
†‡§ See footnotes to Table 2.

cogen, the larger ones did (Fig. 11). Thus, a gradient of transitional stages could be assembled, ranging from enlarged bile duct epithelium to small diploid parenchymal cells (Figs. 8-12). The possibility that newly formed diploid parenchymal cells originate from bile duct epithelium, as postulated by Fishback (14), is supported by these observations.

Ribonucleic acid (RNA).—The TA-affected liver with its increased nuclear ribonucleoprotein provides excellent material for the study of the nucleolus and chromosomal RNA during the mitotic cycle. In prophase, concomitant with the breakdown of the nuclear membrane, the nucleolus fragments, and large and small nucleolar granules were seen scattered among the chromosomes (Fig. 5). At metaphase such ribonucleopro-

these elements were evenly dispersed, giving the cytoplasm a homogeneous appearance. The dispersion of the basophilic material may be correlated to a decrease in glycogen and a disaggregation of the endoplasmic reticulum in the liver cell (3, 10, 13, 44). An increase in the cytoplasmic basophilia (RNA) was evident at 18 hours, reaching a maximum in the 23- and 28-hour samples studied (Fig. 2). The increase in cytoplasmic RNA occurred after noticeable increases in the volume and RNA content of the nucleoli were observed. Photometric measurements of nucleoli and cytoplasmic areas of individual parenchymal cells support these observations (45, 48). The relationship of nuclear and cytoplasmic RNA synthesis does not appear to be a simple one (reviews, 5, 43). Isotope studies of regenerating rat liver have shown that cytoplasmic RNA could not be entirely nuclear in origin (24). Data from various cell systems support the hypothesis that nuclear RNA may be only partly or remotely operative in certain cytoplasmic RNA syntheses (6, 48, 49, 54).

The basophilic elements in TA-affected liver cells are diffusely dispersed throughout the cytoplasm (Fig. 3). This may be correlated with the decrease in stored glycogen (29) and the decrease in the microsomal fraction (31). Nine hours after partial hepatectomy no change in basophilia was apparent. At 18 hours the basophilic elements were greatly increased. Samples taken at 23 and 28 hours continued to show increased density of these elements (Fig. 4) comparable to those of the controls.

**DISCUSSION**

The results of this study indicate that TA, at nontoxic levels, does not interfere with restorative growth. Following partial hepatectomy morphological changes, DNA synthesis, and mitotic rates were comparable to corresponding controls.

It is well known that nuclear volume varies in response to a large assortment of physiological factors influencing chromosome size, number, composition, or nuclear fluid intake (12, 40). The initial increase in nuclear volume resulting from TA treatment appears to be due primarily to water uptake. The enlarged nucleoli and the presence of inclusions (29, 30) also affect the nuclear volume. The DNA per nucleus is not increased (29, 31, 50). Photometric measurements of non-nucleolar protein stained with the Millon reaction or naphthol yellow S show no change per nucleus, and studies of isolated nuclei made with interference microscopy indicate only small increases in non-nucleolar mass (unpublished data, R.G.K.). Following partial hepatectomy the nuclei and the nucleoli show additional volume increases (Table 1). When the increases are calculated as the per cent of the normal mean volumes, they correspond to the increased values found in regenerating liver controls.

Variations in the RNA content of cells have been linked with modifications in the rate, and probably kinds, of proteins synthesized (review, 5). It has been noted that cell growth or increased secretory activity is accompanied by large nuclear and nucleolar size and increased nuclear and cytoplasmic ribonucleoproteins (9). This is the picture one finds in regenerating liver. In the TA-treated liver large increases occur in nucleolar RNA, while cytoplasmic RNA may decrease or remain unchanged (31). Following partial hepatectomy there is an increase in cytoplasmic RNA. The TA-affected liver cell, therefore, remains capable of responding to the biosynthetic needs called forth during restorative growth.

The mechanism of TA action on the cell is still elusive. It is generally believed that a derivative of the drug, rather than the drug itself, induces the cellular changes (33). Sexton (42) observed that TA breaks the dormancy in tubers and suggests that TA may inactivate specific growth-inhibitory substances. Rather (39), following this line of reasoning, proposed that "... the locus of action of thioacetamide in the liver cell is on some inhibitory enzymatic system, within the nucleus, which controls syntheses processes. The effect of thioacetamide might then be to inactivate the inhibitory system and permit less restrained synthesis."

In acute TA intoxication, Gallagher et al. (17) report a change in the permeability of the cell membrane. They claim that calcium ions accumulate in the cell and oxidative phosphorylation is inhibited. Their data are not altogether convincing, yet it is conceivable to suppose that the inflow or loss of certain ions may have wide-reaching effects on certain cellular systems (23, 32, 41).

The changes induced by TA do not have an adverse effect on proliferation. In fact, prolonged administration of the drug often results in malignant lesions (15, 19, 20). In this regard it is pertinent to note the well known antagonism between cell function and mitotic activity (52). Swann (46), in an excellent review, discusses this problem most pointedly. He states that the cell may be stimulated to divide by mechanisms which steer its synthetic activities away from the elaboration of the products of differentiation over toward making the structural and enzymic proteins required for division. The factors concerned in this "redirection" in regenerating liver are not clearly defined (18, 22). Whatever the stimulus, TA-affected cells respond in a manner similar to the controls.

The transition of bile duct cell derivatives to...
form parenchymal cells in rats receiving TA during the regeneration period is strongly suggested in view of the observed morphological features (Figs. 8–12) and the increased diploid cell population (Table 3). Renewed interest in the transition of bile ducts to parenchyma has been stimulated by the recent report of Wilson and Leduc (53). The authors postulate that restoration in a liver subjected to prolonged injury may depend on the transition of "cholangioles" to replace the damaged parenchyma. The hepatic cells of rats receiving TA respond normally during the first and second bursts of division. With continued treatment the bile duct elements become more prominent, and transitional stages are seen. It is possible that TA is more damaging to parenchymal cells which have recently divided, thus providing an environment favoring the transition of bile duct epithelium.

SUMMARY

The effect of thioacetamide on liver restoration after subtotal hepatectomy was investigated. Pretreatment with the drug did not interfere with subsequent restoration; in fact, slightly larger livers resulted at the end of 10 days. Animals treated prior to and following surgery showed some retardative effects, the wet weight gains and mitotic activities averaging slightly lower than corresponding controls.

Thioacetamide reportedly induces striking increases in the nuclear and nucleolar volumes of rat liver cells and stimulates nuclear RNA synthesis. Following partial hepatectomy the cellular changes induced by the drug and those associated with preparation for mitosis occurred concurrently. The nuclear and nucleolar volumes showed further enlargement, and nucleoli assumed irregular shapes. Photometric measurements of Feulgen-stained nuclei showed that DNA synthesis was highest at 20–28 hours in both drug-treated and control animals. The first burst of mitosis occurred between 25 and 30 hours, primarily involving the cells of the periportal areas. Cytoplasmic basophilia, although decreased after TA treatment, increased in density after partial hepatectomy in a pattern identical to that of controls.

Four to 10 days following partial hepatectomy, the percentage of diploid nuclei was decreased while that of the higher polyploids was increased in control animals. In thioacetamide-treated animals, although an increase in the polyploid nuclei occurred, either no change or significant increases in the diploid population were noted. The possible transition of bile duct cell derivatives toward diploid parenchymal cells was discussed.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the skilled technical assistance of Miss Barbara J. Wheaton.

REFERENCES

20. ———. Nodular Cirrhosis and Metastasising Tumours


All preparations were stained with Azure B. X1000.

Fig. 1.—Normal rat liver.

Fig. 2.—Liver from the same rat as shown in Figure 1, 28 hours after partial hepatectomy.

Fig. 3.—Liver from a rat treated with TA for 7 days.

Fig. 4.—Liver from the same rat as shown in Figure 3, 28 hours after partial hepatectomy.

Figs. 5–7.—Mitotic figures from a 48-hour sample of regenerating liver. The rat received TA 7 days prior to and daily treatment following partial hepatectomy. Note the dense nucleolar fragments in the late prophase (Fig. 5) and metaphase (Figs. 6 and 7) figures.

Figs. 8–12.—Liver samples from a rat 10 days after partial hepatectomy. The rat received TA 7 days prior to and daily treatment following partial hepatectomy.

Fig. 8.—Typical bile duct epithelium.

Fig. 9.—Bile duct epithelium with enlarged nuclei and increased cytoplasmic basophilia.

Fig. 10.—"Transitional" cells with enlarged oval nuclei, enlarged nucleoli and dense cytoplasmic basophilia.

Fig. 11.—"Transitional" cells filled with glycogen. Preparation stained with periodic acid-Schiff reagent and Azure B.

Fig. 12.—Group of diploid parenchymal cells believed to have formed from the transition of bile duct epithelial derivatives.
Evidence that the rat tissue produces antibodies specific for the malignancy is lacking. It is possible that the sensitized nodes produce cytotoxic material against all mouse tissues, but the faster growth rate of the tumor renders it more sensitive to this action than the rest of the mouse. The high proportion of deaths in mice receiving rat tissue even before they were challenged with tumor (see Chart 3) appears to represent a cytotoxic death. Pathological confirmation of this phenomenon is at present under investigation and is not considered within the province of the present report. Nevertheless, the ineffectiveness of lymph nodes from rats sensitized only against mouse muscle implies indirectly that specific anti-tumor, as well as anti-mouse, antibodies may be involved in the extension of life of some of the experimental animals.

Lymph node heterografts rather than homografts were selected on the basis of the work of Algire et al. (1). These workers demonstrated that the formation of antibodies against either homotransplant or heterotransplant when enclosed in a Millipore chamber was not sufficient to cause their rejection in an unsensitized animal. However, when a recipient had been previously sensitized by a heterograft, a further heterotransplant in a chamber would be rejected. Using this principle in reverse, then, we have assumed that rat tissue sensitized to mouse, then later again exposed to mouse while embedded in a chamber, would produce a rejection reaction that could effectively cross the Millipore barrier. At the same time this barrier would protect the transplant itself and allow it to survive for an extended period in the nonsensitized host.

While the experiments recorded here cannot be considered proof of continuing antibody production by the transplanted lymphoid tissue, the 50 per cent extension of life in some treated animals (see Charts 3 and 4) suggests an effect beyond that expected from the simple release of antibodies contained in the lymph cells at time of transplanting. When Harris and Harris (5) made suspensions of washed cells from regional nodes draining the site of an injected antigen, they were able to confer adoptive immunity on the recipients; but when the suspensions were treated by a method that killed the cells without destroying the antibodies, no measurable immunity resulted.

Function as well as survival of heterografts within Millipore chambers for extended periods has been reported (3), and histologic examination of the lymphoid tissue 30 days after it was embedded in the chambers in the present experiments has shown healthy cells consistent with continuing function.

SUMMARY

1. Utilizing the Algire Millipore chamber, a technic is described that permits survival of heterografted antibody-producing cells. Rat lymph...
The Effect of Thioacetamide on Rat Liver Regeneration I. Cytological Studies

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