Early Changes in Rat Liver and Kidney Cells
Induced by Thioacetamide*

RUTH G. KLEINFELDT†

(Department of Physiology, Ohio State University, Columbus, Ohio)

Thioacetamide (TA), a drug effective in the control of orange decay (5), was found to induce liver cirrhosis, tumors (9), and cancer of the bile ducts (11) if fed to rats over a period of several months. The most striking effects, first described by Rather (28), are the doubling of nuclear volume, increased nucleolar and cell volumes, and altered basophilia of the cytoplasm. These changes are well developed within a few days of treatment and are largely reversible. Biochemical studies of the early effects of the drug show a considerable increase in nuclear ribonucleic acid (RNA) and protein, while the deoxyribonucleic acid (DNA) per nucleus remains the same (19, 34). Photometric measurements of Feulgen-stained nuclei indicate that the early increase in nuclear volume is not associated with corresponding increases in the DNA content (15). After prolonged TA treatment, i.e., 3–7½ months, the DNA content per nucleus increases in direct proportion to the increased nuclear volume (4, 12).

The recent work of Nygaard et al. (20), using S<sup>35</sup>-labeled TA indicates that TA is rapidly metabolized in the rat. More than 80 per cent of the radioactivity is excreted in the urine during the first 24 hours. This explains the need for continuous treatment (addition of TA to the diet or daily injections) to maintain the effect induced by the drug. The liver, reported to be the only organ showing morphological changes after TA treatment, does not concentrate S<sup>35</sup>-labeled TA. The bone marrow, thyroid, and adrenals do concentrate the labeled drug to a considerable degree temporarily, yet appear to remain unaffected.

The early effect of the drug results in a disturbance of the ribonucleoprotein system of the cell (19), whereas increased DNA synthesis and cell proliferation are responses to chronic intoxication (12). It is rapidly becoming established that RNA serves as an essential element in certain protein syntheses and is a sensitive indicator of many cellular activities. With this in view, a cytochemical study of the early effects of the drug on rat liver and kidney and the cellular recovery upon withdrawal of treatment were undertaken.

MATERIALS AND METHODS

Two preliminary experiments were performed: (a) a test for dosage tolerance and (b) a study of the effect of successive liver biopsies. The results of Experiment 1 showed that the rat could readily tolerate a daily dosage of 5 mg TA/100 gm body weight. Owing to the rapid metabolism of the drug, it was found that the maximum and most uniform response was obtained if tissue samples were taken 4 hours following the last injection. Under these conditions, effects on the proximal convoluted tubules of the kidney as well as the liver were noted. In Experiment 2, a group of rats was biopsied every 10th day for 50 days. Upon being sacrificed 3 days after the third, fourth, or fifth biopsy, no regenerative changes in the liver were found, and the effect of TA and subsequent recovery of the cells from the drug were identical to that in nonbiopsied controls. The results of these experiments determined the procedure of the experiments to be discussed in detail.

Male rats of the Wistar strain, weighing about 250 gm., were used. A liver biopsy was initially obtained from each animal prior to the start of drug administration. Each animal thus provided its own normal control. This enabled a more carefully controlled analysis of change and eliminated, in part, variations among individuals. After 10 days of recovery, daily subcutaneous injections of TA were administered as a 2 per cent solution in isotonic sodium chloride, in a dose of 5 mg/100 gm body weight. A second liver biopsy was taken from two rats for each experimental period of 0, 2, 4, 8, 10, and 14 days of treatment. Each animal continued to receive the drug for a total of 14 days, after which time drug treatment was...
stopped. The animals were sacrificed in pairs 0, 2, 4, 8, 10, and 14 days after withdrawal of drug administration. Each rat thus provided three liver samples: (a) normal control, (b) during a stage of TA treatment, and (c) during a phase of the recovery period.

The study of the effect of TA on the rat kidney entailed the sacrifice of a pair of rats for each experimental period: 4, 10, and 14 days of TA treatment, and 4 and 10 days of recovery after 14 days of treatment. Tissues were fixed in 10 per cent neutral formalin and 3:1 alcohol-acetic acid, processed through the isopropyl alcohol-paraffin schedule (8) and sectioned at appropriate thicknesses.

Biopsy technic.—Liver biopsy was performed under ether anesthesia with the usual sterile precautions. A midline incision was made, beginning at the level of the xiphoid process extending 3–4 cm. posteriorly. A small piece of liver was removed from the edge of the left lateral lobe with sharp scissors and fixed immediately. The operative wound was then closed with two layers of sutures. The wound was reopened in taking subsequent biopsies.

Volume determinations.—Nuclear volumes were determined by direct measurement of whole nuclei in 20-μ sections of Feulgen preparations. The nuclei were grouped according to their degree of ploidy by visual estimation of dye intensity and volume. The observer’s accuracy in allocating nuclei to the proper class, i.e., class I, diploid; class II, tetraploid; class III, octaploid, was tested against photometric determinations. The visual estimate was 100 per cent accurate for normal liver and kidney tissue and 95 per cent accurate for drug-treated liver samples. The frequency of each polyploid class was determined for several samples of normal and TA-treated rat liver by scoring uncut Feulgen-stained nuclei within random fields.

Nuclear volumes were determined by direct measurement of nuclei in 18-μ sections of azure B and Feulgen-fast green preparations. Selection was made of tetraploid liver cell nuclei containing a single nucleolus (fusion complete) in order to standardize the normal range of variation of nucleolar material within the most common liver cell. Diploid nuclei containing a single nucleolus were chosen for measurement in kidney tubules.

Deoxyribonucleic acid (DNA) measurements.—The relative amount of DNA per nucleus was determined by cytophotometric measurements of Feulgen-stained sections. The Feulgen procedure was essentially that described by Stowell (80). Sections to be measured were mounted in oil matched to the refractive index of the cytoplasm (1.556–1.564) to eliminate nonspecific light loss due to refraction and scattering. The relative amount of DNA was estimated by measurement of individual Feulgen-stained nuclei according to the “two-wavelength” method proposed by Ornstein (81) and Patau (82). The computational procedure described by Patau (82) was applied. The large, round and intranuclear inclusions of the TA-treated liver cell made it difficult to measure the nuclear DNA content accurately by the conventional “plug” method (82). The “two-wavelength” method is more reliable and is particularly recommended when the biological material involved presents problems of uneven distribution and concentration. The photometric apparatus used was essentially that described by Pollitzer (83). The two wavelengths were carefully selected after absorption curves were made of homogeneous areas of interphase nuclei in both normal and drug-treated liver samples; 500 μm was selected as λ1, and λ2 = 545 μm was the wavelength found to give an extinction twice that at λ1, (Eλ1 = 2Eλ2). The extinctions were in the range of 0.1–0.4.

Ribonucleic acid (RNA).—The distribution and concentration of cytoplasmic and nucleolar RNA were estimated by azure B dye binding at pH 4.0 (10) in 4-6-μ sections (alcohol-acetic acid-fixed) pretreated with deoxyribonuclease (Worthington, crystalline, 0.15 mg/ml, pH 6.5, 2 hours, 30° C.). The relative amount of RNA/nucleus was estimated by photometric measurements of tetraploid liver nuclei and diploid kidney nuclei, each containing a single nucleolus. Photometric determinations of the azure B-RNA dye complex were made at the absorption peak of 590 μm, with the use of the “plug” method described by Swift (88) for whole nuclei.

Protein.—The Millon reaction (26, 27) and naphthol yellow S (7) staining reactions were used for observations of protein distribution.

Glycogen.—The periodic acid-Schiff reaction for glycogen (14) was carried out on 6-μ sections of each sample of rat liver after formalin fixation, alcohol-acetic acid fixation, and frozen-dried sections floated in absolute alcohol. Saliva controls were used to check specificity.

Lipide.—Sudan black B in 70 per cent ethyl alcohol was used on formalin-fixed frozen sections to demonstrate intranuclear lipides (9).

RESULTS

The present study is a description of the response of maximally affected cells of the liver and kidney to the drug TA and is not representative of the average cellular response. The increased dosage administered and the optimum timing in the removal of tissue samples with respect to the preceding injection of the drug resulted in less variation. However, even the more uniform tissue samples contained some areas with relatively normal-appearing cells. In the liver, approximately 80–95 per cent of the parenchymal cells were affected after 4 days of treatment. The patches of unaffected cells were usually found in the peripheral region of the lobule. The effect of TA on the proximal convoluted tubules of the renal cortex varied in extent from animal to animal. Approximately 70–95 per cent of the tubules showed typical morphological changes after 4 days of treatment. The distribution of unaffected tubules appeared to be randomly scattered through the cortex. In making measurements for the present study the unaffected, normal looking cells of both liver and kidney were selectively omitted.

Liver.—The parenchymal cell of the control rat liver has a round nucleus containing small, dense nucleoli which are rich in RNA and protein. The cytoplasm is characterized by coarsely clumped masses of basophilic material often concentrically arranged around the nucleus (Fig. 1), which are known to be rich in ribonucleoprotein (6, 18). The cytoplasm is normally filled with glycogen, although there may be marked variation from cell to cell. The presence of lipide droplets as revealed after staining with Sudan black B is limited to the
cytoplasm of an occasional cell.

After 2 days of TA treatment, the nuclear, nucleolar, and cell volumes were greatly increased. Nucleoli assumed irregular shapes (Fig. 2), and the basophilic masses of the cytoplasm were dispersed (Figs. 3, 4). Continued treatment resulted in larger nuclei containing large, dense, often vacuolated nucleoli (Fig. 3). In addition to the large, dense nucleoli, intranuclear inclusions enclosed in a membrane appeared (Figs. 4–6). The inclusions contained RNA, protein, and occasionally glycogen and lipide (17). An electron microscope study (16) has revealed that they form by invaginations of the nuclear membrane. Interphase nuclear fusion in binucleate cells occurred by invaginations of the nuclear membrane. In addition, nuclear inclusions contained RNA, protein, and occasionally glycogen and lipide. The inclusions may have lain free within the nucleus (Fig. 11) or have been within the nucleolus or the membrane-bound inclusion (Fig. 12).

Observations of 2-μ sections (fixed in buffered OsO₄ and embedded in methacrylate [22]) with phase contrast microscopy revealed a tendency of the mitochondria to group around the nucleus (Figs. 13, 14).

The ability of the TA-affected cell to recover after withdrawal of drug treatment was almost as dramatic as the onset of morphological changes induced by drug treatment. Within 2 days of recovery the cytoplasm of the liver cell was filled with glycogen. The cytoplasmic basophilia increased in density (Fig. 15), and the characteristic clumped masses reappeared (Figs. 15–17). Nuclei containing inclusions could still be found after 4 days of recovery (Fig. 16). Since cell necrosis and pyknosis were rarely observed, it appears that the cells containing such inclusions eventually recovered. After 10 days of recovery the average nuclear volume returned to the normal range (Table 5).

The effect of fixation.—The increased nuclear volume, induced in the liver cells by TA, resulted in a dilution of the nucleoprotein as evidenced by the decreased staining with the Feulgen reaction and with basic and acid dyes. To determine whether such an increase in nuclear volume would be maintained after different fixatives, measurements were made of normal and TA-treated liver cell nuclei after alcohol-acetic acid fixation and 10 per cent neutral formalin fixation. The results are summarized in Table 1. Both formalin and acetic alcohol fixation maintained the 44–49 per cent increase in nuclear volume of the 4-day TA-treated cells. Furthermore, the swelling caused by acetic-alcohol fixation, which amounts to 26–27 per cent, was the same for both normal and TA-treated cells and uniformly affected the three nuclear classes (diploid, tetraploid, octaploid) found in the liver.

Nuclear volume.—The changes in nuclear volume induced in the liver and kidney cells by TA treatment are summarized in Table 2. The liver cell nuclei showed an average increase from the control of 45.4 per cent after 4 days, 100.6 per cent after 10 days, and 155.7 per cent after 14 days. The percentage increase for each of the three nuclear classes was fairly uniform.

DNA-Feulgen determinations.—Photometric determinations of the relative amount of DNA per nucleus of normal and TA-treated liver cells are summarized in Table 3 and Chart 1. The DNA values may be grouped into diploid, tetraploid, octaploid, and 16-ploid nuclear classes, respec-

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**TABLE 1**

<table>
<thead>
<tr>
<th>NORMAL</th>
<th>4-DAY-TA</th>
<th>AVERAGE INCREASE DUE TO FIXATIVE B (per cent)</th>
<th>AVERAGE INCREASE DUE TO TA (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>30</td>
<td>104</td>
<td>50</td>
</tr>
<tr>
<td>II</td>
<td>100</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>III</td>
<td>20</td>
<td>95</td>
<td>20</td>
</tr>
</tbody>
</table>

* All liver samples were taken from the same rat. Normal, represents the normal biopsy sample taken prior to drug administration; 4-day-TA, represents the liver sample taken after 4 days of thioacetamide administration.

† I, diploid; II, tetraploid; III, octaploid.

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The cytoplasm of an occasional cell.

After 2 days of TA treatment, the nuclear, nucleolar, and cell volumes were greatly increased. Nucleoli assumed irregular shapes (Fig. 2), and the basophilic masses of the cytoplasm were dispersed (Figs. 3, 4). Continued treatment resulted in larger nuclei containing large, dense, often vacuolated nucleoli (Fig. 3). In addition to the large, dense nucleoli, intranuclear inclusions enclosed in a membrane appeared (Figs. 4–6). The inclusions contained RNA, protein, and occasionally glycogen and lipide (17). An electron microscope study (16) has revealed that they form by invaginations of the nuclear membrane. Interphase nuclear fusion in binucleate cells occurred by invaginations of the nuclear membrane. In addition, nuclear inclusions contained RNA, protein, and occasionally glycogen and lipide. The inclusions may have lain free within the nucleus (Fig. 11) or have been within the nucleolus or the membrane-bound inclusion (Fig. 12).

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DNA-Feulgen determinations.—Photometric determinations of the relative amount of DNA per nucleus of normal and TA-treated liver cells are summarized in Table 3 and Chart 1. The DNA values may be grouped into diploid, tetraploid, octaploid, and 16-ploid nuclear classes, respec-
The number of nuclei containing intermediate amounts of DNA was significantly increased in the 4- and 10-day TA livers (Chart 1), indicative of increased DNA synthesis. In the control liver, 1 per cent of the measured nuclei contained intermediate DNA values. This was increased to 8 per cent intermediates in the 4-day TA liver, 10 per cent in the 10-day TA liver, and 4 per cent in the 14-day TA liver.

Associated with the increase in intermediate DNA values was a two- to fivefold increase in the mitotic index (Table 3). Increased DNA synthesis is known to be associated with cells preparing for division, or endo-duplication (32). The process of DNA synthesis leading to chromosomal duplication appears to be a very rapid one in the rat liver (15). The 8–10 per cent intermediate DNA values of the TA rat liver were comparable to the values found in regenerating rat liver, where the mitotic index was more than 10 times higher than that in the 14-day TA rat. This could be explained if TA stimulated DNA synthesis but prolonged the process, or more likely, interfered with the subsequent mitotic processes, thus resulting in endo-duplication. The increased frequency of highly polyploid nuclei strongly suggests that endo-duplication occurs (see Wilson and Leduc [36, 37] for discussion).

Table 4 summarizes the polyploid frequencies in the livers of two animals, prior to drug treatment, after 14 days of drug administration and after 14 days of recovery. TA administration induced a shift toward higher frequencies of the octaploid and 16-ploid nuclei with a decrease in

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**Table 2**

**Effect of Thioacetamide (TA) on the Nuclear Volume of Rat Liver and Kidney Cells**

<table>
<thead>
<tr>
<th>ANIMAL</th>
<th>CLASS I</th>
<th></th>
<th>CLASS II</th>
<th></th>
<th>CLASS III</th>
<th></th>
<th>AVERAGE VOLUME INCREASE (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIVER</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Normal</td>
<td>180 ± 2.9*</td>
<td></td>
<td>287 ± 5.1*</td>
<td></td>
<td>406 ± 7.1*</td>
<td></td>
<td>45.4</td>
</tr>
<tr>
<td>1 4-day-TA</td>
<td>101 ± 3.8</td>
<td></td>
<td>288 ± 8.1</td>
<td></td>
<td>712 ± 9.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Normal</td>
<td>194 ± 5.3</td>
<td></td>
<td>250 ± 2.9</td>
<td></td>
<td>471 ± 6.3</td>
<td></td>
<td>100.6</td>
</tr>
<tr>
<td>2 10-day-TA</td>
<td>264 ± 5.8</td>
<td></td>
<td>476 ± 4.9</td>
<td></td>
<td>935 ± 13.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Normal</td>
<td>185 ± 2.1</td>
<td></td>
<td>245 ± 3.4</td>
<td></td>
<td>473 ± 6.8</td>
<td></td>
<td>155.7</td>
</tr>
<tr>
<td>3 14-day-TA</td>
<td>387 ± 11.4</td>
<td></td>
<td>584 ± 13.0</td>
<td></td>
<td>1190 ± 24.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIDNEY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>121 ± 2.0</td>
<td></td>
<td>121 ± 2.0</td>
<td></td>
<td>53.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 4-day-TA</td>
<td>186 ± 3.6</td>
<td></td>
<td>186 ± 3.6</td>
<td></td>
<td>52.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 10-day-TA</td>
<td>184 ± 4.0</td>
<td></td>
<td>184 ± 4.0</td>
<td></td>
<td>53.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 14-day-TA</td>
<td>240 ± 4.4</td>
<td></td>
<td>240 ± 4.4</td>
<td></td>
<td>53.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* These values are the standard deviations of the mean = σ/√n, where σ is the standard deviation of the observations in the sample and n is the number of observations.

† See footnote to Table 1.

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**Table 3**

**Amount of DNA per Nucleus (Photometric Determinations of Feulgen Preparations) in Liver and Kidney Cells of Normal and Thioacetamide (TA)-Treated Rats**

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>DNA PER NUCLEUS (ARBITRARY UNITS)*</th>
<th>NO. INTERMEDIATE DNA VALUES</th>
<th>MITOSES PER 5,000 CELLS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CLASS I</td>
<td>CLASS II</td>
<td>CLASS III</td>
</tr>
<tr>
<td>LIVER</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0.984 ± 0.083</td>
<td>2.85 ± 0.080</td>
<td>4.08 ± 0.050</td>
</tr>
<tr>
<td>4-Day-TA</td>
<td>0.965 ± 0.045</td>
<td>1.96 ± 0.032</td>
<td>4.05 ± 0.055</td>
</tr>
<tr>
<td>10-Day-TA</td>
<td>1.07 ± 0.084</td>
<td>1.96 ± 0.031</td>
<td>4.01 ± 0.055</td>
</tr>
<tr>
<td>14-Day-TA</td>
<td>1.01 ± 0.087</td>
<td>2.91 ± 0.050</td>
<td>4.22 ± 0.056</td>
</tr>
<tr>
<td>KIDNEY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0.982 ± 0.040</td>
<td>2.01</td>
<td>24</td>
</tr>
<tr>
<td>4-Day-TA</td>
<td>1.02 ± 0.040</td>
<td>2.04</td>
<td>27</td>
</tr>
<tr>
<td>10-Day-TA</td>
<td>0.979 ± 0.041</td>
<td>2.06</td>
<td>25</td>
</tr>
<tr>
<td>14-Day-TA</td>
<td>0.990 ± 0.023</td>
<td>2.14 ± 0.019</td>
<td>40</td>
</tr>
</tbody>
</table>

* Values are given with the standard deviations of the mean = σ/√n, where σ is the standard deviation of the observations in the sample and n is the number of observations.

† See footnote to Table 1.
the frequency of diploid nuclei. Upon withdrawal of the drug for 14 days, the nuclear volumes returned to normal (Table 5), yet the polyploid frequencies remained at approximately the same level as that of the 14-day treated liver (Table 4). However, giant nuclei of 32-ploid or higher, seen in the TA livers, were rarely encountered after drug withdrawal. Such highly polyploid cells may not be able to maintain themselves, and their disappearance may be due to their death. Continued mitosis in cells that have already duplicated chromosomes is suggested as a possible explanation by Heizer (12). The possibility that two

The nucleolar RNA as measured by the azure B dye bound appeared to increase in proportion to the increased nucleolar volume. The concentration of dye bound per volume appeared to remain more or less constant (Table 5), indicating an increase in nucleolar material and not a change in composition. The values in Table 5 were calculated from extinction values of 1.5–1.7, so that only a general approximation can be gained from these data.

Kidney.—The normal, proximal, convoluted tubule cell of the kidney contains a round diploid nucleus with one or more nucleoli. The basophilia of the cytoplasm is moderately dense, with a homogeneous, granular distribution (Fig. 18). An occasional tetraploid and octaploid nucleus may be found in this typically diploid tissue.

Following TA treatment, the nuclear, nucleolar, and cell volumes increased (Fig. 19). The nuclei show an average increase from the control of 1.5-fold after 4 and 10 days, and almost a twofold increase after 14 days (Table 2).

Photometric determinations of the relative amounts of DNA per nucleus of normal and TA-treated kidney cells are summarized in Table 3 and Chart 2. In the normal kidney 4 per cent of the measured nuclei contained intermediate DNA amounts. This increased to 22 per cent in the 4-day kidney sample studied. The 10- and 14-day TA kidney samples, however, showed fewer intermediates than did the controls. The number of tetraploid nuclei in the drug-affected tubules was increased (Chart 2). The increased DNA synthesis was not accompanied by a higher incidence of mitosis. The appearance of polyploid nuclei appeared to be the result of endo-duplication.

The effect of TA on the volume and RNA content of the nucleoli of the kidney cells are summarized in Table 5. The average nucleolar volume increased 5.5-fold after 10 days of treatment, making up 7 per cent of the total nuclear volume as compared with 1.9 per cent for the normal tubule cells. The average RNA content per nucleolus increased in proportion to the increase in volume (Table 5).

The nuclear and nucleolar volumes returned to normal 4 days after withdrawal of the drug (Table 5).

DISCUSSION

TA induces a complex of cellular changes in rat liver and kidney, magnifying certain aspects of nuclear and cytoplasmic activities to extreme levels. The most striking effect is its action on the ribonucleoprotein system of the cell. During the time the nucleoli are growing to enormous propor-
tions (showing a 13-fold increase in volume) and nucleolar ribonucleoprotein is being synthesized, the cytoplasmic RNA becomes diluted and dispersed.

The biochemical and cytochemical evidence accumulated over the past 15 years has led to the general acceptance of the hypothesis that RNA is involved in protein synthesis (Brächet, Review [2]). The presence of conspicuous nucleoli and cytoplasm rich in RNA is typical of cells engaged in active protein synthesis, i.e., regenerating rat liver (81). The TA-affected rat liver cell presents a confused picture. The nucleoli enlarge to gigantic structures, while the concentration of cytoplasmic RNA is decreased (19). Heizer (12) suggests that we are dealing with a “strained protein-synthesizing apparatus” and that the TA-liver cell appears to be engaged in “strenuous protein synthesis.” Although this possibility cannot be excluded, recent observations regarding RNA and protein synthesis suggest another interpretation. Brächet (in his comprehensive review [2]) summarizes the supporting evidence that: (a) nuclear RNA may act as a precursor to cytoplasmic RNA and protein synthesis, (b) the transfer of nuclear RNA may involve complex interrelationships, and (c) RNA synthesis may increase, while protein synthesis is inhibited. Rather than being generally involved in active protein synthesis, the TA liver cell appears to be synthesizing RNA and protein which is accumulating in the nucleolus. Upon withdrawal of drug treatment the cytoplasmic

### TABLE 4

**POLYPLOID CLASS FREQUENCIES BASED ON COUNTS OF 2,000 CELLS**

<table>
<thead>
<tr>
<th>ANIMAL</th>
<th>CLASS I* (per cent)</th>
<th>CLASS II (per cent)</th>
<th>CLASS III (per cent)</th>
<th>CLASS IV (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Normal</td>
<td>8.1</td>
<td>98.0</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>14-Day-TA</td>
<td>4.7</td>
<td>81.3</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>14-Day-Recovery</td>
<td>4.1</td>
<td>92.0</td>
<td>12.0</td>
</tr>
<tr>
<td>4</td>
<td>Normal</td>
<td>8.2</td>
<td>98.6</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>14-Day-TA</td>
<td>5.1</td>
<td>81.0</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>14-Day-Recovery</td>
<td>5.8</td>
<td>80.0</td>
<td>12.8</td>
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</tbody>
</table>

* See footnote † to Table 1. IV = 16-ploid.

### TABLE 5

**EFFECT OF TA AND SUBSEQUENT RECOVERY ON NUCLEOLAR VOLUME AND RNA CONTENT IN RAT LIVER AND KIDNEY CELLS**

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>NO. OF NUCLEI</th>
<th>NUCLEAR VOLUME (μm²)</th>
<th>NUCLEOLAR VOLUME (μm³)</th>
<th>Nuclerar Volume / Nucleolar Volume (per cent)</th>
<th>AMINE B DYE BINDING PER NUCLEOLUS (ARBITRARY UNITS)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LIVER</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>50</td>
<td>272 (199-346)</td>
<td>6.8 (2.2-11.4)</td>
<td>2.5</td>
<td>6.6 ± 0.9†</td>
</tr>
<tr>
<td>10-Day-TA</td>
<td>50</td>
<td>500 (415-729)</td>
<td>18.7 (15.0-20.5)</td>
<td>84.0 ± 4.3</td>
<td></td>
</tr>
<tr>
<td>Recovery phase:</td>
<td>2-Day-recovery</td>
<td>50</td>
<td>390 (325-481)</td>
<td>9.5 (8.0-10.0)</td>
<td>25.0 ± 2.1</td>
</tr>
<tr>
<td>4-Day-recovery</td>
<td>50</td>
<td>240 (280-481)</td>
<td>11.4 (10.0-15.0)</td>
<td>20.4 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>10-Day-recovery</td>
<td>50</td>
<td>290 (250-500)</td>
<td>14.2 (12.0-16.0)</td>
<td>12.8 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>14-Day-recovery</td>
<td>50</td>
<td>275 (205-785)</td>
<td>18.3 (15.0-20.5)</td>
<td>11.0 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>50</td>
<td>129 (97-177)</td>
<td>2.5 (1.5-4.5)</td>
<td>1.9</td>
<td>3.3 ± 0.02†</td>
</tr>
<tr>
<td>10-Day-TA</td>
<td>50</td>
<td>200 (180-260)</td>
<td>13.9 (11.0-15.0)</td>
<td>4.0</td>
<td>16.1 ± 1.2</td>
</tr>
<tr>
<td>Recovery phase:</td>
<td>4-Day-recovery</td>
<td>50</td>
<td>124 (90-180)</td>
<td>6.0 (5.0-9.0)</td>
<td>2.4</td>
</tr>
</tbody>
</table>

* Nuclear and nucleolar volumes are given with their respective range of variation.
† These values are the standard deviations of the mean = σ/√n, where σ is the standard deviation of the observations in the sample and n is the number of observations.
RNA rapidly increases and the nucleolar RNA decreases. This suggests that TA may induce a block in the transfer or assimilation of nuclear ribonucleoprotein to the cytoplasm. Experiments with P\textsuperscript{32} are now in progress\textsuperscript{1} and should throw new light on the effect of TA on the nucleoprotein system of the liver cell.

An attempt to quantitate nuclear protein proved unsuccessful, owing to the low extinctions encountered in the TA-affected nuclei (non-nucleolar protein). The data accumulated do indicate that the increased nuclear volume is not accompanied by a corresponding increase in nuclear protein. The nucleolar protein however, does increase in direct proportion to the increased nucleolar volume. The 2.5-fold increase in total protein nitrogen of the nuclear fraction (including nucleoli) of TA-treated rat liver reported by Laird (19) could not be completely accounted for by the increase in nucleolar protein alone. The failure to detect an increase in nuclear protein cytophotometrically may be due to the loss of certain fractions during the fixation and embedding process.

\textsuperscript{1}This problem has been started by T. Breitman and G. C. Webster, Department of Agricultural Biochemistry, Ohio State University, Columbus, Ohio.

The tendency of the mitochondria to group around the nucleus (Fig. 14) and the reported decrease in the total mitochondrial protein nitrogen per cell (19) soon after TA administration suggest that TA may act as a respiratory inhibitor (35).

Intranuclear lipides are common in the TA-affected liver cell (Figs. 11, 19). Brock et al. (9) have reported increased nuclear lipides in mouse liver after fasting, in regenerating liver and hepatomas. The enzyme systems of the liver apparently adapt to increased demands of lipid metabolism during fasting, restoring the neutral fat content of the cell to normal levels after 48 hours (13). However in the TA-affected liver, the increased lipide droplets continue to be present as long as the drug is administered. This suggests a possible failure of the cell to cope with certain demands: in this case, increased lipid metabolism.

It has been suggested that the presence of glycogen in the normal mammalian liver cell produces the irregular distribution of the basophilic elements (29). In cells free of glycogen, i.e., during fasting and mitosis, the basophilic elements become homogeneously dispersed. In the TA liver, a similar relationship between the disappearance of stored glycogen and the homogeneous dispersion of the cytoplasmic basophilia exists (Fig 4). However, during early recovery (24–30 hours) many cells that are filled with glycogen continue to have a diffuse distribution of cytoplasmic basophilia. Thus, the distribution of basophilic elements is not associated solely with the presence or absence of glycogen.

The nuclei containing DNA values intermediate between diploid and tetraploid, and between tetraploid and octaploid, amounts (Chart 1) are interpreted as representing nuclei in the process of DNA synthesis (33). The number of cells involved in DNA synthesis during the early period of TA treatment is small. Prolonged drug intoxication was found to result in a widespread increase in DNA synthesis (4, 12) and to be roughly proportional to the increased nuclear volume of the cells (12). The low average nuclear volumes of rat liver cells after prolonged treatment reported by Heizer (12) sharply contrast with the values presented in this study. This discrepancy may be explained by: (a) the different method and duration of drug administration, (b) the selection for maximally affected areas in this study, and the indirect selection for more normal nuclei by Heizer, and (c) the possible adaptation of the liver cells to chronic treatment suggested in the biochemical changes reported by Laird (19).

It is of interest to note the similarity of response induced by thioacetamide in the kidney tubule.
cells and liver parenchyma, whereas all other organs remain unaffected (1). Since both the liver and kidney function in detoxification, the specificity of response may offer a clue to the mechanism of TA action. It would appear that it is not TA itself that induces the cytological changes, but rather the detoxified derivatives produced in the liver and kidney cells that do the damage.

SUMMARY

Daily subcutaneous injections of thioacetamide (TA), a hepatic carcinogen, induced characteristic cytological changes in the parenchymal cells of the liver and, to a lesser degree, in the proximal convoluted tubule cells of the kidney of the rat. A gradual increase in nuclear and nucleolar volumes, and a change in the distribution of the cytoplasmic basophilia of the liver cells, were the first cytological changes noted. The increase in nuclear volume of the liver cells reached 2.5-fold after 14 days of treatment. Photometric measurements of Feulgen-stained nuclei indicated that the increase in volume was not associated with increased amounts of DNA. The nucleolar volume and ribonucleoprotein content increased 3-fold after 14 days of treatment. Photometric measurements of Feulgen-stained nuclei indicated that the increase in volume was not associated with increased amounts of DNA. The nucleolar volume and ribonucleoprotein content increased 3-fold after 14 days of treatment. Upon withdrawal of drug treatment, the morphology of the liver and kidney cells returned to normal.

ACKNOWLEDGMENTS

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REFERENCES

37. ———. Abnormal Mitosis in Mouse Liver. Ibid., 86:51-74, 1950.

FIG. 1.—Normal rat liver. The nucleoli are small, and the cytoplasmic basophilia is clumped into large irregular masses. Azure B stain for nucleic acids. X900.

FIG. 2.—Liver taken from the same animal as shown in Figure 1 after 4 days of TA treatment. The nucleoli are enlarged and frequently irregular in shape. The nuclear and cell volumes increase, and the cytoplasmic basophilia is more evenly dispersed. Azure B stain for nucleic acids. X900.

FIG. 3.—Liver taken from a rat treated with TA for 14 days. A high degree of vacuolization occurs in both the large and small nucleoli. Azure B stain for nucleic acids, X900.

FIG. 4.—Liver taken from a rat treated with TA for 14 days. The characteristic morphological changes induced by TA can readily be seen. The nucleoli are enlarged and stain intensely with basic dyes. The cytoplasmic basophilia is homogeneously distributed. A nuclear inclusion and a dense nucleolus can be seen within the same nucleus (arrow). Azure B stain for nucleic acids. X900.

FIGS. 5 and 6.—Liver taken from a rat treated with TA for 14 days. The tissue was stained with the periodic acid-Schiff reaction to localize glycogen, and with azure B for nucleic acids. The inclusion within the enlarged nucleus in Figure 5 is bounded by a basophilic membrane. The cytoplasm and the inclusion of this cell are free of glycogen. All staining is due to RNA. The cytoplasm of the cell pictured in Figure 6 is filled with glycogen, and the nuclear inclusion contains glycogen as well as RNA. The inclusions are believed to form by invaginations of the nuclear membrane with cytoplasmic material being trapped in the process. X900.

FIG. 7.—Normal rat liver stained with the Feulgen reaction. Note the diploid (I), tetraploid (II), and octaploid (III) nuclei in the field. X900.

FIG. 8.—Liver taken from the same rat as shown in Figure 7 after 10 days of TA treatment, illustrating the fusion of two octaploid (III) interphase nuclei. Stained with the Feulgen reaction. X900.

FIG. 9.—Control rat liver stained with the periodic acid-Schiff reaction, showing the normal distribution of glycogen in a well fed rat. X900.

FIG. 10.—Liver taken from the same animal as shown in Figure 9 after 14 days of TA treatment. The cells around the central vein area are depleted of glycogen, while the cells in the peripheral region of the lobule contain glycogen. Periodic acid-Schiff reaction. ×560.
Figs. 11, 12.—Liver from a rat treated with TA for 14 days. The tissue was stained with Sudan black B to illustrate the presence of cytoplasmic and nuclear neutral fat. The lipid droplets may lie free within the nucleus (Fig. 11) or may be associated with a nuclear inclusion (Fig. 12). \( \times 900 \).

Fig. 13.—Phase contrast photomicrograph of normal liver fixed in buffered osmium tetroxide and embedded in methacrylate. The small dense bodies dispersed throughout the cytoplasm are the mitochondria. \( \times 720 \).

Fig. 14.—Phase contrast photomicrograph of liver from the same rat as shown in Figure 13, after 14 days of TA treatment. In the TA-affected cells the mitochondria aggregate around the nucleus. \( \times 720 \).

Fig. 15.—Liver taken 2 days after withdrawal of drug treatment from an animal subjected to 14 days of TA administration. Note the rapid reappearance of the irregular basophilic masses in the cytoplasm. The basophilic masses appear to be in close contact with the nuclear membrane. The nuclear and nucleolar volumes decrease, and the apparent concentration of the cytoplasmic basophilia (RNA) increases. Azure B stain for nucleic acids. \( \times 1300 \).

Fig. 16.—Liver taken 4 days after withdrawal of drug treatment from an animal subjected to 14 days of TA administration. Nuclei containing inclusions (arrow) are often present. The liver cells show no sign of pyknosis or necrosis during recovery. The cytoplasmic basophilia is increased. Azure B stain for nucleic acids. \( \times 1300 \).

Fig. 17.—Liver taken 10 days after withdrawal of drug treatment. The nuclear volumes gradually decrease and are almost normal in size after 10 days of recovery. The nucleoli also rapidly decrease in size but remain larger than normal for a longer period. The cytoplasmic basophilia returns to a more normal distribution and concentration. Azure B stain for nucleic acids. \( \times 1300 \).

Fig. 18.—Cross-section through a proximal convoluted tubule of normal rat kidney. Azure B stain for nucleic acids. \( \times 900 \).

Fig. 19.—Cross-section through a proximal convoluted kidney tubule taken from a rat after 14 days of TA treatment. Note the enlarged, dense nucleoli and the increased nuclear size. Azure B stain for nucleic acids. \( \times 900 \).
Early Changes in Rat Liver and Kidney Cells Induced by Thioacetamide

Ruth G. Kleinfeld

Cancer Res 1957;17:954-962.

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