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Changes in Hepatocyte Ploidy in Response to Chromium, Analyzed by Computer-Assisted Microscopy

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Changes in Hepatocyte Ploidy in Response to Chromium, Analyzed by Computer-Assisted Microscopy. GARRISON, J. C., BISEL, T. U., PETERSON, P., AND UYEKI, E. M. (1990). *Fundam. Appl. Toxicol.* **14,** 346–355. BDF1 mice were given single injections of sodium dichromate (25 mg/kg) on an acute (6 hr to 7 days) or intermediate (2–4 weeks) basis, or multiple injections (12.5 mg/kg) on a chronic (4.5 months) basis. Observed hepatic changes included programmed cell death (apoptosis) in the periportal region with acute exposure and fusion of liver lobes with chronic exposure. Response to chromate exposure was measured by change in hepatocyte nuclear ploidy state (e.g., the proportion of diploid, tetraploid, and octaploid nuclei) based on computer-assisted imaging from histological sections. The computer-assisted imaging system used in this study was superior to traditional methods because it (1) allows rapid ploidy determinations from histological material and (2) can be used to collect regional information. Regional differences in ploidy were seen to occur in a consistent fashion among both control and treated animals. Nuclei adjacent to the portal triad had the lowest ploidy value (highest proportion of diploid nuclei), an intermediate value was found adjacent to the central vein, and the highest ploidy was found in the midzone. These three ploidy-based zones roughly correspond to the three functional zones of A. M. Rappaport (1973, *Microvasc. Res.* **6,** 212–228) and W. H. Lamers *et al.* W. H. Lamers, A. Hilberts, E. Furt, J. Smith, G. N. Jonges, J. F. Van Noorden, J. W. G. Janzen, R. Charles, and A. F. M. Moorman, 1989, *Hepatology,* **10,** 72–76. Temporal changes in ploidy were seen among control animals (all zones), with young animals (56 days) displaying relatively low ploidy values compared to older animals (184 days). Chromate exposure caused increased ploidy (all zones) among animals treated on an acute basis (the youngest animals). Chromate had no apparent effect on ploidy among animals treated for longer periods of time, probably because of age-related factors.

Heavy metal toxicity is an issue of concern, since waste water effluents and airborne particulates originating from industrial sources are often contaminated with these compounds. Among the heavy metals, chromium is a relatively common environmental contaminant because of its use in a variety of industrial applications. The two biologically active forms of chromium are hexavalent [Cr(VI)] and trivalent [Cr(III)], with the hexavalent form being the most toxic, due to its ability to cross cell membranes and cause DNA damage (Nishio and Uyeki, 1985). The cytotoxic effects of chromium include chromosomal damage and induction of sister chromatid exchanges (Uyeki and Nishio, 1983), as well as inhibition of DNA synthesis (Bianchi *et al.,* 1983; Levis *et al.,* 1978; Nishio and Uyeki, 1985). The primary sites of action in the body are reported to be the respiratory tract, with associated respiratory cancers (Norseth, 1981), the kidney, with acute renal tubular necrosis (Langard and Norseth, 1979), and the liver, where a pattern of necrotic and apoptotic cell death is followed by liver regeneration and fibrosis (Uyeki *et al.,* 1990).

In the present study we investigate chromium-induced changes in ploidy state of mouse liver parenchyma nuclei after acute and

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chronic exposure to sodium dichromate [Cr(VI)]. Among untreated mice, hepatocytes are primarily diploid in young animals, but shift to tetraploid (or higher) as the animals age (Brodsky and Uryvaeva, 1985). In addition to age-related changes in ploidy, a pattern of increased ploidy can be induced as a response to partial hepatectomy (Brodsky and Uryvaeva, 1985) and chemical agents that stimulate hepatocyte proliferation and liver hyperplasia (Böhm and Noltemeyer, 1981). Previous work in our laboratory (Uyeki *et al.*, 1990) indicates that most chromium-induced liver damage occurs in a regional fashion, clearly demarcating the periportal from the pericentral region. Since changes in ploidy occur in response to some toxicants, and the pattern of chromium-induced liver damage occurs in a regional fashion, we attempted to see if (1) chromium induces changes in liver ploidy and, (2) if so, do these changes occur in a regional fashion. In the present study we tested the effects of chromium exposure on hepatocyte ploidy in both pericentral and periportal regions of the mouse liver. Ploidy determination was based on a novel computer-assisted microscopy system which allows rapid and accurate measurements from histological material.

MATERIALS AND METHODS

Experimental. Hybrid, 6-week-old female BDF1 mice (C57 black/6 × DBA/2) were obtained from Jackson Laboratories, Bar Harbor, Maine. Mice were acclimated 1 week prior to treatment. Animals were injected ip with sodium dichromate dissolved in neutral, buffered Hanks' balanced salt solution at a concentration of 12.5 or 25 mg/kg. Preliminary studies indicated that 75 mg/kg induces rapid mortality, while there is very little, if any, mortality at 25 mg/kg. Animals were divided into three groups in order to study acute, intermediate, and chronic effects of chromium exposure. Animals in the acute study were treated with 25 mg/kg (single injection) and euthanized on Days 0.25 (6 hr), 1, 2, 3, 4, 5, 6, and 7. Animals in the intermediate study were also treated with a single injection at 25 mg/kg, and euthanized on Days 14, 21, or 28. Those treated on a chronic basis were injected at 4-week intervals with 12.5 mg/kg over a 4.5-month (128 days) period, for a total of five injections prior to termination. Age equivalent controls were collected for all groups. Livers were removed from the mice at termination, cut into sections approximately 3 mm thick, fixed in MFA (methanol:formalin:acetic acid in a ratio of 85:10:5) at 4°C for 24 hr, and transferred to 70% EtOH for temporary storage. Tissue was dehydrated and embedded in paraffin (Paraplast plus), sectioned at 12 μm, and stained for DNA using the Feulgen reaction. Tissue was scanned for indications of toxic injury (necrosis, apoptosis, mitotic figures) using light microscopy (200×) and the region of any such injury was noted. Changes in gross anatomy of the tissue were noted at the time of termination.

Computer-assisted microscopy. Computer-enhanced images were captured at low power (20× objective, 2.5× projection lens) using an RGB CCD video camera (Sony Model DXC-3000) attached to a Nikon Optiphot microscope and analyzed using an interactive computer-assisted imaging system developed by the Cancer Imaging Section of the British Columbia Cancer Research Centre in Vancouver, British Columbia. A highly stable dc power source (Kikusui Model PAD 16-18L) was used to minimize power fluctuations which can affect lighting. An interference filter (560 ± 50 nm) was used to measure the Feulgen reaction product in individual nuclei. Camera gain and microscope stage illumination were set to initially display pixel brightness values ranging from 0 (black) to 255 (white). All captured images were "normalized" relative to the background (empty) field, such that they were displayed at pixel values ranging from 0 to 100 (equivalent to percentage transmittance) (Garrison *et al.*, 1989). Tissues used in the present study were not so intensely stained as to produce 0% transmittance values, which could cause inaccurate optical density measurement. The images were displayed at 512 × 512 pixel resolution on an analog RGB monitor (Sony Model PVM-1271Q) for manipulation and analysis.

Four liver zones were defined for analysis (see Fig. 1): (1) the first 4 cell layers adjacent to the portal triad (periportal 1–4), (2) periportal cell layers 5–10, (3) the first 4 cell layers adjacent to the central vein (pericentral 1–4), and (4) pericentral cell layers 5–10. All complete hepatocyte nuclei within a zone were measured for area and integrated optical density (IOD). Sectioned nuclei (e.g., partial nuclei) were excluded from measurement. The area is defined as the number of pixels making up the image of the nucleus, and the IOD is defined as the summation of optical density values for each pixel position of the image (Joyce-Loebl, 1985). IOD values are a function of both stain intensity and nuclear area (i.e., the amount of Feulgen reaction product bound to the DNA). Forty-nine animals were measured in the present study (3–5 animals per time point). A minimum of 100 nuclei were analyzed from each zone per animal (400 nuclei/animal). In order to collect at least 100 whole nuclei from each of the 4 zones (zones 1–4 and 5–10 from both pericentral and periportal vessels), a minimum of two to

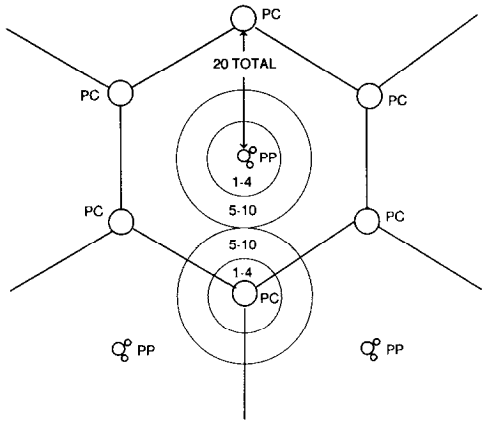

FIG. 1. Diagram of the liver lobule showing the relationship of the pericentral (PC) and periportal (PP) regions. Typically, 15–20 cell layers are present between a central vein and portal triad. A minimum of 100 nuclei/animal were measured from each of the zones demarcated in the diagram (the first 4 cell layers adjacent to the PC and PP, and cell layers 5–10 from the PC and PP).

three pericentral vessels and two to three periportal vessels were measured per animal, and the data from each individual zone pooled for statistical analysis (e.g., four pooled data sets total). In addition, the data from the 4 zones were pooled for each animal for determination of mean liver ploidy (Table 1).

Area and IOD data were sorted and the distribution patterns were plotted in histogram form (Fig. 2). Ploidy classes were determined by histogram peaks. Mouse sperm (haploid nuclei) were measured to confirm the ploidy identifications of the diploid, tetraploid, and octaploid hepatocytes.

Statistics. Data were analyzed using a two-way analysis of variance (ANOVA), comparing (1) differences between liver zones (pericentral 1–4, pericentral 5–10, periportal 1–4, periportal 5–10) and (2) differences related to the time of chromium exposure. Statistics were performed using the SAS statistical software package (SAS Institute, Inc.) and significance was determined at the 0.05% level using Duncan's multiple-comparisons test.

RESULTS

Histology and Pathology

As early as 6 hr, apoptosis (programmed cell death) was observed at the edge of the liver sections, indicating apparent contact injury. Within 24 hr large numbers of oval-shaped, nonparenchymal cells (NPCs) were also present in this region. By 2–3 days apoptotic bodies and NPCs were prevalent in the periportal region (Fig. 3). After Day 3 the number of apoptotic bodies decreased, and none were present by Day 6. Polymorphonuclear (PMN) leukocytes were associated with the apoptotic bodies in both the edge and periportal regions. These PMNs were seen by 24 hr and persisted through Day 5. No obvious signs of cellular injury were seen among animals treated for 2–4 weeks or for 4.5 months. However, the liver lobes from animals treated for 2–4 weeks showed numerous adhesions. Liver lobes from animals treated for 4.5 months had fused and were covered by a tough connective tissue sheath.

Zonal Relationships

The 4 liver zones displayed consistent, significant differences in ploidy from one zone to another (Fig. 4). The periportal zone 1–4 had the lowest ploidy (e.g., the highest percentage of diploid nuclei and the lowest percentage of tetraploid nuclei) of any liver region, regardless of treatment. The pericentral zone 1–4 had the second lowest ploidy. In only one case (4.5 month control) was there any overlap between these values. The periportal zone 5–10 and the pericentral zone 5–10 were statistically identical to one another (based on ploidy), and displayed the highest ploidy values. It should be noted that most of the shift in ploidy values could be related directly to changes in 2N:4N ratios. In all cases, the 8N cells made up a small portion of the overall cell population, and in most cases there were no significant differences in the proportion of 8N cells between zones or treatments.

While treatment of animals with chromium caused shifts in the 2N:4N ratios, the basic zonal relationships remained the same, regardless of treatment. That is, independent of treatment and age, the periportal zone 1–4 always maintained the highest proportion

TABLE 1

CHANGE IN OVERALL LIVER PLOIDY, GIVEN AS PERCENTAGE (±SE) DIPLOID (2N), TETRAPLOID (4N), AND OCTAPLOID (8N) NUCLEI, BASED ON AREA AND IOD[a]

		Area			IOD		
Exposure	*n*	2N	4N	8N	2N	4N	8N
Days							
C	5	58 ± 4.8	39 ± 4.4	3 ± 0.4	56 ± 4.7	41 ± 4.3	3 ± 0.5
0.25	3	59 ± 5.3	39 ± 5.2	1 ± 0.4	57 ± 5.3	41 ± 5.1	2 ± 0.4
1	3	65 ± 5.4	33 ± 5.1	2 ± 0.3	64 ± 5.1	34 ± 4.9	2 ± 0.3
2	4	53 ± 6.5	44 ± 6.1	3 ± 0.6	50 ± 6.1	48 ± 5.8	3 ± 0.6
3	3	47 ± 5.6	48 ± 4.7	5 ± 1.1	42 ± 6.8	52 ± 5.6	6 ± 1.5
4	3	40 ± 4.9	56 ± 4.5	5 ± 1.0	36 ± 4.5	58 ± 4.1	6 ± 1.3
5	3	29 ± 6.4	65 ± 5.6	6 ± 1.0	29 ± 6.2	65 ± 5.4	6 ± 1.1
6	3	12 ± 3.9	72 ± 2.8	16 ± 2.4	11 ± 3.6	72 ± 2.5	17 ± 2.3
7	3	25 ± 6.9	66 ± 5.3	9 ± 2.4	25 ± 6.7	66 ± 5.1	10 ± 2.2
Weeks							
C	3	39 ± 5.4	58 ± 4.8	3 ± 0.8	39 ± 5.4	58 ± 4.8	4 ± 1.0
2	3	38 ± 6.5	59 ± 6.1	3 ± 0.5	37 ± 6.3	60 ± 5.9	3 ± 0.6
3	3	31 ± 6.3	65 ± 5.7	4 ± 0.9	31 ± 6.3	65 ± 5.7	4 ± 1.0
4	3	40 ± 6.4	56 ± 5.8	4 ± 0.9	39 ± 6.5	57 ± 5.9	4 ± 0.9
Months							
C	4	33 ± 5.0	62 ± 4.4	4 ± 0.8	34 ± 5.0	61 ± 4.3	5 ± 1.0
4.5	3	25 ± 4.8	64 ± 3.1	11 ± 2.7	26 ± 5.0	64 ± 3.3	10 ± 2.4

[a] Animals were exposed to chromium on an acute (0.25–7 days), intermediate (2–4 weeks), or chronic (4.5 month) basis. Animal age at termination of the experiment was 56 days in the acute study, 78 days in the intermediate study, and 184 days in the chronic study. Number of animals (*n*) ranged from three to five per group. Control animals (C) were age equivalent to their comparison group.

of 2N cells, the pericentral zone 1–4 always had the second highest proportion, and the periportal and pericentral zones 5–10 always had the lowest.

It is interesting to note that changes in ploidy affected all regions of the liver in a similar fashion. An increase in ploidy that affected any one zone could be noted in the other three zones as well. The mean ploidy values from the pooled data of the four zones showed the same trends as measurements from individual zones; that is, chromium-induced changes in ploidy were not zone specific. For this reason, all subsequent data are here reported as the mean values of the four zones merged.

Chromium-Induced Changes

The primary effect of chromium-treatment was to cause an increase in ploidy among the animals in the acute-treatment group. Animals treated for 0–7 days showed a progressive increase in the 4N:2N ratio (Fig. 5), starting on Day 3. This increase was most marked on Day 6. No changes in this ratio (relative to controls) were seen among animals treated from 2 to 4 weeks or for 4.5 months. Small but significant increases in the percentage of 8N cells were seen among animals treated for 6 and 7 days and those treated for 4.5 months, but not in any other groups. Because 8N cells make up a small proportion of the overall cell population, it is difficult to demonstrate minor changes in the percentage of 8N cells without increasing the sample size.

Age-Related Changes

When the controls from the three different experiments were compared, the age-depen-

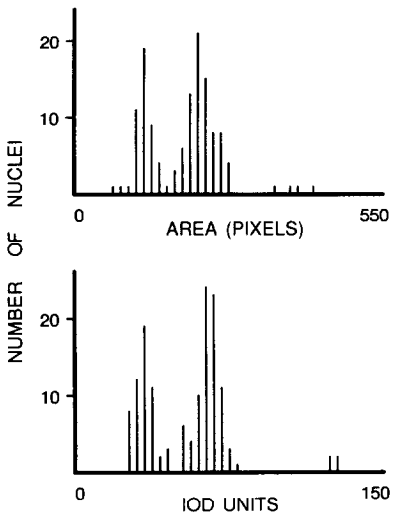

FIG. 2. Representative distribution patterns of diploid, tetraploid, and octaploid nuclei, based on area (number of pixels per nuclear image) and IOD (the summation of optical density values for each pixel position of the image, e.g., a function of both stain intensity and nuclear area). Sample taken from a control central vein, cell layers 5–10.

dent nature of ploidy state became apparent (Fig. 6). The age of the animals in the acute study (0–7 days exposure) was 56 days, the age of the animals in the intermediate-time study (2–4 weeks exposure) was 78 days, while the age of the animals in the chronic study (4.5 month exposure) was 184 days. Fifty-six percent of the nuclei were diploid among the 56-day (acute) group, while only 39% were diploid among the 78-day (intermediate) group and 34% among the 184-day (chronic) group. The only group which showed significant changes in the 2N:4N ratio in response to chromium exposure was the group which had a relatively high percentage of 2N cells (the youngest animals).

Area and IOD as Measuring Criteria

For all practical purposes, area and IOD measurements gave identical ploidy distribution information. Nuclei classified as diploid, tetraploid, or octaploid based on area were classified the same way based on IOD. The similarity in classification between area and IOD is a result of the uniformity of nuclear staining (e.g., most nuclei displayed a similar degree of chromatin condensation). Very little variation in mean OD was observed in the nuclear population.

DISCUSSION

Chromium appears to cause regional toxicity in the liver, on the basis of histological observation, primarily affecting cells in the periportal region. The primary goal of this study was to determine whether chromium exposure also influenced liver ploidy and, if so, whether the response was restricted to the periportal region.

Ploidy of the liver parenchyma appears to be influenced by several factors. Animal age is a major factor. Young animals have a relatively high 2N:4N ratio, which decreases rapidly during the first few months after birth. This age-related change in ploidy has been reported previously and is an irreversible process caused by metaphase or anaphase arrest during attempted cell division (Brodsky and Uryvaeva, 1985).

Chromium appears to accelerate the normal process of polyploidization in the mouse liver. This increase in ploidy state occurred in all regions of the liver and was not limited to the region of obvious cellular damage (e.g., the periportal region). Among animals with a high proportion of diploid cells (e.g., the youngest animals), exposure to chromium for 5–7 days caused a dramatic shift in ploidy. These young animals developed ploidy values similar to control values seen among older animals. It is likely that this change in ploidy is at least partially due to chromium-induced cell proliferation. It has been shown that chromium causes apoptotic cell death in the liver, followed by a compensatory increase in cell proliferation (Uyeki *et al.*, 1990), and that agents such as phenobarbital which induce cell proliferation also cause

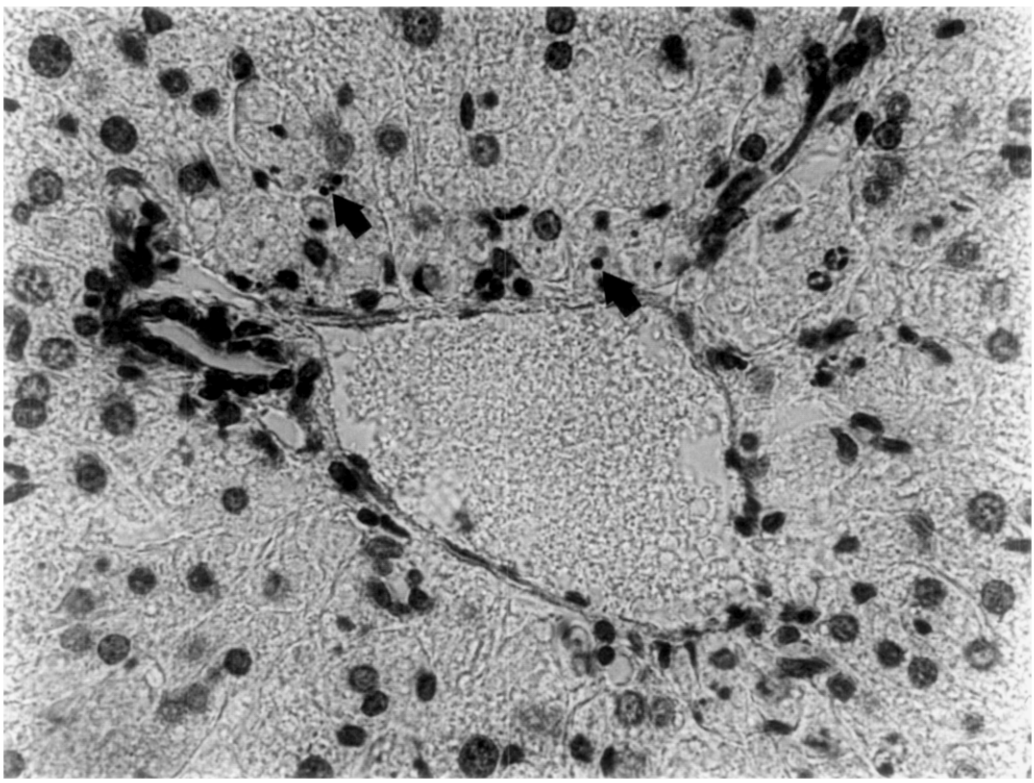

FIG. 3. Periportal region of mouse liver from a 56-day-old animal exposed to chromium for 3 days, demonstrating regional apoptotic cell death (arrows). Feulgen, ×225.

polyploidization (Böhm and Noltemeyer, 1981). Comparison of ploidy among control animals demonstrates that a dramatic shift in ploidy occurs between 56 and 78 days in the BDF1 mouse strain. Further changes with increasing age (184 days) are relatively minor. Brodsky and Uryvaeva (1985) have suggested that these changes are related to the proliferative activity of the developing liver. During the first 1–2 months after birth the parenchyma is highly proliferative, but after two to three division cycles it approaches stasis. It appears that once the hepatocyte population reaches a predominantly tetraploid state, further increases in ploidy (due to aging or chromium exposure) are minimal.

The zonal nature of the liver has been reported before and is based upon a number of different physiological and morphological criteria (Rappaport, 1973; Fawcett, 1986; Lamers *et al.*, 1989). Morphologically, the 3 zones of Rappaport (1973) and Lamers *et al.* (1989) are based on proximity to the portal vein and central vein regions. We have shown that regional differences in ploidy correlate closely with the 3-zone concept. Our periportal zone 1–4 correlates with zone 1, periportal/pericentral zone 5–10 (combined) is the equivalent of zone 2, and pericentral zone 1–4 corresponds to zone 3. It is tempting to speculate that the differences in ploidy among these zones may correlate to differences in functional state of the cell. The observation that cellular damage (apoptosis) occurs as a regional response to chromium supports this idea. According to the "streaming liver" concept of cell renewal (Zajicek *et al.*, 1985), hepatocyte formation from stem cells

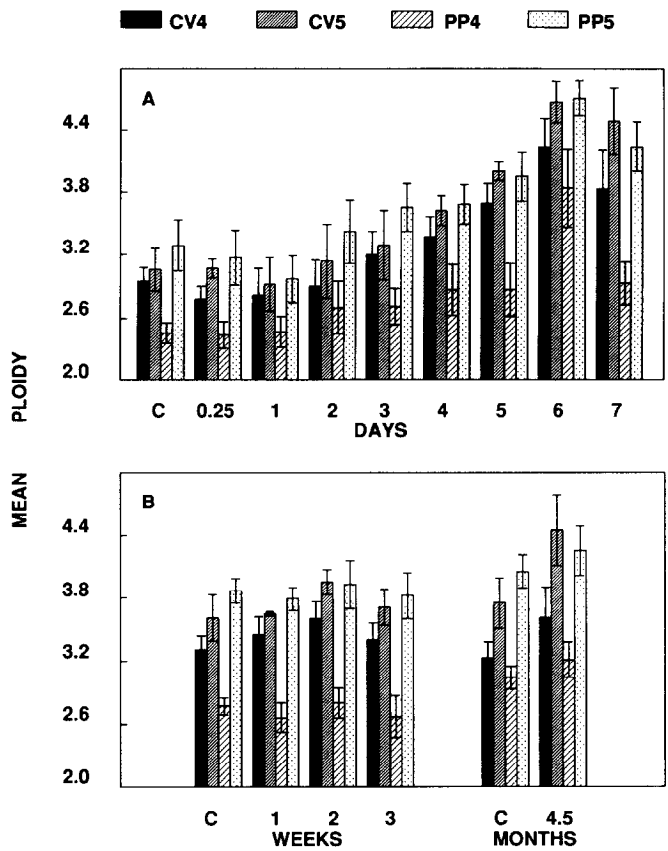

FIG. 4. Mean ploidy (N) of animals treated with chromium for 0.25–7 days, 2–4 weeks, or 4.5 months, based on IOD, demonstrating the relationship of ploidy to liver zone. In all cases but one (4.5-month control), the periportal zone 1–4 (PP4) had the lowest ploidy and the pericentral zone 1–4 (PC4) had the second lowest ploidy (e.g., the lower the ploidy, the higher the percentage of 2N cells). The outer periportal and pericentral zones 5–10 (PP5, PC5) were statistically identical to one another and had the highest ploidy values. Control (C) animals are age equivalent for their reported group (e.g., acute, intermediate, or chronic).

occurs near the portal vein. Older cells are displaced toward the central vein by subsequent cell divisions, with the oldest cells being shed into the central vein. As cells traverse this path through the 3 zones they undergo progressive differentiation related to their physiological function (Zajicek *et al.*, 1985). Our findings indicate that this model may not be able to account for the observed cell distribution pattern. If one assumes that an increase in cell ploidy is an irreversible process (Brodsky and Uryvaeva, 1985), then the lowest ploidy should be found among cells in zone 1 and the highest among cells in zone 3 in a "streaming liver." We found the lowest ploidy values in zone 1, which increased in zone 2, as expected. However, the observed drop in ploidy from zone 2 to zone 3 was unexpected, given that cell removal is reported to occur only at the central vein, and that reduction division of nuclei of high ploidy value to lower ploidy supposedly does not occur. There are several ways to produce the low ploidy observed in zone 3. Ploidy can be reduced by (1) a selective deletion of nuclei of high ploidy prior to reaching the central vein

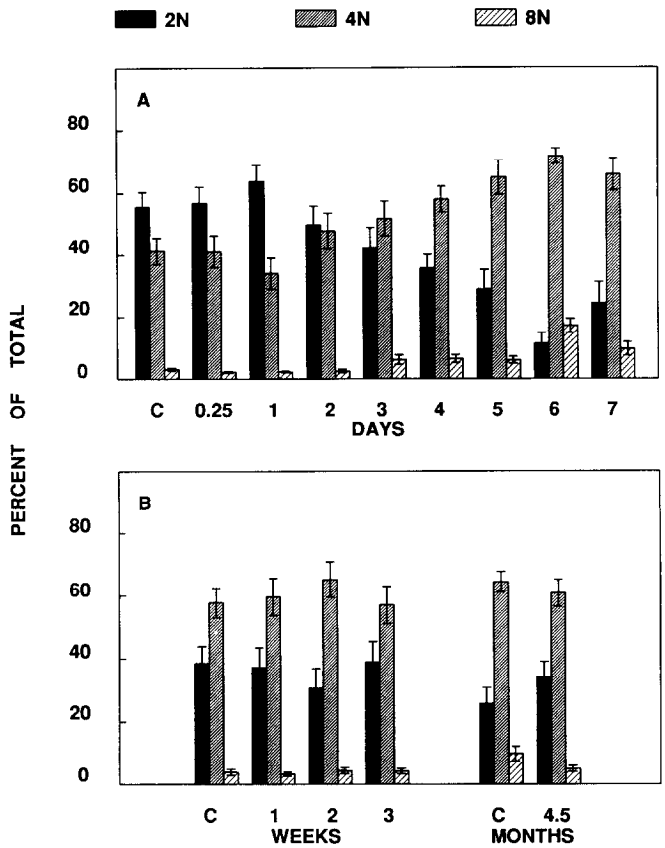

FIG. 5. Ploidy distribution (percentage 2N, 4N, and 8N cells), based on IOD, from chromium-treated mouse liver. Note the progressive decrease in diploid nuclei (and compensatory increase in tetraploid and octaploid nuclei) that occurs among animals treated on an acute basis (0.25–7 days). Animals treated for 4–5 days are different from control values only, those treated for 6–7 days are different from control animals and from animals treated for 0.25–3 days. Values given are the mean values from the four liver zones combined. Control (C) animals are age equivalent for their reported group.

(counter to the claims of Zajicek *et al.*, 1985), (2) the cells undergoing reduction division (counter to the claims of Brodsky and Uryvaeva, 1985), or (3) the 2N cells of zone 2–3 proliferating at a faster rate than the 4N and 8N cells, effecting a lower ploidy in the terminal region (zone 3). At the present time we have no evidence to support one hypothesis over the others. We are currently performing [^{3}H]thymidine labeling studies in an attempt to answer this question.

Not all xenobiotics cause increases in hepatocyte ploidy. Hepatocarcinogens such as phenobarbital have been shown to induce diploidization in liver foci, presumably by selecting for resistant cells (Deleener *et al.*, 1987; Haesen *et al.*, 1988). Thus the question should be addressed as to the role of increased ploidy in the liver as a response to aging, cell proliferation, or selected xenobiotic exposure. It has been suggested that the major advantage of polyploidization is gene amplification, and that polyploidization may increase the resistance of cells to genome injury because of this chromosome redundancy (Brodsky and Uryvaeva, 1985). As the liver is the primary site of detoxication, hepatocytes obviously need some mechanism for dealing

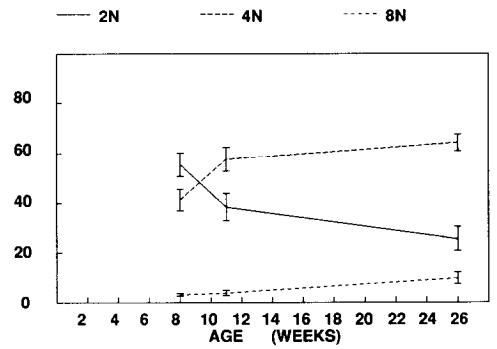

FIG. 6. Effect of age on liver ploidy values among control animals (56, 78, and 184 days) demonstrating the normal drop in percentage of 2N nuclei relative to 4N and 8N nuclei. The rate of polyploidization is relatively high during the first few months after birth (during the first two to three cycles of cell division) and then shows a marked decrease.

with genotoxicants. In addition, it has been shown that increases in ploidy are correlated with increased synthesis of RNA (Morselt and Wigjerden, 1975) and protein (Engelmann *et al.*, 1981), suggesting that polyploidization may also influence the secretory response to xenobiotics or cell metabolism.

A secondary goal of this study was the development of an efficient and objective methodology for determining ploidy from histological material. Invariably there are drawbacks to any procedure for determining ploidy. When working with histological material, many nuclei are incomplete, having been cut during the sectioning procedure. If all nuclei are measured, regardless of sectioning, the resulting distribution histograms are poorly differentiated (see Deleener *et al.*, 1987). Discarding incomplete nuclei from measurement tends to disproportionately reduce the percentage of large nuclei which are counted. Measuring relatively thick histological sections minimizes the percentage of unmeasured large nuclei, but increases the percentage of nuclei which cannot be measured because of overlap. This overlap phenomenon leads to an underestimate of the percentage of small nuclei, since (1) unlike octaploid cells, a large percentage of the diploid cells in the mouse liver are binucleate with the nuclei in close proximity to one another and (2) diploid cells are smaller than tetraploid and octaploid cells and thus more closely packed. We found that 12-μm-thick sections provide the best compromise thickness for dealing with sectioning and overlap problems.

The use of computer-assisted microscopy for measuring ploidy offers several advantages over traditional methods. Ploidy determinations based on flow cytometry or centrifugation/densitometry in combination allow rapid measurement of large numbers of cells from tissue homogenates (Steele *et al.*, 1981). However, these techniques suffer from two major drawbacks, on the basis of their use of homogenates: (1) the inability to derive positional (e.g., zonal) information and (2) the unavoidable contamination of hepatocytes with other cell types found in the liver. Manual microscopic observation, since it is based on histological material, allows regional measurement, but suffers because assignment of nuclei to various ploidy classes is subjective and usually does not take into allowance differences in density. Microspectrophotometry of single cells gives the densitometric values required for accurate DNA measurement and has been used to collect regional data on changes in nuclear ploidy (Haesen *et al.*, 1988). However, this technique is time-consuming and would be difficult to use for analyzing slight changes in ploidy in large cell populations. In contrast, computer-assisted microscopy-based measurement can be performed 100× to 1000× as fast as single-cell spectrophotometry. For these reasons, we feel that computer-assisted microscopy offers significant advantages over traditional methodologies for measuring nuclear ploidy.

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