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Use of Primary Cultures of Human Hepatocytes in Toxicology Studies[1]

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ABSTRACT

Often results from toxicological studies using rodent models cannot be directly extrapolated to probable effects in human beings. In order to examine the genotoxic potential of chemicals in human liver cells, a human hepatocyte DNA repair assay has been defined. Procedures were optimized to prepare primary cultures of human hepatocytes from discarded surgical material. On eight different occasions human hepatocyte cultures of sufficient viability to measure DNA repair were successfully prepared by collagenase perfusion techniques. The cells were allowed to attach to plastic or collagen substrata for periods of 1.5 to 24 h and subsequently incubated with [^{3}H]thymidine and test chemicals for periods of 18 to 24 h. Chemically induced DNA repair, measured as unscheduled DNA synthesis, was quantitated autoradiographically. The following compounds were tested: 2-acetylaminofluorene, aflatoxin B_1, 2-aminobenzyl alcohol, aniline, benzo(*a*)pyrene, carbon tetrachloride, chloroform, 2,4-diaminotoluene, 2,6-diaminotoluene, di(2-ethylhexyl)phthalate, dimethylnitrosamine, 1,6-dinitropyrene, 2,4-dinitrotoluene, 2,6-dinitrotoluene, methyl chloride, 5-methylchrysene, mono(2-ethylhexyl)phthalate, 2-methyl-2-*P*-(1,2,3,4-tetrahydro-1-naphthyl)phenoxypropionic acid (nafenopin), β-naphthylamine, nitrobenzene, 2-nitrobenzyl alcohol, 2-nitrotoluene, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, unleaded gasoline, and 4-chloro-6-(2,3-xylidino)-2-pyrimidinylthioacetic acid (Wy-14,643). In only one of eight cases did some of the chemicals generally regarded as genotoxic fail to give a positive response. For purposes of comparison, all test chemicals were evaluated in the *in vitro* rat hepatocyte DNA repair assay. Individual-to-individual variation in the DNA repair response was far greater for the human cultures than for cultures derived from rats. For only three chemicals was there a qualitative difference in the response between the rodent and the human cells; β-naphthylamine was positive in the rat but in none of the human cultures examined, whereas the opposite was seen for 2,6-diaminotoluene and 5-methylchrysene. Clofibric acid, mono(2-ethylhexyl)phthalate, and Wy-14,643 induced enzymes indicative of peroxisomal proliferation in primary rat hepatocyte cultures, but not in two human hepatocyte cultures. These results indicate that, in general, the *in vitro* rat hepatocyte DNA repair assay is a valid model for predicting potential genotoxic effects in human beings. However, rodent hepatocytes may not be appropriate for assessing the potential of chemicals to elicit nongenotoxic effects in human beings such as the induction of hepatocyte peroxisomal proliferation.

INTRODUCTION

Numerous genotoxicity assays successfully identify genotoxic and potential carcinogenic activities of chemical agents in a variety of cell culture and whole animal systems (1–4). However, most of this work deals with the correlation of genotoxic activity in bacterial and rodent cell culture models with carcinogenic activity observed in rodent bioassays. Given the large degree of species specificity observed in experimental carcinogenesis and the central role that rodent bioassays have in public cancer policy, it is important to establish the degree to which nonhuman models actually predict potential events in human cells. DNA repair assays in primary hepatocyte cultures are particularly valuable in that the cells are metabolically competent and reflect with reasonable accuracy conditions and events that are likely to occur in the intact liver (5, 6). The ability to prepare primary human hepatocyte cultures from discarded surgical material provided the means to compare and contrast the DNA repair response in the rat *versus* the human systems (7).

While genotoxicity assays are very good at detecting carcinogens the primary biological activity of which is alteration of the DNA, they fail to detect numerous distinctly different classes of nongenotoxic carcinogens that appear to be acting primarily through a variety of nongenotoxic mechanisms (4, 8). Differences in tissue and species specificity and carcinogenic potency can be dramatic for these nongenotoxic carcinogens. Use of human cell culture models may be useful in addressing issues of relevance for these agents as well. One class of nongenotoxic hepatocarcinogen is represented by diverse chemicals such as DEHP,[3] clofibric acid, and Wy-14,643 that induce peroxisomal proliferation and liver hyperplasia in treated animals (9–11). It has been suggested that these events play a role in the carcinogenic process for this class of carcinogen. Therefore, the ability of these chemicals to induce enzymes indicative of peroxisomal proliferation was also compared in rat *versus* human primary hepatocyte cultures.

MATERIALS AND METHODS

Chemicals. Concentrated stock solutions of test chemicals were prepared in DMSO or water, depending on solubility. These were added to WEI Flow Laboratories (McLean, VA) containing 10 μCi/ml [^{3}H]-thymidine to make the medium in which the cells were incubated. The highest concentration of DMSO ever used was 1% (v/v). Lower concentrations of the chemical were prepared by serial dilution in WEI containing [^{3}H]thymidine. 2-AAF (reagent grade), 2-ABA (98%), benzo(*a*)pyrene (99 + %), DMN (>99%), 2-nitrobenzyl alcohol (97%), and 2-NT (99%) were from Aldrich (Milwaukee, WI). Aflatoxin B_1 (A grade) was purchased from Calbiochem (San Diego, CA). Aniline HCl was from Eastman (Rochester, NY). Carbon tetrachloride, chloroform, and nitrobenzene (ACS certified) were purchased from Fischer (Raleigh, NC). Methyl chloride (99.9%) was from Matheson Gas Products (Morrow, GA). β-NA (reagent grade) and clofibric acid were from Sigma (St. Louis, MO). DEHP (99.8%) was supplied by Dr. John Hodgson, Tenneco Chemicals (Saddle Brook, NJ). 2,4-DNT (99.98%), 2,6-DNT (99.95%), 2,4-DAT (99.98%), and 2,6-DAT (99.963%) were specially purified and supplied by Air Products and Chemicals, Inc. (Allentown, PA). 1,6-Dinitropyrene (>99%) was a gift of Dr. Robert Mermelstein of Xerox Corporation (Rochester, NY). 5-MC (>99.9%) was from the Bureau of References of the Commission of the European Communities (Brussels, Belgium). MEHP was the generous gift of Dr. Daniel Wierda, West Virginia University (Morgantown, WV). 2-Methyl-2-*P*-(1,2,3,4-tetrahydro-1-naphthyl)phenoxypropionic acid (na-

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[3] The abbreviations used are: DEHP, di(2-ethylhexyl)phthalate; UDS, unscheduled DNA synthesis; 2-AAF, 2-acetylamino-fluorene; 2-ABA, 2-aminobenzyl alcohol; 2,4-DAT, 2,4-diaminotoluene; 2,6-DAT, 2,6-diaminotoluene; 2,4-DNT, 2,4-dinitrotoluene; 2,6-DNT, 2,6-dinitrotoluene; 5-MC, 5-methylchrysene; MEHP, mono(2-ethylhexyl)phthalate; 2-NT, 2-nitrotoluene; Wy-14,643, 4-chloro-6-(2,3-xylodino)-2-pyrimidinylthioacetic acid; β-NA, β-naphthylamine; DMSO, dimethyl sulfoxide; WEI, Williams Medium E without serum; WEC, Williams Medium E containing 10% heat inactivated fetal bovine serum; NG, net grains per nucleus; DMN, dimethylnitrosamine.

Table 1 *DNA repair response*

Chemical	Concentration (mM)	Case 1		Case 2		Case 5	
		NG ± SE[a]	% IR ± SD[b]	NG ± SE[c]	% IR ± SD[b]	NG ± SE[d]	% IR ± SD[b]
Media control		−4.8 ± 3.6[e]	0	−0.5 ± 0.1	3 ± 4	−2.1 ± 0.2	0 ± 0
Solvent control	1% DMSO	−4.6 ± 0.8	1 ± 0	−1.2 ± 0.5	5 ± 1	−4.2 ± 0.2	0 ± 0
2-AAF	0.001	17.8 ± 6.4[f]	82 ± 21	11.2 ± 1.1[f]	81 ± 10[g]	−2.9 ± 0.5	0 ± 0
	0.01	19.1 ± 2.5[f]	90 ± 3	4.4 ± 1.1[f]	41 ± 18	1.7 ± 1.1[f]	27 ± 9
	0.1	12.2 ± 0.3[f]	75 ± 1	6.4 ± 0.4[f]	62 ± 9	Toxic[h]	
Aflatoxin B_1	0.0001			2.7 ± 0.4[f]	34 ± 2	−2.4 ± 0.5	0 ± 0
	0.001	15.5 ± 2.7[f]	79 ± 4	12.1 ± 0.1[f]	86 ± 5	Toxic[i]	
	0.01	25.0 ± 3.6[f]	98 ± 4	16.8 ± 1.2[f]	91 ± 7		
	0.1	25.6 ± 1.4[f]	99 ± 1				
Aniline	0.01			−0.8 ± 0.3	6 ± 4		
	0.1			−1.8 ± 0.5	7 ± 5		
	1.0			−2.6 ± 0.4	3 ± 3		
Benzo(*a*)pyrene	0.001	6.5 ± 8.6[e,f]	49	5.5 ± 1.3[f]	53 ± 10		
	0.01	7.4 ± 7.0[e,f]	61	9.8 ± 0.5[f]	85 ± 5		
	0.1[j]	2.8 ± 0.5[f]	33 ± 13	9.4 ± 0.9[f]	80 ± 10	−2.5 ± 0.1	0 ± 0
2,4-DAT	0.01	−1.5 ± 1.0	6 ± 4	−0.3 ± 0.2	7 ± 5		
	0.1	−1.1 ± 1.6	15 ± 6	2.7 ± 0.7[f]	30 ± 9		
	1.0	11.3 ± 0.1[f]	74 ± 11	9.8 ± 2.1[f]	75 ± 21		
2,6-DAT	0.01	−1.0 ± 0.6	14 ± 1	0.3 ± 0.5[f]	14 ± 12		
	0.1	2.4 ± 1.8[f]	29 ± 16	5.6 ± 1.4[f]	54 ± 18		
	1.0	14.5 ± 1.2[f]	86 ± 2	9.3 ± 0.6[f]	79 ± 4		
DEHP	0.1	−3.3 ± 0.6	4 ± 2	−0.8 ± 0.5	4 ± 5		
	1.0	−5.4 ± 0.6	2 ± 1	−1.1 ± 0.3	6 ± 4		
	10[j]	−4.8 ± 0.6	1 ± 0	−2.5 ± 0.2	5 ± 4		
DMN	0.1	−1.1 ± 5.2[e]	6	7.2 ± 1.2[f]	63 ± 10	1.8 ± 1.2[f]	33 ± 9
	0.5	3.6 ± 1.7[f]	36 ± 13				
	1.0	6.6 ± 0.9[f]	52 ± 7	14.9 ± 0.8[f]	91 ± 6	5.0 ± 1.1[f]	47 ± 19
	10			21.0 ± 1.7[f]	96 ± 2[g]	8.9 ± 1.0[f]	83 ± 5
1,6-Dinitropyrene	0.00005			23.6 ± 1.4[f]	98 ± 2[g]	−0.3 ± 1.0	3 ± 5
	0.0005			34.4 ± 1.8[f]	100 ± 0[g]	5.5 ± 1.6[f]	47 ± 28
	0.005			37.0 ± 4.0[f]	99 ± 1[g]	35.4 ± 7.6[f]	93 ± 9
2,4-DNT	0.01	−4.9 ± 1.0	4 ± 1	−1.9 ± 0.3	4 ± 5		
	0.1	−2.9 ± 1.1	6 ± 1	−0.1 ± 0.1	12 ± 2		
	1.0	−2.5 ± 0.4	0 ± 0	Toxic[i]			
2,6-DNT	0.01	−3.5 ± 0.3	1 ± 1	−0.2 ± 0.6	8 ± 7		
	0.1	−2.7 ± 0.5	4 ± 4	−0.3 ± 1.2	10 ± 10		
	1.0	−2.6 ± 0.5	3 ± 1	−0.7 ± 0.5	5 ± 3		
Methyl chloride	0.1%					−1.0 ± 0.3	3 ± 5
	0.3%			−2.2 ± 0.3	0 ± 0	−1.4 ± 0.2	0 ± 0
	1%			−1.8 ± 0.3	0 ± 0	1.5 ± 0.4[f]	10 ± 5
	3%			Toxic[i]		Toxic[h]	
β-NA	0.01	−5.5 ± 1.0	2 ± 1	−0.8 ± 0.2	5 ± 2		
	0.1	−6.0 ± 0.8	3 ± 2	−2.7 ± 0.7	5 ± 2		
	1.0	Toxic[i]		Toxic[i]			
2-NT	0.01			−1.2 ± 0.3	6 ± 3		
	0.1			−1.2 ± 0.2	7 ± 7		
	1.0			−1.8 ± 0.6	4 ± 4		
Nitrobenzene	0.01			−0.6 ± 0.5	7 ± 4		
	0.1			−1.5 ± 0.4	9 ± 6		
	1.0			−2.1 ± 0.4	11 ± 5		
TCDD[k]	0.001			−1.1 ± 0.6	0 ± 0		
	0.01			−1.8 ± 0.1	0 ± 0		
	0.1[j]			−3.8 ± 0.5	1 ± 1		

[a] One hundred cells were counted for each of two slides. SE is slide-to-slide variation.
[b] An individual cell with ≥5 NG was considered in repair (IR).
[c] Fifty cells were counted for each of three slides. SE is slide-to-slide variation.
[d] Fifteen cells were counted for each of two slides. SE is slide-to-slide variation.
[e] Only one slide counted. Variation is cell-to-cell.
[f] Greater than the average of the control slides by the unpaired *t* test for the equality of two means at $P \leq 0.05$.
[g] Published data from this laboratory (13).
[h] Those few cells remaining did exhibit DNA repair.
[i] Those few cells remaining did not exhibit DNA repair.
[j] Solubility exceeded.
[k] TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

fenopin) was obtained from Ciba-Geigy (Summit, NJ). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin was provided by Drs. Tadashi Sawahata and William Greenlee of this Institute. Unleaded gasoline (PS-6) was provided by the American Petroleum Institute (Washington, DC) and was the same lot as that used to induce kidney tumors in male rats and liver tumors in female mice (12). Wy-14,643 was obtained from Wyeth Laboratories, Inc. (Philadelphia, PA).

Human Hepatocyte Preparation. Fresh human tissue was obtained as excess material from prescribed surgery. Small portions of apparently healthy tissue not needed for pathological examination were placed in ice-cold saline and transported to the laboratory. Catheters were inserted in the larger vessels on the cut surface, the tissue was perfused with a collagenase solution, and a primary hepatocyte culture was established as described previously (7). Details of incubation procedures are presented for each case individually.

UDS Experiments. Induction of DNA repair in the human cultures was performed as described previously (13). Briefly, the hepatocytes were incubated in media containing the test chemical and 10 μCi/ml [^{3}H]thymidine. Details of incubation, washing, and fixing procedures are presented for each case individually. The primary rat hepatocyte DNA repair assays were performed as described previously (6).

Autoradiography and Evaluation of Results. Slides were air-dried, dipped in NTB-2 photographic emulsion (Kodak, Rochester, NY) diluted 1:1 with water and exposed for 8 days at −20°C. Slides were developed and scored as described previously (14). Silver grains over the nucleus minus the grains over an equal-sized area in the cytoplasm was defined as net grains per nucleus and quantitated with an automatic grain counter. A negative number indicates there were more grains per unit area in the cytoplasm than in the nucleus. As a conservative estimate, any individual cell with greater than or equal to 5 NG was considered in repair. The percentage of cells in repair was also calculated as an indication of the extent of the response among the cells. Historical observations with rat hepatocytes indicate that if a chemical induces greater than or equal to 5 NG (population average) and greater than or equal to 20% of cells in repair, the response can be considered positive (6). A population average between 0 NG and 5 NG would be considered a marginal response. Because human samples differ so much from each other it was not possible to establish tight historical controls or strict criteria to define a positive response. Control cells from the same preparation do represent a true concurrent control. The unpaired *t* test for the equality of two means with the NG counts (population average) of the individual slides as the unit of measure is an appropriate statistical test for these data (6). This was the statistical test used in these studies, with a significance level of ≤ 0.05. When using this statistical test for rat hepatocytes, NG values of greater than zero usually score as positive, because control values tend to be about −5 NG and it is rare to see a value above zero. Note, however, that there is greater case-to-case variability in the control NG counts for the human cells. For example, the NG value for the case 1 media control was −4.8 NG, while that for the average of case 2 media control slides was only −0.5 NG. During the course of these experiments, several of the individual control slides scored above zero, but the average for any one experiment was always negative. Thus, other factors beyond strict statistical significance, such as a dose-response relationship, should also be factored into the decision to score a response as positive. The probable reason that control NG values tend to be less than zero is that the cytoplasm (and the components therein producing the cytoplasmic background) is slightly thinner over the nucleus compared to the rest of the cell as it sits on the substrate. NG counts may also vary as the result of compound-related effects on cytoplasmic grain counts. Consequently, no result may be considered positive unless the compound actually produces more grains over the nucleus than over the cytoplasm, *i.e.*, a NG value greater than zero. Knowledge of the biology of this assay dictates that in order to have any confidence in a positive DNA repair response, the treatment must produce nuclear counts beyond the cytoplasmic background. Thus, for any statistical test used, a lower limit of at least 0 NG is required for a positive response (6).

Induction of Peroxisomal Enzymes. Culture conditions were optimized for rat hepatocytes (15). Cells were allowed to attach to collagen-coated 100-mm tissue culture dishes for 24 h and incubated in WEC containing hydrocortisone, insulin, and the test chemical for 48 or 72 h. Cells from 3 dishes were scraped into 1 ml of 154 mM KCl-50 mM Tris buffer, sonicated, and assayed for palmitoyl-CoA oxidase and carnitine acetyltransferase activity as described previously (16). Three replicates were assayed for each data point.

RESULTS

During the course of this investigation various modifications of the procedures were tried in order to optimize the protocol for the human hepatocytes. To more clearly relate the effects of each modification, each procedure is presented with the outcome on the success of the cultures and the DNA repair experiments.

Case 1. The patient was a 30-year-old female admitted for surgical resection of two suspected benign liver tumors. Hepatocyte viability was 78% as measured by trypan blue exclusion. Approximately 15,000 cells in WEC were plated in Linbro 35-mm, 6-well cluster dishes (Flow Laboratories), containing Thermanox plastic coverslips (Miles Laboratories, Naperville, IL). After an attachment period of 3 h the cultures were washed with WEI. Attachment was poor. For comparison, this procedure would have resulted in almost confluent cultures had rat hepatocytes been used. The human hepatocytes were then incubated for 18 h with WEI-containing [^{3}H]thymidine and the test chemical. Cells were washed and fixed as described previously for rat hepatocytes (14). Following this procedure there were insufficient cells to score.

Additionally, approximately 80,000 hepatocytes were plated in 0.3 ml of WEC in each well of a rat-tail collagen-coated 8-well "chamber/slide" (Lab Tech Products, Naperville, IL). Cells were allowed to attach for 2 h. In contrast to the Thermanox coverslips, the attachment in the chamber/slides was very good. Cells were incubated for 18 h in the presence of 10 μCi/ml [^{3}H]thymidine and the test chemical. The slides were washed three times in WEI, dipped in successive containers containing 4 liters of 0.9% saline, swollen for 8 min in 1% sodium citrate, fixed for 2 × 10 min in acetic acid:methanol (1:5), soaked for 1 h in 10% phosphate-buffered formalin, and washed twice with water. Autoradiography and scoring were done as described in "Materials and Methods." Chemically induced DNA repair was clearly induced by a variety of genotoxicants (Table 1).

Case 2. Case 2 was a 6-year-old female who underwent surgery to remove a liver sarcoma. Cells were 86% viable. Approximately 84,000 cells were plated in 0.3 ml WEC in each well of a collagen-coated 8-well chamber/slide. Cells were allowed to attach for 1.5 h. The cells were then washed and incubated for 18 h in WEI with 10 μCi/ml [^{3}H]thymidine and test chemicals. Some slides were sealed into 1.9-liter GasPak chambers (Baltimore Biological Laboratories, Cockeysville, MD) and 9 changes of an atmosphere containing a predetermined concentration of methyl chloride were pumped through the chamber. Diluted methyl chloride was prepared by mixing a measured volume of methyl chloride with a measured volume of 5% CO_2 in air in a Saran bag (Anspec Inc., Ann Arbor, MI). Slides were incubated in these atmospheres in WEI and the [^{3}H]thymidine as described previously (17). Slides were washed and fixed as in case 1. Chemically induced DNA repair was clearly induced by a variety of genotoxicants (Table 1).

Case 3. Case 3 was a 17-year-old motorcycle accident victim who was brain dead and placed on a respirator as a possible organ donor. The cells were 86% viable, but the cytoplasm contained large numbers of fatty vacuoles, presumably because individual was being kept on a respirator. Approximately 120,000 cells/well in 0.3 ml were plated in WEC in each well of a collagen-coated 8-well chamber/slide. Cells were allowed

Table 2 *DNA repair response*

Chemical	Concentration (mM)	Case 12		Case 13		Case 14	
		NG ± SD[a]	% IR ± SD[b]	NG ± SD[a]	% IR ± SD[b]	NG ± SD[a]	% IR ± SD[b]
Media control		−5.5 ± 5.1	2	−1.0 ± 4.3	8	−6.4 ± 5.7	2
Solvent control	1% DMSO	−2.7 ± 4.7	3	−0.3 ± 4.4	12	−5.8 ± 4.4	1
2-AAF	0.001			21.6 ± 11.3[c]	99	1.3 ± 7.4[c]	32
	0.01			33.3 ± 15.0[c]	99	5.6 ± 7.8[c]	47
	0.1	28.8 ± 12.1[c]	100	33.0 ± 11.2[c]	100	2.8 ± 7.7[c]	39
Aflatoxin B_1	0.0001	12.3 ± 16.7[c]	60	15.6 ± 9.0[c]	88	16.4 ± 9.3[c]	91
	0.001	9.1 ± 13.4[c]	58	24.9 ± 13.1[c]	97	59.5 ± 21.0[c]	100
	0.01	31.0 ± 11.9[c]	100	34.6 ± 16.3[c]	99	61.8 ± 20.4[c]	100
2-ABA	0.01	33.5 ± 14.5[c]	100	64.8 ± 21.6[c]	100	7.0 ± 8.7[c]	57
	0.1	25.0 ± 9.7[c]	99	63.8 ± 30.8[c]	100	89.8 ± 30.4[c]	100
	1.0	50.7 ± 19.3[c]	100	56.6 ± 18.6[c]	100	94.9 ± 35.6[c]	100
Aniline	0.01	−4.4 ± 5.6	6	−2.5 ± 5.3	6	−4.3 ± 4.9	3
	0.1	−4.8 ± 4.0	1	−0.6 ± 2.6	7	−7.0 ± 6.8	1
	1.0	−1.3 ± 4.0	5	−1.3 ± 3.0	0	−7.8 ± 9.1	3
Benzo(*a*)pyrene	0.001			11.5 ± 6.7[c]	87	18.7 ± 9.4[c]	94
	0.01			21.6 ± 11.4[c]	95	11.8 ± 8.8[c]	83
	0.1[d]			18.5 ± 9.1[c]	98	26.5 ± 11.6[c]	97
CCl_4	0.01	−4.7 ± 5.0	1	−2.0 ± 6.1	10	−8.1 ± 5.6	1
	0.1	−4.3 ± 6.0	3	−3.2 ± 4.1	1	−10.3 ± 10.1	5
	1.0			−3.5 ± 3.8	3	−3.0 ± 4.3	2
Chloroform	0.01			−1.8 ± 4.3	5	−5.5 ± 4.2	1
	0.1	−4.8 ± 5.2	3	−0.4 ± 3.5	6	−8.0 ± 7.5	1
	1.0	−6.8 ± 5.3	1	−0.1 ± 3.3	5	−9.8 ± 7.4	1
DEHP	0.01	−5.1 ± 5.4	0				
	0.1	−5.2 ± 5.1	2				
	1.0	−4.1 ± 4.8	2				
DMN	0.1	2.8 ± 7.1[c]	37	17.2 ± 9.3[c]	94	20.1 ± 10.3[c]	97
	1.0	27.8 ± 13.8[c]	96	33.2 ± 14.9[c]	99	24.2 ± 13.7[c]	96
	10	31.6 ± 17.9[c]	93	30.8 ± 13.3[c]	98	26.1 ± 13.4[c]	95
1,6-Dinitropyrene	0.000005	−2.7 ± 4.5	3				
	0.00005	8.5 ± 8.3[c]	65	41.2 ± 14.1[c]	100		
	0.0005	39.9 ± 15.2[c]	100	46.2 ± 13.6[c]	100		
	0.005	37.5 ± 12.6[c]	100	72.6 ± 23.5[c]	100		
					100		
Methyl chloride	2%	Toxic (3 h)[e]					
	5%	Toxic (3 h)[f]					
	10%	Toxic (18 h)[f]					
5-MC	0.001					1.3 ± 5.9[c]	29
	0.01			23.4 ± 12.8[c]	97	24.1 ± 10.0[c]	81
	0.1			16.9 ± 10.8[c]	84	8.4 ± 7.5[c]	67
MEHP	0.01					−4.1 ± 5.4	5
	0.1	−6.7 ± 6.2	2	0.0 ± 5.1	14	−5.2 ± 5.9	3
	0.2	−2.4 ± 4.1	3	−0.9 ± 4.5	9		
	0.5	−1.7 ± 3.8	3	Toxic[g]			
	1.0					−2.7 ± 4.3	5
β-NA	0.01	−3.1 ± 4.0	3	0.3 ± 3.3	8	−7.0 ± 6.5	2
	0.1	−3.5 ± 4.3	1	Toxic[g]		−6.4 ± 7.7	7
	1.0	Toxic[f]				Toxic[h]	
Nitrobenzene	0.01	−2.6 ± 4.1	3	−2.8 ± 3.6	2	−5.6 ± 3.7	0
	0.1	−5.9 ± 5.6	0	−1.9 ± 3.6	3	−6.4 ± 3.9	0
	1.0	−6.6 ± 4.6	1	−3.6 ± 3.3	1	−7.1 ± 4.7	1
2-Nitrobenzyl alcohol	0.01	−2.9 ± 6.7	9	−3.7 ± 6.2	7	−2.6 ± 4.5	3
	0.1	−4.4 ± 5.9	4	−3.4 ± 5.4	5	−5.3 ± 5.4	1
	1.0	−4.9 ± 5.7	2	−3.5 ± 6.5	5	−8.0 ± 4.5	0
2-NT	0.01	−6.4 ± 4.8	0	−3.4 ± 6.0	7	−5.8 ± 6.7	5
	0.1	Toxic[f]		Toxic[g]		−5.3 ± 6.4	3
	1.0					−8.0 ± 7.5	3
TCDD[i]	0.000002	−6.8 ± 7.0	3	−0.1 ± 4.0	10	−5.8 ± 4.4	1
	0.00002	−5.0 ± 5.7	4	−2.9 ± 3.3	1	−5.9 ± 4.7	0
	0.0002	−4.5 ± 5.0	3	−1.7 ± 3.9	2	−5.3 ± 4.9	1
Unleaded gasoline	0.1%			Toxic[j]		−5.4 ± 1.3[k]	7
	0.33%					Toxic[j]	
	1%					Toxic[j]	

Table 2—*Continued*

Chemical	Concentration (mM)	Case 12		Case 13		Case 14	
		NG ± SD[a]	% IR ± SD[b]	NG ± SD[a]	% IR ± SD[b]	NG ± SD[a]	% IR ± SD[b]
Media control (unleaded gasoline)						−4.4 ± 5.7[k]	3
DMN (unleaded gasoline control)	10					79.2 ± 18.1[c]	100

[a] One hundred fifty cells were counted on one slide. SD is cell-to-cell variation.
[b] An individual cell with ≥5 NG was considered in repair (IR).
[c] Greater than the average of the cells on the solvent control slide by the unpaired *t* test for the equality of two means at $P \leq 0.05$.
[d] Solubility exceeded.
[e] Those few cells remaining did exhibit DNA repair.
[f] No cells remaining.
[g] Few cells. For those remaining many are pyknotic and/or have missing cytoplasms or very few grains in the cytoplasm. While some cells appeared to be in repair, the effect was probably lowered cytoplasmic counts. The absolute nuclear counts were not elevated relative to the concurrent controls.
[h] While some cells appeared to be in repair, it was difficult to evaluate because cytoplasms were missing.
[i] TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.
[j] Cytoplasmic backgrounds were too high to evaluate the slides. In addition, 0.33 and 1.0% unleaded gasoline cultures were run. With the middle dose, the cytoplasm had abnormal morphology or was missing altogether. With the high dose no cytoplasm or even silver grains were present (other than the background on the slide). With the two lower doses it appeared that many of the cells did exhibit DNA repair, but it was difficult to evaluate because of the toxicity.
[k] Average of 3 slides. SD is slide-to-slide variation.
[l] Pyknotic cells with missing cytoplasms and few grains.

to attach for 18 h. The cells were then washed and incubated for 18 h with [^{3}H]thymidine and test chemicals as in case 2. Slides were washed individually with WEI in a 50-ml centrifuge tube, placed in a slide holder, and washed twice more in WEI. Cells were then washed 6 times for 1 min each in successive containers containing 4 liters of 0.9% saline. Cells were allowed to swell in 1% sodium citrate for 10 min and fixed in 10% phosphate-buffered formalin for 1 h in the cold room. Cells were then fixed for 2 × 10 min in methanol:acetic acid (1:5). Cells were washed twice with distilled water and allowed to air-dry. After autoradiography and development the cultures were very sparse. Those cells present either were pyknotic or had extremely high cytoplasmic grain counts and could not be scored.

Case 4. The patient was a 53-year-old male who underwent a partial liver resection. Cells were only 45% viable. Cells were allowed to attach for 2 h, were exposed to the test chemicals for 21 h, and were prepared as for Case 3. Following development, essentially no cells were observed on any of the slides.

Case 5. Case 5 was a 71-year-old female undergoing prescribed liver surgery. Cells were 71% viable. Approximately 90,000 cells/well were plated and processed as described in case 3. All of the chemicals and doses run for case 2 were run for case 5 in addition to 0.01, 0.1, and 1.0 mM 3-NT and 4-NT and 0.001, 0.01, and 0.1 mM 5-MC. The DNA repair response in this case was very weak. Although attachment was good, very few compounds produced an UDS response. Representative genotoxicants and all compounds that produced any positive response at all (upon visual examination) are shown in Table 1. All other compounds and concentrations were negative (data not shown). Because of the concerns raised by this uneven pattern of responses, this case will be omitted in some of the comparative discussions.

Cases 6 to 11. For cases 6 through 11 experiments were performed using the chemicals and techniques described in case 5. No experiment was acceptable because of failure of the cells to attach, poor morphology, and/or lack of a response to the known genotoxicants used.

Case 12. Case 12 was a 73-year-old male who underwent a left lobectomy for the removal of a metastatic tumor. Cells were 82% viable. It was decided to lengthen the attachment period and alter the substratum to see if better cultures could be achieved. Approximately 400,000 cells were plated in WEC on collagen-coated Thermanox plastic coverslips. After an attachment period of 20 h the cultures were washed with Williams Medium E and incubated for 24 h with the test chemicals and [^{3}H]thymidine. Attachment was good. Although the cytoplasmic backgrounds were elevated relative to the previous conditions, attachment and morphology were good and the slides were easily scorable. Even with the longer culture times, the cells retained the ability to metabolically activate the genotoxic carcinogens tested. Chemically induced DNA repair was induced by a variety of genotoxicants (Table 2).

Case 13. Case 13 was a 25-year-old female who underwent surgery for a colon cancer metastasis. Cells were 84% viable. The procedures for case 12 were used. Attachment was for 18 h in WEC supplemented with 100 μM hydrocortisone and 1 μM insulin (18). Treatment time was 21.5 in WEI supplemented with insulin. For the unleaded gasoline, cells were plated on the collagen-coated coverslips in 30-ml, screw top, round bottles containing 5 ml WEC. After attachment, the medium was aspirated to remove any unattached cells and replaced with 5 ml WEI containing 10 μCi/ml [^{3}H]thymidine. Unleaded gasoline was added directly into the bottles and partitioned into small droplets at the surface of the medium. The culture bottles were immediately sealed and incubated for 18 h. Chemically induced DNA repair was induced by the genotoxicants used (Table 2).

Case 14. Case 14 was a 16-year-old female suicide victim. Cells were 75% viable. Procedures from case 13 were followed. In this case cells were allowed to attach for 24 h in WEC containing a mixture of hormones (18), and incubated for 22 h in WEI containing the hormones. For the unleaded gasoline experiments 5.32 ml of incubation media were used. Chemically induced DNA repair was seen with the genotoxic chemicals used (Table 2).

Case 15. Patient was a 56-year-old male who underwent surgery for the removal of liver metastases. Cells were 87% viable. Procedures were those used in case 14. Cells were allowed to attach for 5 h. Treatment period was for 22 h. Chemically induced DNA repair was observed with a variety of genotoxicants (Table 3).

Case 16. The patient was a female who had undergone a liver resection at University of Pittsburgh, Pittsburgh, PA. Tissue was provided by Dr. T. Starzl of the Department of Surgery. The sample was packed in Euro-Collins media at 11:30 a.m. and transported to Duke University where it arrived to 11:30 p.m. Viability of the primary hepatocyte culture prepared from the tissue was 89%. Attachment was overnight. Exposure was for 18 h. Chemically induced DNA repair was produced by a

Table 3 *DNA repair response*

Chemical	Concentration (mM)	Case 15		Case 16		Rat hepatocytes	
		NG ± SD[a]	% IR ± SD[b]	NG ± SD[a]	% IR ± SD[b]	NG ± SE[c]	% IR ± SD[b]
Media control	1%	−3.9 ± 3.9	0			−8.5 ± 3.8	6 ± 5[d]
Solvent control	DMSO	−4.1 ± 5.5	5	−2.3 ± 3.1	0	−12.5 ± 1.8	0 ± 0[e]
2-AAF	0.001	−1.2 ± 3.6	5	9.8 ± 11.1[f]	67	26.4 ± 5.5[f]	97 ± 4[g]
	0.01	0.9 ± 3.9[f]	15	32.1 ± 20.5[f]	94	52.2 ± 0.9[f]	100 ± 0[g]
	0.1	Toxic[h]		24.9 ± 11.4[f]	96	65.6 ± 0.3[f]	99 ± 1[g]
Aflatoxin B_1	0.0001	5.9 ± 8.0[f]	54	22.4 ± 17.7[f]	90	28.5 ± 4.0[f]	99 ± 1[g]
	0.001	12.3 ± 6.4[f]	91	20.2 ± 10.7[f]	93	42.8 ± 7.4[f]	99 ± 1[g]
	0.01	129.7 ± 74.7[f]	100	47.9 ± 18.1[f]	99	47.1 ± 7.8[f]	100 ± 0[g]
2-ABA	0.001					−2.2 ± 1.8	16 ± 9[i]
	0.01					35.2 ± 14.4[f]	96 ± 10[j]
Aniline	0.01	−2.2 ± 3.1	0	−1.9 ± 3.2	0	−7.2 ± 4.5	0[g, j]
	0.1	−4.0 ± 4.6	3	−1.3 ± 3.2	4	−6.3 ± 0.7	4 ± 4[g]
	1.0	−0.6 ± 3.5	6	−0.3 ± 3.3	6	−7.1 ± 1.0	3 ± 3[g]
Benzo(*a*)pyrene	0.001	Toxic[k]		0.9 ± 3.6[f]	13	−0.1 ± 4.2	12[g, j]
	0.01	Toxic[k]		27.9 ± 20.5[f]	89	9.3 ± 1.2[e]	60 ± 3[g]
	0.1[l]	8.6 ± 12.7[f]	70	15.2 ± 12.2[f]	79	20.1 ± 6.2[e]	87 ± 17[g]
CCl_4	0.01	−2.0 ± 3.0	3				
	0.1	−2.0 ± 3.5	1				
	1.0	−0.6 ± 2.6	4				
Chloroform	0.01	−2.1 ± 3.7	5				
	0.1	−2.3 ± 3.8	3				
	1.0	−3.1 ± 4.2	1				
2,4-DAT	0.01					8.1 ± 0.5[f]	70[m]
	0.1					7.6 ± 1.3[f]	60[m]
	1.0					0.7 ± 4.2	35[m]
2,6-DAT	0.01					−8.1 ± 1.0	7 ± 4[e]
	0.1					−9.3 ± 2.0	4 ± 7[e]
	1.0					Toxic[e]	
DEHP	0.1					−7.3 ± 0.8	5 ± 1[n]
	1.0					−8.7 ± 6.3	8 ± 3[n]
	10.0[l]					−7.7 ± 6.2	6 ± 3[n]
DMN	0.1	15.5 ± 18.0[f]	78	39.7 ± 18.2[f]	99	3.7 ± 1.3[f]	38 ± 6[e]
	1.0	53.8 ± 35.3[f]	91	43.8 ± 23.8[f]	99	22.4 ± 2.3[f]	91 ± 1[e]
	10	42.2 ± 19.5[f]	100	71.7 ± 37.8[f]	100	32.1 ± 2.6[f]	96 ± 5[e]
1,6-Dinitropyrene	0.00005	73.9 ± 33.7[f]	100			28.6 ± 8.4[f]	93 ± 5[o]
	0.0005					49.0 ± 6.9[f]	99 ± 1[o]
	0.005	165.1 ± 52.6[f]	100			56.9 ± 0.2[f]	98 ± 2[o]
2,4-DNT	0.1					−2.9 ± 2.2	20[m]
	1.0					−0.6 ± 2.3	22[m]
2,6-DNT	0.1					−3.4 ± 1.8	6[m]
	1.0					−1.8 ± 1.7	12[m]
Methyl chloride	0.1%					−6.7 ± 3.0	7 ± 5[p]
	0.3%					−2.6 ± 1.6	16 ± 5[p]
	1.0%					3.7 ± 2.6[f]	40 ± 18[p]
	3.0%					Toxic[p]	
5-MC	0.001	Toxic[k]				−11.1 ± 3.6	4 ± 4[e]
	0.01	1.7 ± 5.5	28			−4.7 ± 0.6	11 ± 10[e]
	0.1	Toxic[k]				−4.8 ± 1.2	18 ± 11[e]
MEHP	0.01	−2.1 ± 3.5	2				
	0.1	−2.8 ± 3.7	3			−11.8 ± 7.2	2[q]
	0.2					−12.5 ± 7.5	4[q]
	0.5					−8.6 ± 6.2	2[q]
	1.0	Toxic[k]					
Nafenopin	0.001	−3.3 ± 3.9	1			−2.1 ± 3.8	4[q]
	0.01	−1.0 ± 2.8	2			−2.2 ± 4.6	3[q]
	0.1	Toxic[k]				Toxic[q]	
β-NA	0.01	−0.4 ± 3.3	8			−1.9 ± 2.2	22 ± 19[d]
	0.1	−0.2 ± 3.7	9			15.4 ± 7.3[f]	73 ± 18[d]
	1.0	−0.9 ± 4.1	5			Toxic[d]	
Nitrobenzene	0.01					−6.1 ± 3.0	7 ± 8[g]
	0.1					−9.3 ± 4.7	1 ± 1[g]
	1.0	−2.9 ± 3.6	1			−6.0 ± 1.0	5 ± 5[g]

Table 3—*Continued*

Chemical	Concentration (mM)	Case 15		Case 16		Rat hepatocytes	
		NG ± SD[a]	% IR ± SD[b]	NG ± SD[a]	% IR ± SD[b]	NG ± SE[c]	% IR ± SD[b]
2-Nitrobenzyl alcohol	0.01					−4.0 ± 2.2	11 ± 2[i]
	0.1					−3.5 ± 2.9	9 ± 2[i]
	1.0					−1.5 ± 1.1	11 ± 6[i]
2-NT	0.01	Toxic[k]		−2.3 ± 3.7	0	−3.0 ± 1.4	7 ± 6[r]
	0.1	−1.6 ± 4.7	10	−2.2 ± 3.0	1	−8.0 ± 2.5	4 ± 5[r]
	1.0	−2.5 ± 4.3	4	−0.7 ± 2.8	5	Toxic[r]	
TCDD[s]	0.000002	−1.8 ± 3.3	0				
	0.00002	Toxic[k]					
	0.0002	−2.0 ± 4.6	5				
	0.001					−7.6 ± 1.5	7 ± 6[e]
	0.01					−8.2 ± 2.9	7 ± 6[e]
	0.1[l]					−11.8 ± 2.8	2 ± 2[e]
Unleaded gasoline	0.01%	1.3 ± 0.6[f,t,m]	25	−2.4 ± 0.5[v]	1	−13.5 ± 0.8	2[n]
	0.05%	Toxic[k,m]		−2.0 ± 0.9[v]	0	6.8 ± 3.0[f]	53[n]
	0.1%	Toxic[k]		−1.5 ± 0.5[v]	0	17.6 ± 1.6[f]	78[n]
	0.2%	Toxic[k]				Toxic[v]	
Media control (unleaded gasoline)		−5.4 ± 1.0[f,m]	1	−1.4 ± 0.9[f]	0	−13.3 ± 1.2	2[n]
DMN (unleaded gasoline control)	10	63.8 ± 23.2[f]	100			201 ± 8[e]	100[n]
Wy-14,643	0.001	−2.5 ± 3.7	1			−1.9 ± 4.0	6[q]
	0.01	−3.0 ± 4.4	1				
	0.1	−3.2 ± 3.4	0			−3.0 ± 3.3	1[q]
	0.5	−2.7 ± 2.7	0				
	1.0	Toxic[k]				−4.2 ± 4.2	0[q]

[a] One hundred cells were counted on one slide. SD is cell-to-cell variation.
[b] An individual cell with ≥5 NG was considered in repair (IR).
[c] Fifty cells were counted on one slide for each of three slides. SE is slide-to-slide variation. Presented are typical results selected from several experiments performed during the course of the human experiments. The same source of chemical was used in these and the human studies. Sometimes the rat experiments were performed the day following one of the experiments with the human hepatocytes, so that the same DMSO stock solutions of chemicals could be used.
[d] Data from this laboratory (experiment H7).
[e] Data from this laboratory (experiment H9).
[f] Greater than the average of the cells on the control slides by the unpaired *t* test for the equality of two means at $P \leq 0.05$.
[g] Data from this laboratory (experiment H8).
[h] Difficult to evaluate because cytoplasms were missing.
[i] Data from this laboratory (experiment H117).
[j] Only one slide counted. SD is cell-to-cell variation.
[k] Cells pyknotic and sparse with few grains.
[l] Solubility exceeded.
[m] Published data from this laboratory (32).
[n] Published data from this laboratory (16).
[o] Published data from this laboratory (13).
[p] Published data from this laboratory (17).
[q] Published data from this laboratory (36).
[r] Published data from this laboratory (31).
[s] TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.
[t] Average of three slides. SE is slide-to-slide variation.
[u] Published data from this laboratory (35).
[v] Average of four slides. SE is slide-to-slide variation.

variety of genotoxicants (Table 3).

Rat Hepatocyte Experiments. DNA repair experiments using rat hepatocytes were conducted throughout the course of this study. Table 3 presents typical results selected from several such experiments. The same source of chemical was used in these and the human studies. In some cases the rat experiments were performed the day following one of the human experiments, so that the same DMSO stock solution of chemical could be used. Results for 0.001 mM 2-AAF are presented for three typical rat hepatocyte experiments to contrast animal-to-animal variability with that observed for the human samples (Table 4).

Table 4 *DNA repair induced by 0.001 mM 2-AAF in rat hepatocytes*

Experiment	NG ± SE[a]	% IR ± SD[b]
H7	21.4 ± 6.8	80 ± 23
H8	26.4 ± 5.5	97 ± 4
H9	20.0 ± 1.4	93 ± 5

[a] Fifty cells were counted on one slide for each of three slides. SE is slide-to-slide variation. Presented are results for 0.001 mM 2-AAF from several experiments performed during the course of the human experiments. The same source of chemical was used throughout.
[b] An individual cell with ≥5 NG was considered in repair (IR).

Induction of Peroxisomal Enzymes. The induction of peroxisomal enzymes by various peroxisomal proliferators was measured in primary rat hepatocytes and in primary human hepatocyte cultures from cases 14 and 15 (Table 5). Activities of palmitoyl-CoA oxidase, a peroxisome-specific enzyme, and carnitine acetyltransferase, a peroxisomal enzyme also found in mitochondria, were both examined. No enzyme induction was observed for the human cultures, while the rat hepatocytes all responded. These results indicate that the human hepatocytes are less responsive to peroxisomal enzyme induction by these agents than the rat hepatocytes under the conditions used.

DISCUSSION

The following factors affected the probable success of the primary human hepatocyte cultures. In general, cells from

Table 5 *Peroxisomal enzymes in primary hepatocyte cultures*

Sample	Treatment	Palmitoyl-CoA oxidase (units/g)	Carnitine acetyl-transferase (units/g)
Human	0.5 mM clofibric acid	—[a]	69 ± 2[b]
Case 14	0.2 mM MEHP[c]	—	64 ± 4
48 h treatment	0.05 mM Wy-14,643	—	66 ± 3
	0.1% DMSO	—	65 ± 1
	Freshly isolated	2.5[d]	107
Human	0.2 mM MEHP	1.0 ± 0.2	54 ± 2
Case 15	0.05 mM Wy-14,643	0.8 ± 0.2	53 ± 5
72 h treatment	1% DMSO	1.2 ± 0.5	51 ± 3
	Nothing	1.3 ± 0.5	49 ± 5
Rat	0.5 mM clofibric acid	2.0 ± 0.3	55 ± 4
48 h treatment	0.2 mM MEHP	3.4 ± 0.2	37 ± 4
Experiment 1	0.05 mM Wy-14,643	3.7 ± 0.6	67 ± 3
	1% DMSO	1.5 ± 0.4	5 ± 1
	Nothing	1.4[d]	4
Rat	0.5 mM clofibric acid	9.1 ± 0.2	85 ± 1
48 h treatment	1% DMSO	3.1 ± 0.1	8 ± 1
Experiment 2	Nothing	2.5 ± 0.6	6 ± 1
	Freshly isolated	6.7[d]	9
Rat	0.05 mM Wy-14,643	6.9 ± 0.1	61 ± 1
48 h treatment	1% DMSO	2.4 ± 0.1	7 ± 1
Experiment 3	Nothing	1.5 ± 0.3	7 ± 1
	Freshly isolated	8.3[d]	11

[a] —, insufficient sample.
[b] $N = 3$ with each replicate consisting of 3–4 pooled plates. Mean ± SD.
[c] DEHP is immediately hydrolyzed to MEHP in the body and is better for cell culture experiments because of its greater water solubility.
[d] Single sample.

Table 6 *Qualitative DNA repair response*

Chemical	Case 1	2	5	12	13	14	15	16	Rat
AAF	+	+	+	+	+	+	+	+	+
Aflatoxin B_1	+	+	−	+	+	+	+	+	+
2-ABA				+	+	+			+
Aniline		−		−	−	−	−	−	−
Benzo(*a*)pyrene	+	+	−		+	+	+	+	+
CCl_4				−	−	−	−		
Chloroform				−	−	−	−		
2,4-DAT	+	+	−						+
2,6-DAT	+	+	−						−
DEHP	−	−		−					−
DMN	+	+	+	+	+	+	+		+
1,6-Dinitropyrene		+	+	+	+		+		+
2,4-DNT	−	−							−
2,6-DNT	−	−							−
Methyl chloride		−	+						+
5-MC			−		+	+	+		−
MEHP				−	−	−	−		−
Nafenopin							−		−
β-NA	−	−		−	−	−	−		+
Nitrobenzene		−		−	−	−	−		−
2-Nitrobenzyl alcohol				−	−	−			−
2-NT		−		−	−	−	−	−	−
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin		−		−	−	−	−		−
Unleaded gasoline						−	+	−	+
Wy-14,643							−		−

younger patients attached more quickly and yielded better cultures. Attachment to rat-tail collagen-coated plastic coverslips or "chamber/slides" was far better (in some cases obligatory) than to untreated plastic coverslips. In most cases attachment periods from 12 to 24 h were required. This is in contrast to rat cells, which attach in as little as 1.5 h. The longer attachment periods still yielded cultures that retained the ability to metabolically activate the genotoxic carcinogens tested and to repair their DNA. The fact that all of the cells were in repair following treatment with DMN indicates that it was the viable cells that attached during the longer attachment periods (Table 3). Supplementing the media with hormones such as hydrocortisone and insulin seemed to yield healthier cultures (18).

With few exceptions, cells from all eight human cases responded qualitatively the same and yielded a DNA repair response to the genotoxicants used (Table 6). Case 5 was different than the rest in failing to respond to several of the genotoxicants, suggesting that results from this case be viewed with caution. Note also that these cells were from an elderly patient. The historical data base presented here and by others should be helpful in assessing whether future preparations are providing a typical profile of activity for human cells (19–21). Individual-to-individual variation in the DNA repair response was greater than for the rat assay. Table 4 presents the values for the UDS response in rat hepatocytes exposed to 0.001 mM 2-AAF for three separate experiments and confirms that animal-to-animal variation is only on the order of 30%. In contrast, the equivalent UDS response in the human cultures ranged from −1.2 NG (case 15) to 21.6 NG (case 13). Some of this variation may be due to the different culture conditions used. Given the scarcity of available tissue, the difficulty in establishing satisfactory primary cultures, and the great individual-to-individual variability, this assay is not adaptable for the routine screening of chemicals. Cryopreservation of viable, metabolically competent hepatocytes may be a means of making the assay available on a limited basis, particularly in cases where differences in the human and rodent response are suspected.

A major conclusion to be drawn from these data is that the *in vitro* rat hepatocyte DNA repair assay is a valid model for predicting potential genotoxic effects in human beings. Both the rat and human cells yielded an UDS response for a variety of genotoxicants that require metabolic activation including 2-AAF, aflatoxin B_1, 2-ABA, benzo(*a*)pyrene, 2,4-DAT, DMN, and 1,6-dinitropyrene (Table 6), consistent with similar observations by others using human hepatocytes (19–21).

The observation that 2-AAF elicited a DNA repair response in both rat and human hepatocytes is consistent with comparative metabolism studies that show that the metabolites of this carcinogen produced by human and rat hepatocytes were similar (22). In contrast, 2-AAF does not induce DNA repair in male C57BL/6 × C3H F_1 (hereafter called B6C3F_1) mouse hepatocytes *in vivo* or *in vitro* (20, 23). While 2-AAF is carcinogenic in both the rat and mouse, substantial differences in species, strain, and target organ susceptibility as well as time to tumor have been observed with this compound (24). If such differences are related to the different profiles of metabolites produced and to the biological activity of those metabolites, then the DNA repair results suggest that the human tumor response to 2-AAF would be expected to be more like that of the F-344 rat rather than the B6C3F_1 mouse.

β-NA is a human bladder carcinogen (25). Yet, it was positive only in the rat hepatocytes and not in any of the human cultures examined. Numerous metabolites of β-NA have been identified including glucuronic acid conjugates of the *N*-hydroxy metabolites (25, 26). A possible reason for the pattern of activity observed with β-NA is that conjugating reactions may predominate in the human hepatocyte to the extent that insufficient reactive metabolites reach the DNA to elicit a measurable DNA response. Deconjugation and further reactions leading to genotoxic metabolites subsequently occur in human bladder cells (25, 26). This example illustrates the problems inherent in extrapolating from cell cultures to the whole animal, from one target organ to another, and from the rodent model to humans.

5-MC produced a DNA repair response in the human but

not the rat hepatocyte cultures. In contrast, there is a report that 5-MC is positive in the primary rat hepatocyte DNA repair assay at doses similar to those used in this study (27).

Methyl chloride appears to be a weak, direct-acting genotoxicant. While DNA repair activity can be measured in hepatocytes and spermatocytes directly *in vitro*, only extremely high concentrations of inhaled methyl chloride elicit a response in the whole animal, and then only in hepatocytes (17). Exposure of rat hepatocytes *in vitro* to a 1% atmosphere of methyl chloride for 18 h resulted in an UDS response of only 3.7 NG. A similar exposure to human hepatocytes yielded no response in case 2 and 1.5 NG in case 5, confirming a weak susceptibility to the genotoxicity of this agent in both species.

Metabolic activation of 2-nitrotoluene and 2,6-dinitrotoluene involves formation of the benzyl alcohol derivative, conjugation with glucuronic acid, excretion in the bile, reduction by intestinal flora of a nitrogroup to form an amine, reabsorption, and activation in the liver to the ultimate DNA reactive species (28, 29). The extremely potent DNA repair response elicited by 2-ABA in both human and rat hepatocytes is consistent with the above proposal that the 2-ABA metabolite of 2-nitrotoluene represents a proximate genotoxicant/carcinogen. The lack of genotoxic activity of 2,4-DNT, 2,6-DNT, 2-NT, and 2-nitrobenzyl alcohol is also in accord with the concept that reduction by intestinal flora is required in addition to hepatic metabolism for activation (30, 31). 2,4-DAT was genotoxic in both the human and rat hepatocytes, while 2,6-DAT elicited a DNA repair response only in the human cells (14, 32). With the exception of 2,6-DAT, all of the nitroaromatic and aromatic amine compounds discussed here responded qualitatively the same in both the human and rat hepatocytes (Table 6).

Exposure of rodents to concentrations of 2000 ppm unleaded gasoline yielded male rat kidney tumors and female mouse liver tumors (12). The mechanism of action of production of the male rat kidney tumors does not appear to be related to direct genotoxicity, but rather to promotional effects associated with sustained hyperplasia resulting from tubule cell accumulation of the male rat specific urinary protein $\alpha_{2\mu}$-globulin (33, 34). Weak genotoxic activity in cells in culture, however, is associated with unleaded gasoline as evidenced by the observation that 0.05 and 0.1% (v/v) suspensions of unleaded gasoline elicit a DNA repair response in primary rat hepatocytes (35). In general, human and mouse hepatocytes are more susceptible to the cytotoxic effects of unleaded gasoline, so that quantitative comparisons to the rat are difficult. A concentration of 0.1% unleaded gasoline yielded no response in case 14; 0.01% unleaded gasoline produced 1.3 NG in case 15, with 0.05% being cytotoxic; and 0.1% unleaded gasoline did not induce an UDS response in case 16. In summary, human hepatocytes appear to be less susceptible to the genotoxic effects of unleaded gasoline relative to rat hepatocytes as evidenced by a minimal UDS response in the human cells. Those components in unleaded gasoline responsible for this genotoxic activity remain to be identified.

None of the chemicals such as CCl_4, chloroform, DEHP, MEHP, nafenopin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, or Wy-14,643, that appear to exert their carcinogenic effects primarily through various nongenotoxic actions, produced a genotoxic response in the human or rodent hepatocytes, indicating the importance of identifying those mechanisms at work in the production of cancer by these agents (8). Knowledge of those mechanisms will provide the basis for new predictive assays and more rational risk assessment models.

The peroxisomal proliferating carcinogens are represented by a diverse group of chemicals that exhibit no DNA binding or mutagenic activity but are characterized by their ability to induce hepatic peroxisomes, mitogenic hyperplasia associated with increased liver size, lipofusion accumulation, and sustained regenerative hyperplasia, possibly resulting from oxidative damage (9–11, 36). It is possible that genotoxic and promoting activities of these agents are secondary to oxidative damage, with the implication that doses or species that do not yield peroxisome proliferation would not be at increased risk for tumor formation. Consequently, risk assessment for the peroxisome proliferator hepatocarcinogens is complicated by the often questioned relevance of rodent studies to humans. Quantitative evaluation of liver tissue from patients receiving such drugs has yielded both positive and negative results relative to peroxisomal proliferation (37, 38). Those responses reported in humans and in some primates are consistently much weaker than observed in rodents (11). Results from the human hepatocyte experiments presented here confirm that DEHP, MEHP, nafenopin, and Wy-14,643 are not directly genotoxic in human hepatocytes as evidenced by lack of a DNA repair response. Enzyme activity indicative of peroxisomal proliferation could be induced in rat, but not human, primary hepatocyte cultures incubated with MEHP, Wy-14,643, and clofibric acid. Similar results have been reported for MEHP (39). The human cell cultures used were viable as evidenced by their ability to metabolize the procarcinogens and to repair their DNA. Further work may be needed to optimize conditions for human hepatocytes and caution should be applied in using this information since there is no direct evidence that peroxisome proliferation and hepatocarcinogenesis are causally linked. Nevertheless, these data suggest fundamental differences between human and rodent hepatocytes in their response to peroxisome proliferators.

In summary, these studies indicate that, with few exceptions, the *in vitro* rat hepatocyte DNA repair assay is a valid model for predicting potential genotoxic effects in human beings. However, rodent hepatocytes may not be appropriate for assessing the potential of chemicals to elicit some nongenotoxic effects related to cancer production in people, such as the induction of hepatocyte peroxisomal proliferation.

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