**Table 1. Composition of the Diets**

| Ingredient* | without β-carotene g/kg | with β-carotene g/kg |
|---|---|---|
| casein | 200 | 200 |
| corn starch | 590 | 590 |
| soybean oil | 50 | 49.9 |
| α-cellulose | 10 | 10 |
| D,L-methionine | 3 | 3 |
| choline bitartrate | 2 | 2 |
| mineral mixture | 35 | 35 |
| vitamin mixture | 10 | 10 |
| β-carotene | 0 | 0.1 |

*Corn starch was obtained from Roquette Freres, France. Soybean oil was procured from Tung Yi Co. Taipei, Taiwan. Casein, α-cellulose, AIN-76 mineral mixture, and AIN-76 vitamin mixture were procured from ICN Biochemicals (Costa Mesa, CA, USA). DL-Methionine, choline bitartrate, and β-carotene were procured from Sigma Chemical (St. Louis, MO, USA).

fore the final day of the experiment.

### Cell isolation and Culture

Rat hepatocytes were isolated using a 2-step collagenase perfusion as described previously.[20] After isolation, hepatocytes were resuspended in L-15 culture medium (pH 7.6), containing 18 mM (HEPES), 0.2% BSA, 5 μg/mL each of insulin and transferrin, 5 ng/mL selenium as sodium selenite, 1 mg/mL galactose, 1 μM dexamethasone, 100 IU/mL penicillin, and 100 μg/mL streptomycin, in a final volume of $0.5 \times 10^6$ cells/ml. Five milliliters of the cell suspension was plated onto collagen-precoated 60-mm plastic tissue culture dishes (Falcon Labware, Franklin Lakes, NJ, USA) with a total of $2.5 \times 10^6$ cells in each dish.

Cells were incubated at 37 °C in a humidified incubator with an air atmosphere. The first change of medium was performed 4 h after plating and, subsequently, once a day. Twenty-four hours before the end of the experiment, the medium was removed and replaced with incubation medium supplemented with 0, 0.05, 0.1, or 0.2 mM $FeCl_3$. Lactate dehydrogenase (LDH; *EC* 1.1.1.27) release was determined in all in vitro preparations as an index of cytotoxicity using the method of Vassault.[21] LDH release was expressed as a percentage of the total LDH released from cells.

### Hepatotoxicity Assay

Hepatocytes were pretreated for 24 h at a final concentration of 0, 0.05, 0.1, or 0.2 mM $FeCl_3$ respectively added for 30 or 60 min, then an aliquot (0.1 mL) of the culture medium was removed and analyzed for LDH with kits from Sigma.

### Lipid peroxidation assay

Lipid peroxidation products were assayed according to an improved thiobarbituric acid (TBAR) reactive substances (malondialdehyde, MDA) fluorometric method emission at 553 nm with excitation at 515 nm.[22] 1,1,3,3-Tetramethoxypropane was used as the standard.

### Antioxidant Enzymes Assay

CAT activity in primary rat hepatocytes was determined on the same day as harvesting using the method of Baudhuin et al.[23] The remaining supernatant fractions were stored at -20 °C until SOD activity was determined by the method of McCord and Fridovich,[24] and GSH-Px activity were measured by the method of Lawrence and Burk.[25]

### Total Protein Assay

Total protein was determined in each dish using the method of Lowry et al.[26] Protein concentration was determined using a standard commercial kit with BSA (Sigma) as the standard.

### Determination of β-carotene in Primary Rat Hepatocytes or Liver

Cell monolayers were washed 3 times with phosphate-buffered saline (PBS), detached with 0.25% trypsin, and resuspended in PBS. The cells were then pelleted at 200 ×g for 10 min at 4 °C. Precautions were taken throughout the procedure to avoid exposure of the β-carotene solution samples to direct lighting.

Each 1 mL of PBS contained $1 \times 10^6$ cells, and 3 mL of absolute alcohol was added. This mixture containing 0.0025% butylated hydroxytoluene (BHT) was then extracted twice by adding 6 mL of hexane and mixing for 1 min. The hexane layers were combined and evaporated under $N_2$. A 25-μl aliquot of the reconstituted extract was injected into HPLC.