ULTRASTRUCTURAL AND ENZYME-CYTOCHEMICAL STUDIES OF HEPATOCYTES IN RAT AND HUMAN HEPATOMA AND AFLATOXIN-B₁-TREATED RAT LIVER

JAANG J. WANG^{1,2}, CHIUNG H. CHENG², NING K. CHEN¹, JIE S. HWU¹,
SHU H. WU², DANNY L. WANG² AND JEFFREY P. CHANG³

*Department of Biology and Anatomy¹, National Defense Medical Center, Taipei, Taiwan,
Republic of China, Institute of Biomedical Sciences² and Institute of Zoology³,
Academia Sinica, Taipei, Taiwan, Republic of China*

Received for publication November 4, 1992

Ultrastructural changes and enzyme-cytochemical localization of Chang hepatoma in rat, human hepatoma, and aflatoxin B₁ (AFB₁)-treated rat hepatocytes are demonstrated by electron microscopy. Both dark and light cell types appear in immersion-fixed samples, as well as in perfusion-fixed ones, in normal and experimental samples. The light cell contains pale cytoplasm rich in glycogen, less G6Pase activity in ER, and much sensitivity to AFB₁-treatments. Dark hepatocytes make up the major cell population in ascites-form and solid-form of Chang hepatoma, induced by chronic feeding with 3'-methyl-4-dimethyl-aminoazobenzene. Dense cytoplasmic ground substances, ER, Golgi apparatus, and normal mitochondria are encountered in dense cells. Enzyme-cytochemical labelings indicate that each of the activities of acid phosphatase, glucose-6-phosphatase, and thiamine pyrophosphatase are localized in both cell types. This work confirmed the existence of two populations of dark and light hepatocytes in normal and abnormal livers.

Dark and light cell populations have been frequently observed in a variety of normal (2) and abnormal tissues (10, 12). The dark cell contains electron-dense cytoplasm with compact organelles (1, 22). However, the light cell contains pale cytoplasm, dispersed organelles, and is rich in glycogen (1). The cell damage and enhanced appearance of dark and light cells induced by aflatoxin B₁ (AFB₁) were observed in duckling (22) and rat livers (20). Other poisons also caused dark and light cell phenomenon in the liver (15, 19). The debate concerning dark and light cells lies mainly in their co-existence in experimentally injured livers (2, 12, 13, 19), as well as in normal livers (10). Dark and light hepatocytes in preneoplastic and malignant livers have been reported by Ogawa (19). It has been suggested that dark cells are artifacts caused by uneven infiltration of fixatives during immersion-fixation (9, 21, 23). According to our previous studies, dark and light cell phenomenon was reduced

to the minimum in thin-sliced tissue blocks, after immersion-fixation (17, 23). However, dark and light cell phenomenon appeared in ascites form of Chang hepatoma cells, when fixed by immersion method (equal to the perfusion method for individual cells in tissue block) (24, 25).

The cytochemical and ultrastructural changes have been reported mostly in the light cells of human hepatoma (19, 23), rat hepatoma ascites cells (16, 17, 26), and rat livers after AFB₁-treatment (13). The diverse morphological and histochemical characteristics of dark and light cells indicate that there are two different cell populations in the liver (10). However, the origin and enzyme-cytochemical properties of the major cell type, dark hepatocytes, and the difference between the light cells remains unclear.

Enzyme-cytochemical changes that happened prior to the ultrastructural changes were demonstrated previously (16, 25). The localization of AcPase, TPase, and G6Pase at the subcellular level in the target organelle indicate their functional states during natural, pathological, or experimental manipulations. In the present study, we have reported the ex-

Correspondence Author: Jaang J. Wang, Ph. D., Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, R.O.C. 11529.

istence of dark cells and light cells under diverse conditions, and the cytochemical and ultrastructural properties of the dark and light hepatocytes.

MATERIALS AND METHODS

Chang hepatoma cells, ascites and solid form

Chang hepatoma ascites cells, induced by feeding 3'-methyl-4-dimethyl-aminoazobenzene, have been routinely maintained (4, 26). The *In Vivo* and *In Vitro* ascites cells were collected and washed with 0.1 M phosphate-buffered saline (PBS) at pH 7.2, and then diluted to 5×10^6 cells/ml (16). Solid form Chang hepatoma cells were induced in the air pouch on the back of the rat's neck, by injecting ascites cells 1-2 weeks before use. The ascites and solid forms of Chang hepatoma cells were immersion-fixed as before (23-25).

Single dose of AFB₁-treatments

AFB₁ was purchased from Makor Chemicals, Ltd., Israel. A single dose of half LD₅₀ (3 mg/ml) was dissolved in n, n-dimethyl-formamide and propylene glycol (1 : 1 by volume), and IP injected (25). Young male Sprague-Dawley (SD) rats, weighing 120-150 gm, were divided into 9 groups. Two to three animals were used for each group. In control group 1, no AFB₁ was injected; control group 2 was injected with solvent containing no AFB₁. After single doses of AFB₁-injection, the control and AFB₁-treated animals (groups 3-9) were sacrificed after 3, 6, 12, and 24 hr, and 1, 2, and up to 5 weeks. Liver samples were processed for morphological and enzyme-cytochemical studies of G6Pase, AcPase and TPPase. The experiments were repeated three times.

Human hepatoma

Three human hepatoma samples of male patients aging 50, 52, and 65 years were obtained from the Department of Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei. The perihepatoma normal liver blocks were used as controls.

Perfusion and immersion-fixation methods

Normal and AFB₁-treated SD rats, under ether-anesthesia, were perfused with 0.2 ml heparin (1000 U/ml), through the femoral vein. The left lateral lobe of the liver was ligated before perfusion and cut off, then fixed immediately for control of immersion-fixation. Perfusion was performed with a blunt-end needle through the common carotid artery with 4% paraformaldehyde and 1% glutaraldehyde fixative in PBS for 10 min. The flow rate was 1.91 ml/min using a 50 ml syringe. Ten minutes later, when flow was cleared from the femoral artery, liver samples from the perfused liver lobes were sliced into 0.5-1.0

mm³ blocks for further immersion fixation of another 60 min at room temperature.

Enzyme-cytochemistry of AcPase, G6Pase and TPPase activities

Enzyme-cytochemical methods of our previous work (25) were followed. Briefly, liver samples were fixed and washed in PBS overnight. Frozen sections of 40 μ m thickness were made and washed in 10% dimethyl sulfoxide (DMSO), before enzyme incubations. AcPase incubation medium contains 1 ml of 3% disodium glycerophosphate (Sigma), 10 mg lead nitrate, 10 ml 50 mM acetate buffer, pH 5.0, and 6% sucrose. G6Pase incubation medium contains 6.3 mg monosodium glucose-6-phosphate (Sigma), 5 ml 0.2 M tris-maleate buffer, pH 6.7, 15 mg lead nitrate, 6% sucrose, and 7.5 ml distilled water. The samples for AcPase and G6Pase were incubated for 20 min at 37°C. For TPPase activity, 10 mg thiamine pyrophosphate was added in 10 ml fixative solution for substrate protection. After washing overnight in substrate-free buffer, the samples were incubated in a medium containing 10 mg thiamine pyrophosphate, 2 ml 25 mM manganese chloride, 12 mg lead nitrate, 4 ml 0.2 M tris-maleate buffer, pH 7.2, 4 ml distilled water, and 6% sucrose. A substrate-free medium, containing 10 mM sodium fluoride, was used as a control. The samples were washed, post-fixed in 1% osmium tetroxide, dehydrated with graded ethanol, embedded in Spurr, and processed for electron microscopy. Thin sections were obtained and double stained with uranyl acetate and lead citrate for ultrastructure. Thin sections, for enzyme-cytochemistry, were single stained with lead citrate or uranyl acetate.

RESULTS

Chang hepatoma cells in ascites and solid-form: Ultrastructural changes, observed in dark and light cells of Chang hepatoma in ascites form (Figs. 1, 2), were similar to cells in solid form (Fig. 3). Light cells, with pale cytoplasm and round nucleus, were observed in less number than dark ones. Chang hepatoma ascites cells, obtained from either *in vivo* or *in vitro*, showed a similar ultrastructural appearance. The thinly sliced and immersion-fixed solid form of Chang hepatoma cells showed dark and light cell types as well (Fig. 3). Both AcPase (Fig. 1) and TPPase (Fig. 2) activities were seen in the trans of Golgi complex and small vesicles, nearby, in the compact cytoplasm of dark ascites cells. AcPase deposits were seen in the Golgi saccules and in the Golgi endoplasmic reticulum and lysosome area (Fig. 1). TPPase activity was mostly

found in the trans face saccules of Golgi apparatus (Fig. 2). G6Pase activity was detected in the ER and nuclear envelope as before (16).

Aflatoxin B₁-treated rat livers: The dark cell/light cell phenomenon was remarkable in AFB₁-treated rat livers, with either immersion-fixation or perfusion-fixation. The dark cells showed dense irregular-shaped nuclei, compact ER, mitochondria, and active Golgi complexes. Much less glycogen granule was observed in the cytoplasm of light cells. The nucleoli and mitochondria were similar to those in the controls. The fine structural changes in the dark and light cells

of the rat liver were remarkably observed after 24 hr of AFB₁-treatment (Figs. 4, 5). The fibrillar and granular portion of the nucleolus became condensed after 3 hr of AFB₁-treatment. Vacuoles appeared in nucleolus after 6 hr to 24 hr AFB₁-treatment (Fig. 4). Parallel rough endoplasmic reticulum (RER), regularly seen in normal hepatocytes, disappeared in light cells after 24 hr treatment (Fig. 4). The hepatocytes of the control group treated with solvent for 1–2 weeks showed normal structure in dark and light cells (Fig. 6). Two weeks after a single-dose AFB₁-treatment, the hepatocytes reversed from the toxin shock to normal

- FIG. 1. Dark (D) and light (L) Chang hepatoma ascites cells were found in cell suspension from the ascites fluid of a tumor-bearing rat. The Golgi apparatus (G) of a dark cell with AcPase reaction product was shown as a circle. The sections were single stained with uranyl acetate. $\times 16,500$ Bar = $0.5 \mu\text{m}$.
- FIG. 2. TPPase activity in a dark Chang hepatoma ascites cell was shown at the trans-face (arrowhead) of a Golgi apparatus (G). The densely packed cytoplasm was compacted with ribosomal granules, mitochondria, vesicles and Golgi apparatus. The sections were stained with uranyl acetate only. $\times 31,300$ Bar = $0.5 \mu\text{m}$.
- FIG. 3. Dark and light cell phenomenon was found in solid form of Chang hepatoma. Bile canaliculi-like intercellular spaces (Is) were seen between dark (D) and light (L) cells. The thin sections were double stained with uranyl acetate and lead citrate. $\times 5,000$ Bar = $1.0 \mu\text{m}$.
- FIG. 4. Micrograph shows dark (D) and light (L) cells in rat liver, 24 hr after AFB₁ treatment. Pale cytoplasm and randomly distributed ERs (arrowhead) were seen in the light cell on the right. Degradation of the nucleolus (Nu) was seen in the dark cell. Golgi saccules (G) near a bile canaliculi (b), ER (arrowhead), and darkly stained mitochondria (M) were observed. $\times 6,900$ Bar = $1.0 \mu\text{m}$.
- FIG. 5. A severely damaged light cell (L) was found in liver treated by 24 hr AFB₁-injection. Degenerating area with deformed nucleus (N1) and cytoplasmic residues were seen in the pale cytoplasm of the light cell. Cytoplasm with scattered ER (arrowhead) and mitochondria were seen around the degenerating area. The dark cell (D) at the bottom looked unharmed with densely stained cytoplasm and irregular nucleus (Nd). Bile canaliculi (b) is seen between dark and light cells. $\times 8,250$ Bar = $1.0 \mu\text{m}$.
- FIG. 6. Control rat liver, treated with solvent for two weeks, showed dark (D) and light (L) cells. Pale cytoplasm with Golgi apparatus (G), scattered ERs (arrowhead), and glycogens were seen in the light cell. Dark cell showed Golgi apparatus (G), dense cytoplasm among parallel ERs (arrowhead), glycogens, and mitochondria. Bile canaliculi (b) is seen between dark and light cells. Thin sections were double stained with uranyl acetate and lead citrate. $\times 11,000$ Bar = $0.5 \mu\text{m}$.
- FIG. 7. After two weeks of single-dosed AFB₁ treatment, dark (D) and light (L) cells with active Golgi apparatus (G), normal ERs (arrowheads), dense glycogen granules, and mitochondria were seen. Bile canaliculi (b) is seen between two types of cells. The thin section is double stained. $\times 16,500$ Bar = $0.5 \mu\text{m}$.
- FIG. 8. Enzyme-cytochemical expression of glucose-6-phosphatase (G6Pase) activity in the dark (D) and light (L) hepatocytes of rat liver after 2 weeks of AFB₁-treatment. Recovery of the light cell is observed. G6Pase activity is seen in the cisternae of ERs (arrowheads) in both dark and light cells as dense deposits. G6Pase activity in more densely stained in the dark cell than that in the light cell. Note the Golgi apparatus (G) and bile canaliculi (b) are negatively stained. ER array are similarly arranged in both cell types. $\times 15,000$ Bar = $0.5 \mu\text{m}$.
- FIGS. 9–12. Micrographs show cytochemistry of human hepatoma cells. The sections of Figs. 9 and 10 were stained with lead citrate only. Figs. 11 and 12 were stained with uranyl acetate only.
- FIG. 9. AcPase activity was shown in three layers of trans Golgi saccules (G) in a dark human hepatoma cell. The lysosome (L) and bile canaliculi (arrowhead) were also positive of AcPase activity. $\times 16,500$ Bar = $0.5 \mu\text{m}$.
- FIG. 10. TPPase activity (arrowhead) was shown in the trans-face of Golgi saccules (G) in a dark human hepatocyte. The bile canaliculi (b) was also TPPase positive. Note the different patterns of TPPase and AcPase activity (see Fig. 9) in the Golgi saccules. $\times 27,500$ Bar = $0.5 \mu\text{m}$.
- FIG. 11. Dense cytoplasm between the ER profiles of the dark cells was observed. G6Pase deposits (arrowhead) were seen in the cisternae of ER and nuclear envelope. The Golgi cisternae (G) was negatively stained. The nucleus (N) was densely stained and irregular in shape. Glycogen granules were washed away and pale areas were left in the cytoplasm. Swollen mitochondria (M) were seen. $\times 22,850$ Bar = $0.5 \mu\text{m}$.
- FIG. 12. The G6Pase activities were seen in the ER cisternae (arrowhead) of a light cell with round nucleus (N) at the left lower corner. The glycogen granules were washed away and left pale areas in the cytoplasm. The cytoplasm among the ER profiles was pale when compared to the dark cell in Fig. 11. Mitochondria (M) were observed similar to that in Fig. 11. $\times 16,500$ Bar = $0.5 \mu\text{m}$.

appearance in both dark and light cells (Fig. 7). The light cell always showed more intensive damage than the dark cell. In later AFB₁-treatment, degenerated nucleus, residues of cytoplasmic components, and randomly arranged ER were observed in the pale cytoplasm of light cells, but not in the dark cells (Fig. 5). Both dark and light cells showed positive AcPase, G6Pase, and TPPase activities during the carcinogenesis of AFB₁-treatments. Similar locations of the three enzyme activities were seen in dark and light cells, as in Chang hepatoma and human hepatoma cells. However, after 24 hr to 2 weeks of AFB₁-treatment, G6Pase activity became weaker in the light cells (Fig. 8).

Human hepatoma: Dark and light hepatocytes were observed in two out of three samples of the human hepatoma. Both dark and light cells showed similar fine structures. AcPase activity was detected in the lipofuscin granule and also in the Golgi saccules similar to the light cells. The bile canaliculi and Golgi saccules of the dark cells were AcPase (Fig. 9) and TPPase (Fig. 10) positive. These enzymes were seen distributed in the GERL areas. The nuclei of dark cells were irregular in shape, and were deeply stained in a manner similar to that in AFB₁-treated murine dark hepatocytes. G6Pase activity and glycogen granules were seen in both dark and light cells (Figs. 11, 12). However, the Golgi apparatus was G6Pase negative. The cytoplasm between the ERs was more densely stained in the dark cell (Fig. 11) than in the light one (Fig. 12).

DISCUSSION

Dark cell and light cell phenomenon have been an interesting problem encountered in the morphological study of diverse tissues. In spite of the different methods applied, we found that light cells always exist among dark cells in normal and experimental livers. The ascites form of Chang hepatoma cells fixed by immersion-method is the same as tissue block treated by perfusion-fixation. However, light cells are still found among dark cells, which are the major cell population in Chang hepatoma (4, 26). It is obvious that the phenomena of dark cells and light cells in ascites cells are not caused by the methods of fixation. In addition, perfusion-fixed normal livers, as well as thinly sliced immersion-fixed solid Chang hepatoma, showed light cells among dark cells. Since human hepatoma could not be prepared by perfusion-fixation, tissue was processed by slicing into 0.5–1.0 mm thick strips for immersion-fixation. In contrast to the artifacts caused by immersion-fixation (9, 21), our results suggested

that both dark and light cells appeared in samples prepared by either perfusion or immersion-fixation.

Light cells, with enriched glycogen agglomerates, have been described in human hepatoma in preneoplastic liver (1, 27), and also in AFB₁-treated rat liver (23). The dark hepatocytes with less glycogen are suggested to be poorly differentiated hepatocellular carcinomas (1). Dark hepatocytes increase significantly in liver after irradiation (12) and after aflatoxin treatments. The main difference between the fine structural components of dark and light cells seems to be the cytoplasmic density (9, 21). Dark and light cells with gradient cytoplasmic densities and large mitochondria, with a few numbers of cristae and dense bodies in the matrix, were found. Increasing amounts of lysosomal granules with positive AcPase activity near the bile canaliculi were observed in dark cells. The ER system looks normal, and no ER cisternal dilations are observed in dark and light cells. This result indicates that gradual changes may take place between dark and light cells.

The light cells prepared by the electron microscopy are acidophilic, and they are eosinophilic by light microscopy (22, 27). The dark and light phenomenon can be easily demonstrated in the semithin resin sections stained with toluidine blue. AFB₁-induced hyperchromatic characteristics in duck hepatocellular carcinoma, by hematoxylin-eosin staining, have been reported (22). AFB₁-induced disorganization of the fine structures in hepatocytes of rat liver have been reported (24). Recently, dark and light cells have also been found in single-dosed AFB₁ treated duckling livers (7). Similar fine structural changes induced by AFB₁ in dark and light cells have been described (20). The appearance of two populations of dark and light cells is enhanced after AFB₁ dosing. Following the disorganization of the fibrillar and granular portions in the nucleoli (6, 24), the distribution of AFB₁ in the target hepatocytes has been localized in the granular portion by immuno-gold staining (unpublished data). Disarrangement of ER profiles were observed in the light cell, and then cell degeneration follows.

It has been reported that a single dose of AFB₁-treatment can not induce carcinogenesis (3). Our enzyme-cytochemical findings have indicated that AcPase, TPPase, and G6Pase activities are shown in both dark and light hepatocytes of Chang hepatoma, human hepatoma, and AFB₁-treated rat liver. However, weak activity or absence of G6Pase activity in light cells indicates a state of neoplasm (23, 24, 27), and decreased G6Pase activity indicates an initiation of hepatocarcinogenesis (8, 18). Less G6Pase activity

expressed in light cells then in dark cells, after 6–24 hr of a single AFB₁-treatment, suggested a possible pathway of transformation of carcinogenesis. Although G6Pase activity has been shown to be regularly expressed at the periportal area in the liver after AFB₁ treatment, after 1–5 weeks of application, the morphology of hepatocytes recovered, after AFB₁-treatment and G6Pase activity reappeared. Similar results were observed in AFB₁-induced hepatocarcinoma after 15 weeks (14). In our results, the G6Pase activity was found to be back to normal after 5 weeks of single AFB₁-dose.

Light cells are more sensitive to the treatment of AFB₁ than dark cells, since G6Pase activity in light cells is weaker than that in dark ones. Dark cells in human and rat hepatocytes showed irregular-shaped nuclei, dense granules in mitochondria, densely packaged Golgi apparatus, and positive AcPase, TPase, and G6Pase activities. These results indicated that the dark cell belongs to a dedifferentiated cell population which survives single-dosed AFB₁-treatment. AFB₁-induced ultrastructural and cytochemical changes in the rat liver were not observed in mouse liver treated with the same doses and time periods of AFB₁. However, dark and light cells are normally seen in the mouse liver and kidney before and after AFB₁-treatments (5). These results are consistent with our previous observations that suggest the mouse liver is AFB₁-resistant ultrastructurally and cytochemically. In conclusion, we suggest the existence of dark and light cell populations in a variety of liver tissues.

ACKNOWLEDGEMENTS

The accomplishment of this study is partially supported by the National Science Council of the Republic of China by grants NSC-79-0412-B-016-70 and NSC-79-0412-B-016-79.

REFERENCES

1. Bannasch, P., Mayer, D. and Hacker, H. J.: Hepatocellular glycogenesis and hepatocarcinogenesis. *Biochim. Biophys. Acta* 605; 217–245, 1980.
2. Bruni, C. and Porter, K. P.: The fine structure of the parenchymal cell of the normal rat liver. I. General observations. *Am. J. Path.* 46; 691–755, 1965.
3. Butler, W. H. and Hemsall, V.: Histochemical studies of hepatocellular carcinomas in the rat induced by aflatoxin. *J. Path.* 134; 157–170, 1981.
4. Chang, J. P., Gibley, Jr. C. W. and Ichinoe, K.: Establishment of transplantable hepatoma induced by 3'-methyl-4-dimethyl-aminoazobenzene with special reference to the histologic features of the transplants of early passages. *Cancer Res.* 27; 2065–2071, 1967.
5. Chen, N. K., Huang, S. C., Chou, S. H., Hwu, J. S. and Wang, J. J.: Ultrastructural changes of rat kidney induced by a single-dose of AFB₁-injection. The Sixth Joint Annual Conference of Biomedical Sciences. Taipei, Taiwan, R. O. C., 1991.
6. Cole, K. E., Jones, T. W., Lipsky, M. M., Trump, B. F. and Hsu, I. C.: Comparative effects of three carcinogens on human, rat and mouse hepatocytes. *Carcinogenesis* 10; 139–143, 1989.
7. Cova, L., Wild, C. P., Mehrotra, R., Turusov, V., Shirai, T., Lambert, V., Jacquet, C., Tomatis, L., Trepo, C. and Montesano, R.: Contribution of aflatoxin B₁ and hepatitis B virus infection in the induction of liver tumors in ducts. *Cancer Res.* 50; 2156–2163, 1990.
8. Farber, E.: The sequential analysis of liver cancer induction. *Biochim. Biophys. Acta* 605; 149–166, 1980.
9. Ganote, C. E. and Moses, H. L.: Light and dark cells as artifacts of liver fixation. *Lab. Invest.* 18; 740–745, 1968.
10. Ghadially, F. N.: Dark cell-light cell phenomenon. In "Ultrastructural Pathology of the Cell and Matrix", ed. by F. N. Ghadially, New York, 1988, pp. 954–961.
11. Ghadially, F. N. and Parry, E. W. and Parry, E. W.: Ultrastructure of a human hepatocellular carcinoma and surrounding non-neoplastic liver. *Cancer* 19; 1989–2004, 1964.
12. Ghidoni, J. J.: Light and electron microscopic study of primate liver 36 to 48 hr after high doses of 32-million-electron-volt protons. *Lab. Invest.* 16; 268–286, 1967.
13. Herdson, F. B., Garvin, P. J. and Jennings, R. B.: Fine structural changes produced in rat liver by partial starvation. *Am. J. Path.* 45; 157–181, 1964.
14. Kalengayi, M. M. R. and Desmet, V. J.: Sequential histological and histochemical study of the rat liver during aflatoxin B₁-induced carcinogenesis. *Cancer Res.* 35; 2845–2852, 1975.
15. Lafontaine, J. G. and Allard, C.: A light and electron microscope study of the morphological changes induced in rat liver cells by the azo dye 2-Me-DAB. *J. Cell Biol.* 22; 143–148, 1964.
16. Moller, P. C., Yokoyama, M. and Chang, J. P.: Ultracytochemical localization of glucose-6-phosphatase in Chang rat hepatoma *in vivo* and *in vitro*. *J. Natl. Cancer Inst.* 58; 1401–1405, 1977.
17. Moller, P. C., Wang, J. J., Yokoyama, M. and Chang, J. P.: Cytochemical localization of lectin labeled vesicles in GERL region of hepatoma ascites cells. *Histochem.* 62; 289–297, 1979.
18. Moulin, M. C. and Daoust, R.: Glucose-6-phosphatase activity in rat liver parenchyma during azodye carcinogenesis. *Int. J. Cancer* 8; 81–85, 1971.
19. Ogawa, K., Medline, A. and Farber, E.: Sequential analysis of hepatic carcinogenesis. A comparative study of the ultrastructure of preneoplastic, malignant, prenatal, postnatal, and regenerating liver. *Lab. Invest.* 41; 22–34, 1979.
20. Pritchard, D. J. and Butler, W. H.: The ultrastructural features of aflatoxin B₁-induced lesions in the rat liver. *Br.*

- J. Expt. Pathol.* 69; 793-804, 1988.
21. Roberts, J. C., MacCrossan, M. V. and Jones, H. B.: The case for perfusion of large tissue samples for ultrastructural pathology. *Ultrastruct. Pathol.* 14; 177-191, 1990.
 22. Theron, J. J.: Acute liver injury in ducklings as a result of aflatoxin poisoning. *Lab. Invest.* 14; 1586-1603, 1965.
 23. Wang, J. J.: Ultrastructures and localizations of acid phosphatase, thiamine pyrophosphatase and glucose-6-phosphatase activities in human hepatoma. *Proc. Natl. Sci. Counc. B.* 6; 235-246, 1982.
 24. Wang, J. J., Liu, J. C. and Chang, J. P.: Cytochemical and ultrastructural changes in aflatoxin-induced injury to rat liver cells. *Proc. Natl. Sci. Counc. B.* 8; 135-142, 1984.
 25. Wang, J. J., Chang, J. P., Hsu, C. Y., Liang, H. M. and Moller, P. C.: A cytochemical study of acid phosphatase, thiamine pyrophosphatase and horseradish peroxidase in the Golgi-GERL complex of hepatoma ascites cells. *Cell Tissue Res.* 233; 367-376, 1983.
 26. Wang, J. J., Chang, J. P. and Teng, C. S.: Immunocytochemical demonstration of the binding and internalization of growth hormone in GERL of Chang hepatoma cells. *Cell Tissue Res.* 262; 273-281, 1990.
 27. Williams, G. M.: The pathogenesis of rat liver cancer caused by chemical carcinogens. *Biochim. Biophys. Acta* 605; 167-189, 1980.