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# Effects of Carcinogens and Partial Hepatectomy on the Nitrogen Utilizing and the Xenobiotic Metabolizing Enzymes in the Hepatic Tissues of Rats

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The biochemical changes of the hepatic tissues, induced by the carcinogen treatment such as diethylnitrosamine and acetamidofluorene in combination with the partial hepatectomy after Solt and Farber, were determined for the characterization of the induction of the proliferative capacity and the environmental adaptability of the carcinogenic tissues during the malignant transformation process. For the study of the proliferative capacity of the tissues, the activities of the enzymes, related with the nitrogen trapping mechanism, such as glutamine synthetase and gamma-glutamyltranspeptidase, were monitored, while the contents of cytochrome P450's and their isozymic patterns as well as the activities of the glutathione S-transferase were determined in the function of time after the hepatocarcinogenic stimuli. The induction of the preneoplastic hyperplastic nodules by the procedure was confirmed by the immunohistochemical analysis with GST-P antibody. During the process of hepatocarcinogenesis, the activities of glutamine synthetase and glutathione S-transferase showed the increasing pattern concomittantly with the appearance of the hyperplastic nodule. However; the contents of cytochrome P450's as well as cytochrome b5 and gamma-glutamyltranspeptidase activities did not change significantly. From these results, it could be concluded that the proliferative capacity of the hepatocarcinogenic tissues could be obtained through the acquisition of the high glutamine synthetase activity, and the cellular chemical resistency, through the induction of glutathione-S transferase, especially of its acidic isozyme, GST-P. However, the characteristic pattern of the high level of gamma-glutamyltranspeptidase with the lower level of cytochrome P450's in the cancer tissues was not prominent at the preneoplastic stage of the hepatocarcinogenesis.

## INTRODUCTION

The chemical carcinogenesis in the experimental animals usually comprises the multistep stimuli, such as initiation and promotion (Schechter *et al.*, 1985; Weinstein *et al.*, 1985). The standard hepatocarcinogenic model, originally

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developed by Solt and Farber, included the initiation step with diethylnitrosamine treatment, followed by the promotion step with acetamidofluorene and by the growth stimuli through the induction of regeneration with partial hepatectomy (Solt & Farber 1976; Solt *et al.*, 1977, 1983). This protocol of the hepatocarcinogenesis has been widely used for the research of enzymatic changes and genetic alteration in relation with the hepatocarcinogenic mechanism, and for *in vivo* screening of tumor promoting agents, as well as for the study of biochemical changes in the preneoplastic lesion, which contributed abundant accumulation of data to the carcinogenesis research (Pitot & Sirica 1980; Farber 1984). The biological principles of the cancer, such as proliferation, invasion and metastasis, and environmental adaptability, lead the carcinogenesis research to study on the mechanism of induction of such behavior during the carcinogenic period. Excluding the tumor characters of invasion and metastasis, whose mechanisms are still remained to be elucidated in terms of biochemistry, the proliferation ability and the environmental adaptability of the cancer could be partially explained through the nitrogen trapping mechanism and the chemical resistency (Park *et al.*, 1987; Meister 1976). For the nitrogen trapping mechanism, the role of glutamine synthetase positioned the prime importance, while there were many candidate systems for the chemical resistency of the cancer, such as the amplification of the genes of the target molecules, the oxidative and conjugation mechanism of the chemicals, and the pumping-out system of the xenobiotics (Fujino *et al.*, 1982; Satoh *et al.*, 1985; Fiala *et al.*, 1976; Scotto *et al.*, 1986).

In the present experiment, we determined the levels of glutamine synthetase and gamma-glutamyltranspeptidase for the characterization of nitrogen uptake of the cancer tissues and the activities of glutathione S-transferase as well as the contents of cytochrome b5 and cytochrome P450's for the ability of chemical resistency. Especially, we have focused our attention on the temporal induction pattern of these biochemical parameters in the liver tissues by the treatments of DEN, AAF and partial hepatectomy, because it was our assumption that the neoplastic transformation should have the super-power of proliferation and the chemical adaptation, which should be induced during the carcinogenic process.

## METHODS AND MATERIALS

### *Reagents and monoclonal antibodies*

Chemicals were purchased from the following sources: dithiothreitol, 1 chloro-2,4 dinitrobenzene, *r*-glutamyl p-nitroanilide, glycylglycine, sodium dodecyl sulfate, glutamine, adenosine triphosphate(ATP), adenosine diphosphate (ADP), from Sigma Chemical Co. (St. Louis, MO. USA); alkaline phosphatase conjugated-anti rabbit IgG sheep antibody and alkaline phosphatase Elisa kit from Kirkegaard & Perry Laboratories, Inc (Gaithersburg, MD. USA). And other chemicals of analytical grade were obtained from the available commercial sources. The monoclonal antibodies respectively specific to T.C.D.D.-dependent and phenobarbital dependent cytochrome P450's were kindly donated from Dr. Sang Shin Park and those respectively specific to ethanol-dependent, pregnenolone dependent, and

c-AMP-inducible cytochrome P450's, generously from Dr. Byung June Song of National Institutes of Health (Bethesda, MD. USA). And the monospecific antibody to GST-P was generously given by Professor K. Sato from 2nd Department of Biochemistry, Medical School, Hirosaki University (Japan).

*Animals*

Male Sprague-Dawley albino rats were obtained from Seoul National University animal breeding house at approximately 200 g of body weight. Animals were treated with diethylnitrosamine(DEN), dissolved in 0.9% NaCl, intraperitoneally at necrogenic dose (200 mg/kg). After two weeks on basal diet, the rats received the acetamidofluorene (AAF) intragastrically, daily at dose of 1 mg/head in corn oil for further two weeks. Then the animals were subjected to two-thirds partial hepatectomy under the penthothal anesthesia. Rats were sacrificed, eight heads for the respective group, under the following time schedule: control (Group 1), two weeks after DEN treatment (Group 2), two weeks after AAF treatment (Group 3), 30 minutes after partial hepatectomy (Group 4), 4 hours after PH (Group 5), 1 day after PH (Group 6), 3 days after PH (Group 7), 1 month after PH (Group 8), 2 months after PH (Group 9), 6 months after PH (Group 10) (Fig. 1).

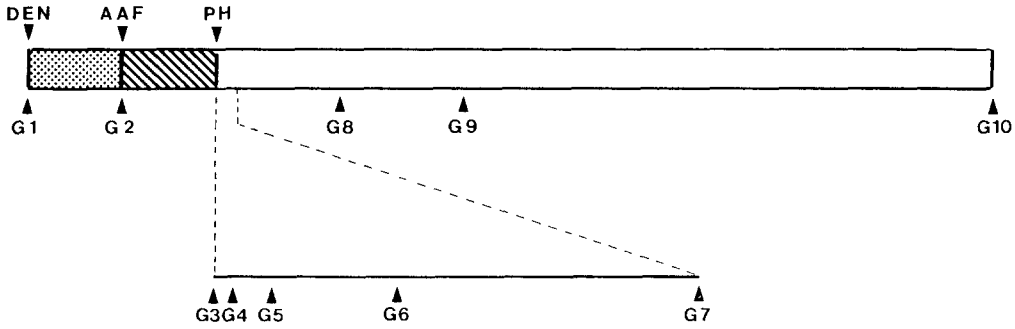


**Fig. 1. Hepatocarcinogensis model after Solt and Farber: Group 1, control: Group 2, two weeks after DEN: Group 3, two weeks after AAF: Group 4, 30 minutes after pH: Group 5, two hours after PH: Group 6, one day after PH: Group 7, three days after PH: Group 8, one month after PH: Group 9, two month after PH: Group 10, six months after PH.**

*Collection and preparation of samples*

The samples were divided into two fractions: one fraction was rapidly frozen in liquid nitrogen tank and stored in the deep freezer at -70°C until analysis and the other fraction was immersed into the formalin solution for histological and immunohistochemical analysis. The samples were homogenized with polytron homogenizer (Biotron, Swiss) in 5 volumes of Tris-HCl buffer (pH 7.4, 10 mM). The homogenates were centrifuged at 10,000 x g for 30 min at 4°C and the supernatants were recentrifuged at 105,000 x g for 1 hour at 4°C with Beckman ultracentrifuge. The supernatants were divided into 100 μl aliquots and used as cytosol fraction for GS and GST analysis. The precipitates were suspended in potassium phosphate buffer (pH 7.4, 0.15 M, 0.1 mM PMSF), added glycerol to 20% solution and used as microsomal fraction for GGTP, cytochrome b5 and, cytochrome P450's analysis.

*Enzymatic analysis*

Various enzyme activities in the samples were monitored after the respective standard method: namely, gamma-glutamyltranspeptidase after Szasz (1974), glutamine synthetase after Meister (1976), and glutathione S-transferase after Habig *et al* (1974). And the protein concentration was determined by Lowry's method (1951). The content of cytochrome b5 was estimated from its redox spectrum of NADH reduced versus oxidized cytochrome and cytochrome P450 was determined by spectral change at 450 nm induced by CO exposure (Lake 1987).

*Enzyme linked immunoassay for cytochrome P450's*

Each 10 μg protein of the microsomal fractions from the varying sources was applied on the 96 well plate (Costar). After four hours incubation, the nonadsorbed samples were washed out three times with potassium phosphate buffer (pH 7.4, 100 mM). The monoclonal antibodies to the type-specific cytochrome P450's were 500-fold diluted and each 100 μl of antibody solution was added to each well and the mixture was incubated for the next four hours. Then the well plate was again washed with potassium phosphate buffer three times. Finally the alkaline phosphatase - conjugated second antibody solution (1/1000 dilution) was added and incubated overnight. After overnight incubation, the plates were washed now with Tris-HCl buffer (pH 7.4, 100 mM). The washed plates were incubated for alkaline phosphatase activity expression with enzyme analysis kit solution, containing p-nitrophenyl phosphate. And the absorbances were monitored at 540 nm.

*Immunohistochemical analysis*

Sections (3 mm thick) of the livers were immediately fixed in formalin solution and embedded in paraffin. Serial sections were prepared and each section was subjected to standard hematoxylin-eosin staining or immunohistochemistry for GST-P using peroxidase-anti-peroxidase complex, visualized with diaminobenzidine oxidation. The relative ratio of GST-P positive foci to the whole section was calculated with the image analyzer.

## RESULTS

### *Activities of glutamine synthetase and gamma-glutamyl transpeptidase*

The enzymic activities of glutamine synthetase (GS) in the cytosol fractions of the hepatocarcinogenic tissues were rather decreased by DEN and AAF treatment, which were not recovered to the normal level even through the partial hepatectomy. The control GS activities of the hepatic tissues were 0.566 I.U./mg protein, while GS activities in the carcinogen treated and regenerating liver tissues were in the range of 0.313 to 0.629 I.U./mg protein until 1 month of posthepatectomy. Only after 2 months, the GS activities in the hepatic tissues were restored and rather increased further on (Table 1). In case of gamma-glutamyltranspeptidase (GGTP), its activity at normal control liver tissues was 5.43 I.U./mg protein, which was increased to 13.12 I.U./mg protein, approximately to 2.5 fold, by treatment with DEN and AAF, and the following partial hepatectomy kept its activity at higher level than the normal, but with decreasing tendency (Table 1). GGPT activity was highest after AAF treatment and immediately after partial hepatectomy (Table 1).

Table 1. Change of enzymic activities in the hepatic tissues of Sprague-Dawley rats, modified by carcinogen treatment and partial hepatectomy.

| Groups / Enzymes | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 | Group 8 | Group 9 | Group 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Glutamine synthetase (I.U./mg protein) | 0.566 ±0.026 | 0.499 ±0.038 | 0.509 ±0.048 | 0.603 ±0.046 | 0.629 ±0.053 | 0.481** ±0.048 | 0.313 ±0.026 | 0.471*** ±0.030 | 0.862*** ±0.061 | 0.926*** ±0.071 |
| (percentage) | 100 | 88.2 | 89.9 | 106.5 | 110.8 | 70.8 | 55.3 | 83.2 | 152.3 | 163.6 |
| γ-Glutamyl transpeptidase | 5.43 ±0.65 | 8.325 ±1.997 | 13.12*** ±0.82 | 10.06** ±1.77 | 6.575 ±1.305 | 9.525* ± 1.64 | 9.357* ±1.15 | 5.612 ±1.104 | 7.5 ±0.84 | 6.1 ±1.3 |
| (percentage) | 100 | 153.2 | 241.6 | 185.3 | 121.1 | 175.4 | 172.3 | 103.4 | 138.1 | 112.3 |
| Glutathione S-transferase (I.U./mg protein) | 0.349 ±0.027 | 0.482*** ±0.018 | 0.503*** ±0.042 | 0.461*** ±0.016 | 0.410 ±0.031 | 0.310 ±0.020 | 0.315 ±0.035 | 0.416 ±0.016 | 0.669*** ±0.042 | 0.836*** ±0.024 |
| (percentage) | 100 | 138.1 | 144.1 | 132.1 | 117.5 | 88.8 | 90.3 | 119.2 | 191.7 | 239.5 |

* All the values are means ± S.E.M. (n=8), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ by *t* test

Table 2. **Content changes of cytochrome b5 and cytochrome P450's in the hepatic tissues of rats in response to carcinogen treatment and partial hepatectomy.**

| Classes / Enzyme | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 | Group 8 | Group 9 | Group 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Cytochrome b5 (nmole/mg microsomal proteins) | 0.69 ±0.06 | 0.73 ±0.09 | 0.70 ±0.01 | 0.786 ±0.09 | 0.681 ±0.03 | 0.72 ±0.03 | 0.82 ±0.06 | 0.58 ±0.09 | 0.54 ±0.08 | 0.98** ±0.04 |
| (percentage) | 100 | 105.8 | 101.4 | 113.9 | 98.7 | 104.3 | 118.8 | 83.9 | 78.4 | 142.0 |
| Cytochrome P450 (nmole/mg microsomal proteins) | 1.75 ±1.0 | 1.29 ±0.5 | 1.8 ± 0.8 | 3.4 ±0.4 | 4.0 ±0.5 | 1.6 ±0.8 | 3.1 ±0.5 | 1.9 ±0.3 | 1.3 ±0.3 | 3.0 ±0.5 |
| (percentage) | 100 | 71.4 | 102.8 | 193.7 | 230.3 | 93.7 | 176.6 | 109.1 | 73.7 | 170.3 |

* All the values are means ± S.E.M. (n=8), ** $P < 0.005$ by $t$ test

*Activities of glutathione S-transferase*

The enzymatic activities of glutathione S-transferase (GST) were 0.349 I.U./mg protein at control liver tissues, which were increased to 0.503 I.U./mg protein (1.5 fold to the normal) by treatment with DEN and AAF. However, the partial hepatectomy caused its activity to decrease below the normal level, one day after the operation. But after one month of partial hepatectomy, GST activities in the hepatic tissues started to increase continuously, which marked 0.836 I.U./mg protein, 2.4 fold of the normal GST activity at 6 months after (Table 1).

*Contents of cytochrome b5 and cytochrome P450's*

The contents of cytochrome b5 in the hepatic tissues of Sprague-Dawley rats were almost constant in spite of the consecutive treatments with DEN, AAF and partial hepatectomy (Table 2). However, in case of cytochrome P450's, its contents were increased upto two fold by carcinogen treatment in contrast to the decrease following partial hepatectomy (Table 2). The variation in the contents of P450 isozymes, monitored by the respective type specific monoclonal antibodies, showed the fluctuation pattern. In case of T.C.D.D.-

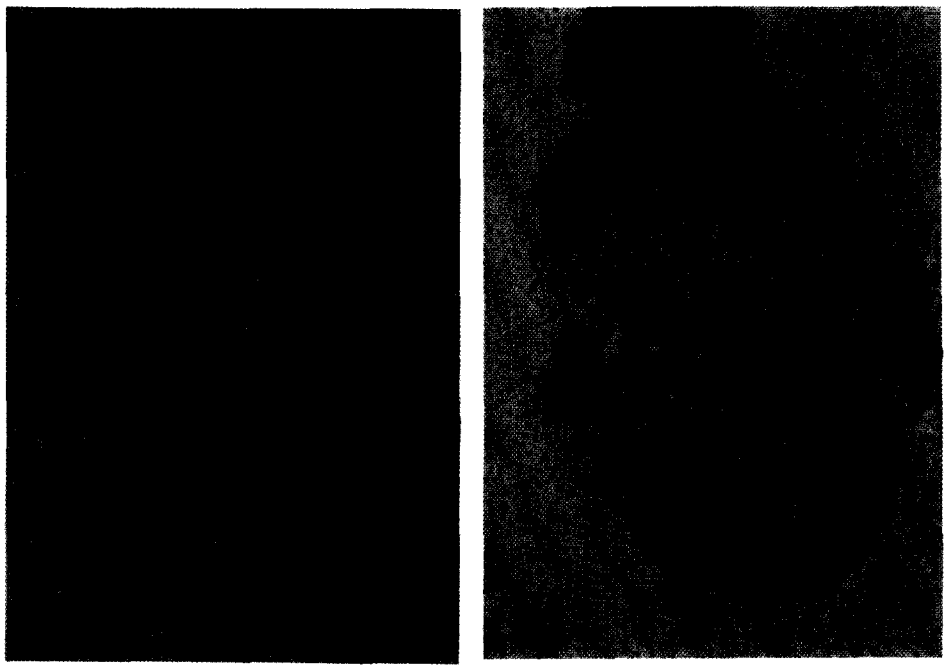

**Fig. 2. Immunohistochemical staining of GST-P positive foci with peroxidase-antiperoxidase complex, visualized by diaminobenzidine oxidation (400 x). (a) two weeks after DEN treatment (b) 6 months after partial hepatectomy.**

**Table 3. Changes of isozymic pattern of rat hepatic cytochrome P450's in response to carcinogen treatment and partial hepatectomy.**

| Groups<br>P450 isozymes | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 | Group 8 | Group 9 | Group 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| T.C.D.D. P450 | 0.173 | 0.173 | 0.170 | 0.197 | 0.195 | 0.205 | 0.151 | 0.143 | 0.145 | 0.177 |
| | ±0.034 | ±0.028 | ±0.025 | ±0.024 | ±0.017 | ±0.087 | ±0.020 | ±0.830 | ±0.023 | ±0.016 |
| (percentage) | 100 | 100 | 98.3 | 113.8 | 112.7 | 118.5 | 87.3 | 82.7 | 83.8 | 102.3 |
| Pb–P450 | 0.212 | 0.235 | 0.192 | 0.199 | 0.179 | 0.329 | 0.234 | 0.207 | 0.223 | 0.342 |
| | ±0.036 | ±0.039 | ±0.032 | ±0.045 | ±0.054 | ±0.047 | ±0.038 | ±0.038 | ±0.044 | ±0.051 |
| (percentage) | 100 | 110.8 | 90.6 | 93.9 | 84.4 | 155.2 | 110.4 | 97.6 | 105.1 | 161.3 |
| Et-P450 | 0.114 | 0.100 | 0.153 | 0.133 | 0.125 | 0.127 | 0.118 | 0.123 | 0.109 | 0.135 |
| | ±0.009 | ±0.011 | ±0.025 | ±0.021 | ±0.018 | ±0.016 | ±0.016 | ±0.016 | ±0.017 | ±0.019 |
| (percentage) | 100 | 87.7 | 134 | 116.7 | 109.6 | 111.4 | 103.5 | 107.8 | 95.6 | 118.4 |
| PCN–P450 | 0.118 | 0.127 | 0.115 | 0.109 | 0.088 | 0.124 | 0.095 | 0.108 | 0.094 | 0.128 |
| | ±0.006 | ±0.009 | ±0.015 | ±0.007 | ±0.012 | ±0.016 | ±0.016 | ±0.009 | ±0.014 | ±0.015 |
| (percentage) | 100 | 107.6 | 97.5 | 92.4 | 74.6 | 105.1 | 80.5 | 91.5 | 79.7 | 108.5 |
| cAMP–P450 | 0.050 | 0.072 | 0.082* | 0.078 | 0.069 | 0.112* | 0.093* | 0.069 | 0.097* | 0.091* |
| | ±0.004 | ±0.016 | ±0.009 | ±0.013 | ±0.013 | ±0.017 | ±0.010 | ±0.010 | ±0.815 | ±0.015 |
| (percentage) | 100 | 144 | 164 | 156 | 138 | 224 | 186 | 138 | 194 | 182 |

All the values are means ± S.E.M. (n=8), which were obtained as O.D. values at 540 nm in Elisa assay using the type-specific monoclonal antibodies. T.C.D.D.–P450 stands for 2,3,7,8-tetrachlorodibenzo-P-dioxin dependent cytochrome P450: Pb-P450, for phenobarbital-inducible P450; Et–P450, for ethanol-inducible P450: PCN-P450, for pregnenolone-inducible P450; cAMP-P450, for cAMP-inducible P450. *$P < 0.01$ by $t$ test.

P450, its contents were not much variable, while the contents of Pb-P450 were increased at later stage following partial hepatectomy. In cases of Et-P450 and PCN-P450, their content variations were rather similar to those of T.C.D.D.-P450. But the contents of cAMP-P450 in the hepatic tissues were elevated approximately two fold by the treatments of carcinogen and partial hepatectomy (Table 3).

*Appearance of hyperplastic nodule*

The hyperplastic nodule, detected with GST-P antibody by immunohistochemical analysis appeared first, two weeks after DEN treatment as a single cell foci (Fig. 2a). Following the treatments with AAF and partial hepatectomy, the GST-P positive foci were enlarged in area and increased in number (Fig. 2b). The relative ratio of GST-P positive area to the whole section of the specimens was increasing: 0.004 at 1 month following hepatectomy, 0.013 at 2 months after and 0.036 at 6 months after (Table 4).

**Table 4. Area ratio of GST-P positive foci in the rat hepatocarcinogenic tissues**

| Group | Area ratio of GST-P positive foci |
|---|---|
| Group 1. | N.D.* |
| Group 2. | 0.002 ± 0.001 |
| Group 9. | 0.013 ± 0.005** |
| Group 10. | 0.036 ± 0.005*** |

*N.D.: non-detectable
All the values are means ± S.E.M. (standard error of mean)
** $P < 0.05$, *** $P < 0.001$ by *t* test

## DISCUSSION

Glutamine synthetase (GS) plays the major role in the nitrogen metabolism, since its product, glutamine is the most important starting molecule for biosynthesis of amino acids, nucleotides and other nitrogenous compounds (Meister, 1976). Therefore, its activity could be correlated with the capacity of positive nitrogen balance of the tissue, the proliferating capability. In order to gain the property of proliferation as one of the malignant phenotypes, the carcinogenic tissue should be imposed on with the higher induction of glutamine synthetase. Actually, its activity in some cancer tissue was higher more than five fold to that in the normal control tissue (Park *et al.*, 1987). In case of the hepatocarcinogenic model, the direct effect of carcinogen administra-

tion on GS activity in the liver tissues was rather suppressive (Table 1). The regenerating stimuli by partial hepatectomy resumed the tissue GS activity, but which was less than expected from the data of GS activity change in the simple regeneration process (Koh *et al.*, 1987). But it is noteworthy that the tissue GS activity was increased at later stage after two months of partial hepatectomy, when was consistent with the period of the hyperplastic nodule appearance (Table 1, Table 4).

On the other hand, the role of gamma-glutamyltranspeptidase (GGTP) has been related with amino acid uptake and glutatione utilization (Orlowsky & Meister, 1970; Meister, 1976). Therefore, GGTP was suggested as the additional indicator of nitrogen balance and also as a preneoplastic marker in the experimental hepatoma study (Jalanko & Ruoslahti, 1979; Fiala *et al.*, 1972; Fiala & Fiala, 1973). GGTP activity was increased several fold in the human hepatoma and the cervical cancer tissues in comparison with those in the normal tissues (Roomi *et al.*, 1985; Park *et al.*, 1987). However, the present data from the hepatocarcinogenic model showed the high GGTP activity immediately after partial hepatectomy, which decreased later on (Table 1). These result was contrasting to our assumption, because GGTP was believed to be the preneoplastic marker with the increasing activity in response to carcinogenic stimuli. Considering that the data of the present experiment were monitored in the microsomal fraction of the whole liver tissues, different from the other studies, where the tissues of the hyperplastic nodules were collected, it was deviated from our assumption that GGTP activity would increase at the later stage when the hyperplastic nodules appeared (Table 4, Fig. 2b).

In the process of chemical carcinogenesis, the appearance of the hyperplastic nodules were deeply related with the biochemical adaptability, that is, the chemical resistancy of the carcinogenic tissues. In order to overcome the environmental load, the tissue resumes the capability to detoxify the toxic xenobiotics and to pump them out of the tissues. Actually, the malignant tumor tissues showed the resistency inducibility to anti-cancer drugs through the higher potential for drug-inactivation, reduction of drug-accumulation, and amplification of target molecules of the anticancer drugs (Fojo *et al.*, 1985; Kaufman, 1984; Scotto, 1986; Tupule *et al.*, 1986), resulting in low levels of cytochrome P450's, high activities of glutathione S-transferase and UDP-glucuronyl transferase (Eriksson *et al.*, 1983; Astrom *et al.*, 1983). But in the present experiment, the total contents of cytochrome P450's and cytochrome b5 did not show the significant change in the process of the carcinogenesis of the hepatic tissues (Table 2), in contrast to the decrease of hemoproteins in the cancer tissues (Park *et al.*, 1987; Stout & Becker, 1987). In cases of the isozymes of the cytochrome P450's, the contents of Pb-P450 and cAMP-P450 were slightly increased while those of other P-450's were remained at steady state during the carcinogenic period (Table 3). These results were different from the reports, which showed the markedly reduced activities of mixed function oxidase in the hepatocarcinogenic model (Astrom *et al.*, 1983). However, in case of the conjugation enzymes, the glutathione S-transferase (GST) was initially increased in activity after carcinogenic stimuli, which

was decreased after 24 hours of partial hepatectomy, but followed by the high increase after 1 month (Table 1). The high increase of GST matched with the previous reports of the higher activity in hyperplastic nodule (Levin *et al* 1978; Astrom *et al.,* 1983), where it was reported that the specific isozyme of GST, the acidic form, was induced. By use of the immunohistochemical assay with the specific antibody to GST-P, we could monitored the increase of the hyperplastic nudule as GST-P positive foci (Kitahara *et al.,* 1984; Moore *et al.,* 1985), from which we could prove that the hepatocarcinogenic model in the present experiment was working in the carcinogenic direction (Table 4).

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# 발암원과 부분간절제술 처리에 의한 백서 간 조직중 질소이용계 및 이물질 대사계 효소의 변화

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Solt와 Farber씨의 방법에 따라 간암유발 자극을 준 흰쥐간조직중의 경시적 생화학적 변화를 구명해본 결과, 질소이용계 효소중 중추적 역할을 하는 glutamine synthetase의 활성은 부분간절제에 의하여 약간 증가되고, 절제술후 2개월이후 유의하게 증가되어 증식성 결절의 출현시기와 일치되는 소견을 보였으나, gamma - glutamyl transpeptidase는 암화자극의 초기에 증가되었을뿐 지속적 증가 현상을 보이지 않았다. 한편 이물질 대사의 phase Ⅰ 효소계인 cytochrome P450의 총량 및 단세포군 항체를 이용한 특정 isozyme의 변화 pattern 분석결과, cytochrome P450의 총량이나, 대부분의 cytochrome P450 isozyme pattern에는 유의한 변화가 없었으며 또다른 hemoprotein의 일종인 cytochrome b5의 양에도 유의한 변화가 없었다. 반면 phase Ⅱ 효소계인 glutathione S-transferase의 활성은 간부분절제술후 2개월이후부터 증가되었을 뿐 아니라, 간조직의 면역조직화학적 방법에 따라 분석해본 결과, 증식성 결절들이 GST-P양성 결절로 출현함을 확인하였다. 이상의 결과로서 암조직에서 일반적으로 보이는 GGTP의 높은 활성, cytochrome P450의 함량저하 등의 현상은 본 전암성 병변에서 아직 관찰되지 않았으나, 증식성 결절의 출현과 일치되어 나타나는 glutamine synthetase 와 glutathione S-transferase의 활성증가는 암화조직의 질소이용계의 촉진과 이물질 또는 화학물질들에 대한 세포의 저항기능의 항진의 바탕을 이루고 있음을 시사해 주고 있다.