CORRELATIVE CYTOCHEMICAL AND BIOCHEMICAL STUDIES OF CARBOHYDRATE METABOLISM DURING HEPATOCARCINOGENESIS. D.Mayer, H.J.Hacker, F.Klimek and P.Bannasch. Institute for Experimental Pathology, German Cancer Research Center, Heidelberg, F.R.G.

Treatment of rats with hepatocarcinogens results in the appearance of putative preneoplastic foci storing glycogen in excess, which later develop into so-called mixed cell foci with variable glycogen content. Different methods were used to investigate some aspects of carbohydrate metabolism in these lesions. Histochemically both types of foci usually reveal typical patterns of alterations in the activities of glucose-6-Pase (decrease), glucose-6-P-dehydrogenase (increase) and glycogen phosphorylase (decrease). As a rule, the lesions occupy only a few percent of the total liver parenchyma. Therefore, most of the alterations in glycogen content and enzyme activities could not be measured in liver homogenates. However, with micro-dissection techniques and biochemical microanalyses it could be shown that the glycogen storage foci contain up to 100% more glycogen than the surrounding tissue or control liver, and that glucose-6-P-dehydrogenase activity was up to 15 fold increased in the mixed cell foci, while it was only slightly elevated in the glycogen storage foci.

THE ROLE OF GLUTATHIONE IN THE DETOXICATION OF REACTIVE METABOLITES GENERATED FROM THE CARCINOGEN N-HYDROXY-2-ACETYLAMINOFLUORENE (N-OH-AAF) IN THE RAT *IN VIVO*. J.H.N.Meerman and G.J.Mulder. Department of Pharmacology, State University of Groningen, Groningen, The Netherlands.

We have shown previously that reactive metabolites formed from the carcinogen N-OH-AAF in the rat which can bind covalently to cellular macromolecules, react with glutathione and are excreted into bile as glutathione conjugates (Meerman *et al.*, Chem.-Biol. Interact. 39, 149, 1982). However, we now report that when glutathione was depleted with diethylmaleate, covalent binding of (ring-^{3}H)-N-OH-AAF to cellular macromolecules was not increased as compared to that in the livers of control rats. This suggests that glutathione does not trap the reactive metabolites of N-OH-AAF that react with cellular macromolecules. This is supported by the observation that the covalent binding of N-acetoxy-2-acetylaminofluorene (a synthetic analogue of the N-sulphate metabolite of N-OH-AAF, which is the main reactive metabolite formed *in vivo*) to RNA *in vitro* could not be prevented by trapping of the reactive nitrenium ion with glutathione or similar nucleophiles. More effective is glutathione catalysed hydrolysis by a SN_2 mechanism back to N-OH-AAF. These results indicate that reactive metabolites of N-OH-AAF formed *in vivo* preferentially react with nucleophiles other than glutathione and that glutathione protects cellular macromolecules from damage by N-OH-AAF to only a very limited extent.

IMMUNOHISTOCHEMICAL LOCALIZATION OF CELLS CONTAINING O^6-ETHYLDEOXYGUANOSINE (O^6-EtdGuo) IN LIVER SECTIONS OF DEN- AND ENU-TREATED RATS. G.J.Menkveld, C.J.Van Der Laken, G.Hermsen, E.Kriek, E.Scherer and L.Den Engelse. The Netherlands Cancer Institute, Division of Chemical Carcinogenesis, Amsterdam.

Very sensitive immunological assays for the detection of carcinogen-DNA adducts in isolated DNA have been developed in the last few years. In our Division a highly sensitive immunoassay for O^6-EtdGuo and AAF-guanosin-8-yl in DNA has been developed recently (Van Der Laken *et al.*, Carcinogenesis, 3, 569, 1982). Furthermore, an immunohistochemical procedure has been introduced by Heyting *et al.* (Cancer Res., in press) for the detection of O^6-EtdGuo in rat brain sections. In the experiments reported here this procedure has been adapted to rat liver. Localization of O^6-EtdGuo was visualized in frozen liver sections by double PAP staining after treatment with RNAse and NaOH. O^6-EtdGuo was found in nuclei of parenchymal cells after injection with ENU (3 hr after 140 mg/kg) or DEN (5 hr after 50, 25 and 12, but not after 6 mg/kg). A striking heterogeneity in staining pattern was observed after DEN: centrilobular regions were stained much more than peripheral zones. After ENU no such heterogeneity was observed. In the case of DEN the intensity of the brown precipitate was clearly and positively dose-related. Intensity also decreased with time after DEN (50 mg/kg) injection: strong at 5 hr, weak at 24 hr, absent at 7 days. Using rabbit anti-AAF-guanosin-8-yl antiserum, positive results were also obtained in liver sections of AAF-treated (65 hr after 8 mg/kg) rats.