alone being designated as the substantia. Others, SZENTAGOTHAI[6], and HEIMER and WALL[7], grant that while there is a slight difference in the cytoarchitectonics of laminae II and III the fiber connections of these 2 laminae are so similar that they both must be included in a definition of the substantia. The results of the present study add little clarification to this problem except to indicate that the area of the chick cord designated the substantia can also be subdivided by differences in morphology and development into discrete lateral and medial regions.

While the boundaries of the substantia remain in question, there appears to be general agreement with the theory, postulated by SZENTAGOTHAI[6], that the substantia is an intrinsic system. The findings of this study, however, reveal that the large neurons, found in the lateral region of the substantia, send their axons into the posterior commissure in the area of the cornu commissuralis of Marie (CAJAL[8]). That these axons may simply re-enter the dorsal horn at a more rostral level cannot at this time be disproved but it is interesting to speculate that the large neurons may in fact send their axons to higher brain centers. At present experiments, utilizing degeneration techniques, are underway to test this possibility[9].

Résumé. Basée selon des critères morphologiques et embryologiques, la substance gélatineuse de la moëlle épinière des Oiseaux peut se diviser en régions latérale et médiale. On suppose que la substance gélatineuse peut être connectée avec les centres supérieurs du cerveau par les grands neurones que l'on a trouvé dans la région latérale.

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Acquisition of an Embryonal Biochemical Feature by Rat Hepatomas

Glutathionase splits glutathione (GSH) by γ-glutamyl transpeptidation[1,2]. The enzyme is present in high activity in the liver of some species, e.g. rabbits or guinea-pigs, but in the liver of some other species as e.g. rat, GSH-ase activity was not detected[3,4]. We demonstrated recently[5,6] a very high GSH-ase activity in various rat hepatomas, in contrast to normal adult rat liver where the activity was very low. Two methods were used for the assay of GSH-ase: 1) polarographic, using GSH as substrate and glycylglycine as the acceptor of the γ-glutamyl group[5]; 2) colorimetric, using a synthetic substrate γ-glutamyl-*p*-nitroanilide (Boehringer Mannheim Corp.), which liberates after the transfer of the γ-glutamyl group, a colored product *p*-nitroaniline[7]. The fact that high GSH-ase activity was observed in rat hepatomas which differed not only in regard to the chemical nature of the inducing carcinogens (3′-methyl, 4-dimethyl aminoazobenzene, DAB, and derivatives of 2-acetyl aminofluorene), but also in regard to the rate of growth (from 4 days to 7 months between transfers) and the degree of differentiation, suggested that the activation of GSH-ase may be an intrinsic feature of rat liver carcinogenesis ('carcinotropic effect')[8]. In accord with this contention, no activation of GSH-ase was observed in regenerating or tumor-bearing rat liver[5,6,8]. Feeding carcinogenic 3′-Me, DAB (0.06%) in a semisynthetic diet increased GSH-ase activity markedly, while feeding non-carcinogenic 2-Me, DAB did not product any substantial change in this activity. Figure 1 shows these effects after 20 days of azodye feeding, using a polarographic assay.

Looking for the nature of GSH-ase activation during carcinogenesis in rat liver, we found that in distinction to adult rat, the embryonal and neonatal livers contained a very high GSH-ase. At 1 h after birth the activity on unit of weight basis was as high, and on unit of DNA almost as high, as in Morris Hepatoma 5123A; at 16 h it was even higher on dry weight basis, while on DNA basis it was slightly lower. The activity between 24–48 h seemed not to change much, but then the activity rapidly declined; at 5 days after birth it was still several times higher than in normal adult rat liver, but 10 days after birth the activity was as low as in adult rat. Figure 2 illustrates this trend, using colorimetric method; polarographic method gave qualitatively the same results.

These observations indicate that as a result of malignant transformation, a neonatal biochemical feature is reactivated in rat liver, whereas differentiation during normal development leads to the suppression of this embryonic character. We do not know yet whether the activation of GSH-ase during rat liver carcinogenesis is simply a manifestation of the proliferation of primitive cells ('stem cells') which cannot differentiate[9] or whether there is a reactivation (derepression) in the parenchymal cells of a latent property which is normally repressed in the adult state. The first alternative is accessible to testing with a new histochemical reaction for γ-glutamyl transpeptidase[10] which showed some γ-G-ase (γ-glutamyl transpeptidase, GSH-ase) activity in endothelial cells of periportal vessels, in the bile duct epithelium and in Kupffer cells, while no activity was detected in hepatic

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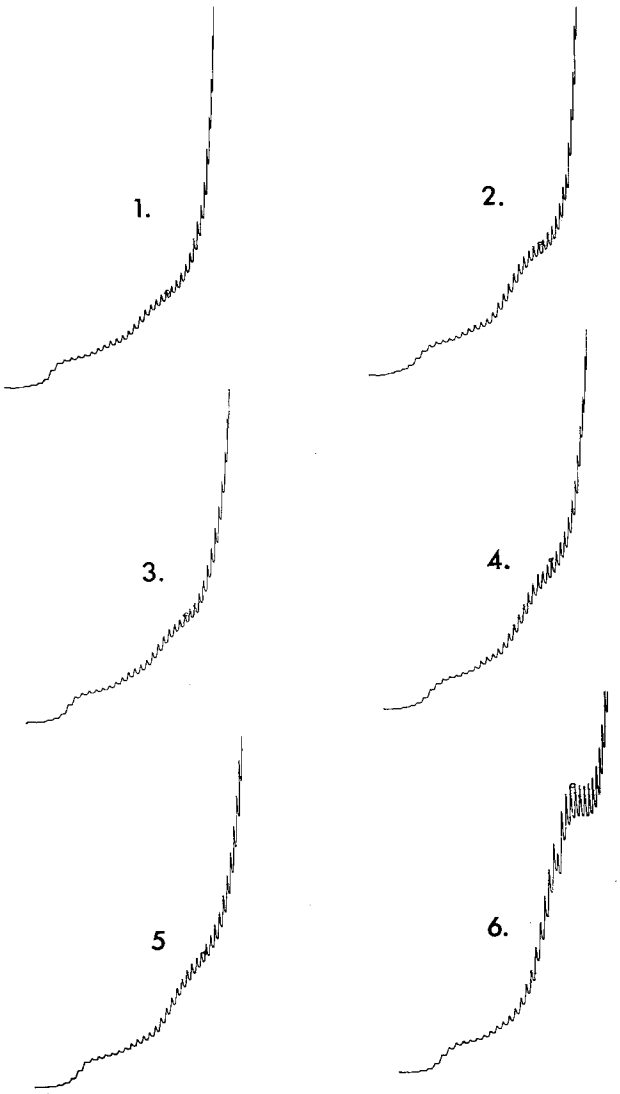

Fig. 1. Activation of GSH-ase in rat liver by carcinogenic azodye 3'-Me,DAB. Lack of activation by non-carcinogenic 2-Me,DAB. Polarographic assay. Enzymatic splitting of GSH manifested by a high maximum at -1.6 V (L-cysteine), following the reduction step of cobaltous ion at -1.2 V. Recorded from -0.8 V at the sensitivity 1:50. Acetone powdered tissue (50 mg) resuspended with stirring in 0.5 ml distilled water, incubated for 0 min and 30 min with 3.25 μmoles GSH and 55 μmoles glycylglycine, in phosphate (0.1 *M*) buffer pH 7.2. Total volume 1.2 ml. Reaction stopped with 0.1 ml 70% perchloric acid. After centrifuging 0.1 ml of the supernatant added to 5 ml of Brdička's solution ($CoCl_2$, $1\times10^{-3}M$; NH_4OH 0.1 *N*, NH_4OH 0.1 *N*). (1 and 2) Basal diet, 20 days, 0 min and 30 min. (3 and 4) Basal diet + 2-Me,DAB, 20 days, 0 min and 30 min. (5 and 6) Basal diet + 3'-Me,DAB, 20 days, 0 min and 30 min.

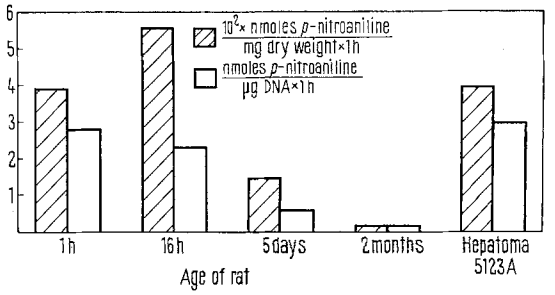

Fig. 2. γ-Glutamyl Transpeptidase (Glutathionase) in neonatal rat liver, adult (2 months) rat liver and in transplantable rat hepatoma 5123 A (Morris). Colorimetric assay. Bars illustrate milimicromoles *p*-nitroaniline liberated from γ-glutamyl-*p*-nitroanilide. Suspension of 5 mg acetone powdered tissue in 1.0 ml *Tris* buffer pH 7.4 was incubated with or without substrate in a system containing 22 μmoles of glycylglycine, 3 ml 0.1 *M Tris* buffer pH 7.6 and 5 μmoles L-γ-glutamyl-*p*-nitroanilid. Total volume was 4 ml. Reaction stopped by heating to 100 °C for 2 min. After centrifugation, the amount of liberated *p*-nitroaniline was measured as the difference in optical density at 410 nm between samples with and without substrate. For calculation, the color of substrate incubated in absence of enzyme was subtracted from the difference.

cells[10]. The facts, however, seem to be more compatible with the second alternative: A very high GSH-ase activity was found in REUBER's hepatoma H-139[6] which like REUBER's H-35[11] is a well-differentiated tumor containing glycogen, producing bile and containing the level of glucose-6-phosphatase activity as high as in normal adult rat liver[6]. All this testifies to the origin of this tumor from parenchymatous liver cells by partial dedifferentiation. The second reason speaking for the second alternative is the fact that the withdrawal of the carcinogen from the food, provided it was done before the 'critical stage of carcinogenesis'[12], led to a substantial decrease, if not a complete loss, of enhanced GSH-ase activity, paralleling roughly the loss of protein-bound carcinogen. It appears, thus, that the acquisition of high GSH-ase activity by rat hepatomas is a derepression, induced by hepatocarcinogens, of an embryonal property which is normally repressed in the adult state. It is known that in many tumors new antigens appear[13]. In tumours of viral origin some of these antigens belong to viral genome. But in other cases these newly appearing antigens are not really new. They correspond to antigens which were present in cells during embryonal development, the elaboration of which was repressed in the fully differentiated state. This is well documented in the case of mouse hepatomas[14]. The relationship of activated GSH-ase to the antigenic structure of rat hepatomas and of embryonal rat liver tissue is presently under investigation.

Zusammenfassung. Nachweis, dass experimentelle Hepatome in der Ratte eine hohe Glutathionase-Aktivität bewirken. Dasselbe gilt auch für die embryonale, nicht aber für die adulte Rattenleber.

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