STUDY ON ORGANIC ANIONS BINDING WITH ISOLATED LIVER SURFACE MEMBRANES

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Plasma membranes were isolated from the rat liver by the differential centrifugation. Then the ability of plasma membranes to bind 35S-BSP or 131 I-BSP was investigated in the presence of the serum protein. Furthermore, the BSP binding proteir was isolated from membranes of rat liver with Desoxycholic acid. (DOC) Gelfiltration experiments on columns of sepha G 200 was performed at the various concentration of DOC.

Results 1) The binding capacity of liver plasma membranes was destroyed by only trypstic digestion. Digestion of liver plasma membranes with phospholipase A,C and neuraminidase did not affect its capacity to bind 131 I-BSP. 2) High and low association constants and maximal binding capacity for BSP of rat liver plasma membranes are $K=9.18X10^{7}M^{-1}$, $Q=5.31X10^{-2}$uM/mg, $K=1.37X10^{6}M^{-1}$, $Q=7.85X10^{-1}$uM/mg respectively.

3) The sephadex G 200 gelfiltration chromatography pattern of soluble liver plasma membranes did vary with the concentration of DOC in bulfer. In the absence of detergent hightly aggregated forms of the receptor is observed. The 35S-BSP that appeared with void volume on gelfiltration if the detergent is omitted entirely. The highest activity of 35S-BSP appeared in the second peak of solubilyed protein on gelfiltration at 0.2% of DOC in buffer. The 35S-BSP peak that appeared in the second peak in gelfiltration disappeared if the concentration of DOC increased more than 0.35%. Under this circumstance, DOC appeared to have in inhibitive property to bind 35S-BSP with liver plasma membranes.

ANALYSIS OF RADIORESPIROMETRIC PATTERN OF GLUCOSE(U-^{14}C) DURING THE FEEDING WITH 3'-Me-DAB IN RAT

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Respirometric pattern in rat during the feeding with 3'-Me-DAB for radioactivity from glucose(U-^{14}C) was studied associating with α-fetoprotein(AFP) and glycolytic enzyme activity.

Male Donryu rats(Nihon Rat Co.,Urawa),5 weeks old were maitained on the basal diet(CE-2,Clea Japan Inc.,Tokyo),until they were 7 weeks old weighing approximately 200 to 250g.Hepatic tumor was induced by the feeding with the diet containing 0.06% 3'-Me-DAB (Oriental Yeast Co.Ltd.,Tokyo) continuously for 6 weeks,and the control rats of comparable age were fed on CE-2 only. After the intraperitoneal administration of glucose(U-^{14}C)(2.5 μCi),the differential radioactivity of respiratory $^{14}CO_2$ was measured continuously for 2 hrs.Analysis of radiorespirometric pattern was determined with three parameters,peak time(PT),peak height(PH) and yield value(YV) by the method of Matsuoka et al. Glycolytic enzyme activity was carried out by the use of the supernatant centrifuged at 30,000g for 1 hr. The results were as follows:

(1) On radiorespirometry,PT was hastened at 2nd week, reached to the maximum at 4th week and returned to delay remarkably at 5th week.YV was occurred to be slightly lower level than that of control.

(2) AFP appeared in rat serum as early as the 3rd week after the administration of 3'-Me-DAB.

(3) Glycolytic enzyme ,glucose-6-phosphate dehydrogenase was markedly activated at 4th week. And hexokinase was also increased at 4th week.While pyruvate kinase was inactivated at a rate of 70% of control immediately after the feeding with 3'-Me-DAB. These activities recovered to the control levels at 6th week.

(4) From these experimental data,it seems that the acceleration of glucose oxidation at an early time of α-fetoprotein appearance could be well reflected with the changes of glucose-6-phosphate dehydrogenase and hexokinase activities.