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RADIORESPIROMETRY USING [1-C-14]GLUCOSE IN THE RAT DURING FEEDING WITH 3'-ME-DAB. S.Kojima,Y.Shiki and A.Kubodera. Faculty of Pharmaceutical Sciences,Teikyo University and Sciece University of Tokyo. Kanagawa and Tokyo.

We have estimated that the earlier peak time in radiorespirometry using [U-C-14]glucose as a substrate during the initial stage of 3'-Me-DAB feeding is due to activation of Hexose monophosphate(HMP) pathway. In order to determine whether the HMP of glucose metabolism is activated at this stage during feeding with 3'-Me-DAB, we carried out radiorespirometry using [1-C-14]glucose.

The use of this labelled compound as a substrate gave more clear cut results than that of [U-C-14]glucose. That is to say,peak time was delayed up to the 2nd week,but was early by the 4th week and was the same as the control at the 5th week and thereafter. Peak height and yield value both changed in almost the same way; they were lower than that of the control until 2nd week,began to increase from 3rd ,reach maximum at the 4th week and remained at the control level at the 5th week and thereafter. These changes well coincided with that of glucose-6-phosphate dehydrogenase activity.

As the result of this study, radiorespirometry using [1-C-14]glucose can be a useful method for an early diagnosis of this kind of carcinogenesis

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QUANTITATION OF DIGITOXIN METABOLITES BY RAT LIVER USING H-3 DIGITOXIN. Y.Soekawa, M.Ohta,M.Nokubo and K.Kitani. Tokyo Metropolitan Institute of Gerontology, Tokyo

H-3 digitoxin (dt3) and its metabolites by rat liver were successfully purified, separated and quantitated by column chromatography (XAD-2) and thin layer chromatography (TLC). Dt3 and its metabolites were extracted by ethanol from the sample and eluted from the column with ethanol after washing column with water. The ethanol fraction was furhter subjected to TLC (cyclohexane:acetone:glacial acetic acid, 49:49:2, 1 time, and isopropyl ether: methanol, 6:1, 4 times). Each metabolite fraction was quantitated from its radioactivity. The major metabolite by isolated hepatocytes preparation was digitoxigenin bisdigitoxoside (dt2) which consisted about 85 % of all metabolites after 1hr incubation. Digoxin (dg3) and digoxigenin bisdigitoxoside (dg2) were also found (6 % and 3 % respectively). On the other hand, in the bile obtained from rat previously iv injected with H-3 dt3, dg3 (44 %) and dg2 (22 %) and conjugated metabolites (17 %) were major metabolites. In conclusion, the presently reported method is relatively simple and efficient to qaunti tate dt3 metabolites in the sample and the results suggest the difference in the dt3 metabolism between vivo and vitro systems.

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STUDY ON ORGANIC ANIONS BINDING WITH ISOLATED LIVER SURFACE MEMBRANES: BSP BINDING TO SOLUBLE MATERIALS FROM TRYPSIN DIGESTED LIVER SURFACE MEMBRANE. M.TANNO**,H.YAMADA*,** C.TOBARI**, S.KAWAGUCHI**, K.CHIBA**, H.MURATA**, M.IIO** *Jikei University School of Medicine. ** Tokyo Metropolitan Geriatric Hospital. Tokyo.

The specific binding of organic anions such as BSP, bilirubin and ICG to the liver plasma membranes(LPM) has been extensively studied in many laboratories. Evidences from these studies has been suggested that the uptake of these dyes in LPM is a carrier mediated process. However, it has not yet known exactly what kind of nature does it have. The present studies demonstrate more detailed properties of BSP binding material released from LPM with trypsic digestion. (RESULTS) 1) The loss of binding of BSP to membranes observed upon extraction with trypsin is accompanied by the appearance of BSP binding materials in the high speed(100,000g) supernatant of the extract. 2) On gelfiltration of I-131 BSP high speed supernatant of trypsin digested LPM, 131-BSP count that appers in the void volume and retarded peak.(second peak) Relative high specific activity (count/protein) was observed at the second peak.3) The analysis of this specific BSP binding materials of LPM contained sialic acid, pentoses, hexoses and protein suggesting one fragment of glycoprotein.

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THE EFFECT OF SPIRONOLACTONE PRETREATMENT ON THE BILIARY EXCRETION OF H-3 DIGITOXIN IN THE RAT. S.Kanai,K.Kitani and Y.Minoda Tokyo Metropolitan Institute of Gerontology Tokyo.

Biliary excretion of radioactivity was compared for control rats(C) and Spironolactone (Sp) pretreated rats (10mg/100g, twice daily for 4 days, orally) of both sexes after H-3 digitoxin injection into the vein. Bile flow rate (ul/100g/min, mean±SD) increased almost two times in both male and female rats pretreated with Sp compared with respective control rats. Male (C,4.72 +0.33,n=6 ; Sp, 9.26 +1.29,n=5), female (C,4.40+ 0.60,n=6; Sp,8.39 +1.29,n=7). The 2 hr biliary recovery of the injected dose (%, mean + SD) also increased 4 to 5 times in both Sp treated groups compared with respective controls. Male (C, 13.76 +2.89; Sp,49.98 + 5.89), female (C, 4.49 + 0.60; Sp, 29.97 + 4.50). Since we previously reported that canalicular choleritic, bucolome, did not increase the biliary excretion of digitoxin, the observed increase in the biliary excretion of digitoxin activity was suggested to be due to the increase in the hepatic metabolic activity increased by Sp pretreatment. The increase in the conjugates fraction in the bile in Sp treated rats supported this interpretation.

Ref. S.Kanai et al. Jap.J.Nucl.Med.14:508, 1977. The effect of bucolome (canalicular choleretic) on the biliary excretion of H-3 digitoxin in the rat.