p-NITROPHENYLGLUCURONIDE FORMATION BY HOMOZYGOUS ADULT GUNN RATS

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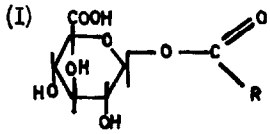
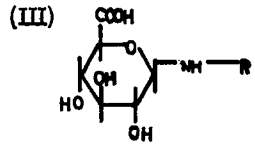
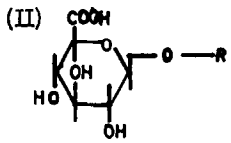
Bilirubin glucuronyltransferase, which is normally found in mammalian liver microsomes, is absent in similar preparations of homozygous Gunn rats (jj) (Schmid et al., 1957; Arias, 1959). Own experiments confirm these observations.

There has been reported also a low o-aminophenol-glucuronyltransferase activity with microsomal fractions of homozygous Gunn rats. Also no anthranilic acid and menthol is conjugated in Gunn rats (Schmid et al., Arias loc. cit.).

Recently Isselbacher (1961) distinguishes between glucuronyltransferases for the formation of N-Glycosides (III) in contrast to those for O-Glycosides (II) and Glycoside-esters (I).







The above scheme received further support by the observation that liver slices of (jj)-rats are able to form aniline glucuronide (Arias, 1961).

We now wish to report that the microsomal fraction from livers of (icteric), adult homozygous Gunn rats (jj) possesses approximately the same glucuronyltransferase activity for p-nitrophenol as similar preparations from normal, non-icteric Wistar rats (JJ).

Preparation of microsomes: Immediately after decapitation and exsanguination the livers were homogenized in 3 vol. of 0,1 M KCl (Elvehjem-Potter). After centrifugation for 10 min. at 15.000 x g. the microsomes were sedimented by centrifugation for 60 min. at 105.000 x g. and resuspended in water. All steps were carried out at 0-4°C.

TABLE I

Formation of p-nitrophenylglucuronide by microsomes from normal Wistar rats (JJ) versus homozygous (jj) Gunn rat livers.

μmoles p-nitrophenylglucuronide/mgN/ 30 min.	
♂ (JJ) normal rats (3 month of age)	♂ (jj)-Gunn-rats (3 month of age)
0,030	0,068
0,022	0,043
0,034	0,027
0,048	0,030
	0,047

Incubation system (according to Isselbacher (1961), slightly modified): 0,15 μm p-nitrophenol; 0,06 μm UDPGA (Sigma Chemical Co.); 26 μm Tris buffer, pH 7,4; 1 μm 1,4-saccharolactone; 0,03 ml microsomal suspension (Tot. vol. 0,305 ml). Incubation temperature: 37°C. Reaction was stopped by addition of ethanol. Aliquots were added to 10 N KOH solution for spectrophotometric assay at λ = 400 mμ.

All figures represent determinations in duplo.

Liver homogenates from newborn, homozygous Gunn rats (jj) exhibit a low activity for the glucuronidation of p-nitrophenol as compared with newborn normal rats. The latter conjugate p-nitrophenol at the adult level.

With liver homogenates (four hours, resp. immediately post mortem) of normal newborn and immature (26 weeks) children, we found essentially no formation of p-nitrophenylglucuronide.

This may suggest, that adult homozygous Gunn rats cannot be compared with newborn children, with respect to p-nitrophenolglucuronidation.

Our results demonstrate that phenolglucuronidation is possible in adult, homozygous Gunn rats. In as much as the different glucuronyltransferase activity may be separable from each other, one may be justified in assuming separate enzymes for N-, O- and ester-glycoside formation.

ACKNOWLEDGMENTS

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REFERENCES

Arias, I. M., N. Y. State J. Med., 3759 (1959); Biochem. and Biophys. Res. Comm., 6, 81 (1961).

Isselbacher, K., Biochem. and Biophys. Res. Comm., 5, 243 (1961).

Schmid, R., Axelrod, J., Hammaker, L. and Rosenthal, I. M., J. Clin. Invest., 36, 927 (1957).