# ETHEREAL AND N-LINKED GLUCURONIDE FORMATION BY NORMAL AND GUNN RATS IN VITRO AND IN VIVO *

Irwin M. Arias

Department of Medicine, Albert Einstein College
of Medicine and Bronx Municipal Hospital Center
New York, New York

Received September 18, 1961

The biosynthesis of ester, ethereal and N-linked glucuronides involves the transfer of glucuronic acid from uridine diphosphate glucuronic acid (UDPGA) to suitable receptors. This reaction is catalyzed by glucuronyl transferase activity[1] which is associated with the microsomal fraction of mammalian liver homogenates and is also present in other tissues (Axelrod et al., 1958; Dutton and Stevenson, 1959; Dutton and Storey, 1954). Axelrod et al. (1958) suggested that a single glucuronyl transferase catalyzes the formation of ester, ethereal and N-linked glucuronides. Instability of the enzymatic activity in liver microsomes has heretofore made it difficult to determine if there is more than one glucuronyl transferase or if a single enzyme exists which demonstrates varied affinity for different substrates. Isselbacher (1961) recently reported the solubilization of glucuronyl transferase from rabbit liver microsomes after treatment with snake venom. This enzyme preparation readily catalyzed the formation of ester and ethereal glucuronides but did not catalyze the formation of aniline glucuronide, an N-linked glucuronide. This observation suggests that different glucuronyl transferases may catalyze the formation of ester and ethereal and N-linked glucuronides.

* This work was supported by a grant (A-2019) from the National Institutes of Health

1 This enzyme system has also been referred to as UDP-transglucuronylase.

The studies to be reported support this postulate.

Homozygous Gunn rats have nonhemolytic acholuric jaundice due to an inability to form bilirubin glucuronide which is an ester glucuronide. Deficient hepatic and extrahepatic glucuronyl transferase activity has been demonstrated in vitro in homozygous Gunn rats using, as glucuronide receptors, bilirubin or O-aminobenzoate (which form ester glucuronides) and O-aminophenol or 4 methyl umbelliferone (which form ethereal glucuronides) (Arias, et al., 1960; Carbone and Grodsky, 1957; Lathe and Walker, 1957; Schmid et al., 1958). In vivo, homozygous Gunn rats excrete less n-acetyl-p-aminophenol glucuronide in the urine than do normal rats after treatment with n-acetyl-p-aminophenol (Schmid et al., 1958).

The urinary excretion of aniline glucuronide was estimated in male normal Wistar and homozygous Gunn rats weighing from 140-165 Gms. following the intraperitoneal injection of 5 mgm aniline hydrochloride/100 Gm of body weight. Sodium acetate (1 Gm) was previously administered by stomach tube to maintain the excretion of an alkaline urine. Urine was quantitatively collected in 2.0 ml of 0.1 N Na OH. Free aniline was extracted and acid hydrolyzable aniline glucuronide was estimated using the method of Brodie and Axelrod (1948). The results are presented in Table I. No significant difference was observed in the percentage of administered aniline which was excreted in the urine as aniline glucuronide in the subsequent 24 hours by normal Wistar or homozygous Gunn rats. No significant difference was observed when the dosage of aniline was doubled.

The formation of aniline glucuronide by slices of liver from normal male and homozygous Gunn rats weighing 140-165 Gms was estimated (TABLE II). The method of Brodie & Axelrod (1948) was used to extract and identify aniline glucuronide. Each estimation was performed in quadruplicate with

TABLE I Urinary excretion of aniline glucuronide after intraperitoneal injection of aniline hydrochloride in male normal Wistar (JJ) and homozygous Gunn (jj) rats.

| Dosage of aniline administered | % aniline excreted in the urine as aniline glucuronide |
|---|---|
| (1) 5 mgm/100 Gm body wt. | |
| JJ (6)* | 23 ± 6.7 |
| jj (6) | 21 ∓ 5.8 |
| (2) 10 mgm/100 Gm body wt. | |
| JJ (4) | 28 ± 6.2 |
| jj (4) | 24 ∓ 8.9 |

( (*) number of animals)

TABLE II In vitro formation of aniline and O-aminophenol glucuronides by slices of male normal Wistar (JJ) and homozygous Gunn (jj) rat liver

| | μm aniline glucuronide formed/ Gm. dry weight of liver |
|---|---|
| JJ (12) (*) | 1.27 ± 0.06 |
| JJ plus OAP (6) | .15 ∓ 0.03 |
| jj (10) | 1.54 ∓ 0.18 |
| jj plus OAP (9) | 1.34 ∓ 0.14 |
| | μm O-aminophenol glucuronide formed/GM dry weight of liver |
| JJ (6) | 4.21 ± 0.21 |
| jj (4) | .32 ∓ 0.08 |

( (*) number of animals)

Incubation system: liver slices (60-120 mgm dry weight): 0.5 M Tris buffer pM 7.6 0.2 ml; 0.5 M $MgCl_2$ 0.1 ml; aniline hydrochloride 0.3 μm; O-aminophenol 0.60 μm; total volume 2.0 ml; incubation for 45 minutes in the dark with shaking in air at 37 degrees C.

suitable controls., The amount of aniline added to the incubation medium resulted in optimal formation of an acid labile aniline conjugate. No significant difference was observed between the amount of aniline glucuronide formed by slices of normal Wistar and homozygous Gunn rat liver. O-aminophenol glucuronide formation was markedly decreased in slices of homozygous Gunn rat liver as compared with slices of normal Wistar rat liver. The

method of Levvy and Storey (1949) was used to estimate O-aminophenol glucuronide formation,

The addition of O-aminophenol to slices of normal Wistar rat liver in the presence of aniline partially inhibited formation of aniline glucuronide. This observation suggests that O-aminophenol may compete with aniline for available UDPGA in normal Wistar rat liver slices. The formation of aniline glucuronide by slices of homozygous Gunn rat liver was not significantly inhibited by O-aminophenol. The conjugation of O-aminophenol with glucuronic acid is defective in homozygous Gunn rat liver slices; therefore, O-aminophenol presumably does not compete with aniline for UDPGA in Gunn rat liver slices.

These observations are consistent with the postulate that the enzyme which catalyzes the formation of N-linked glucuronides is different from that which catalyzes the formation of ester and ethereal glucuronides. Whether the formation of ester and ethereal glucuronides also involves different glucuronyl transferases remains to be elucidated.

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