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DEVELOPMENTAL CHANGES IN *p*-NITROPHENOL GLUCURONIDATION IN THE GUNN RAT*

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Abstract—Non-icteric heterozygous Gunn rats conjugated *p*-nitrophenol more readily than icteric homozygous rats at 2, 14 and 28 days of age. Heterozygous females, 65–70 days old, also formed the glucuronide more readily than homozygous recessives of the same age, but the results for males were not statistically significant. Both genotypes had comparatively low levels of enzyme activity at birth, with absolute levels and rate of maturation lowest in the icteric homozygous recessives.

THE GUNN rat has been a useful laboratory animal for studying the biochemistry of UDPglucuronyltransferase [UDPglucuronate glucuronyltransferase (acceptor-non-specific) IUB 2.4.1.17] deficiency and its role in post-parturient hyperbilirubinemia and kernicterus in the neonate.[1] This mutant of the Wistar strain was first described by Gunn,[2] who found nonhemolytic icterus to be inherited as a recessive characteristic. Subsequently, it was shown that the icterus resulted from the inability to conjugate bilirubin with glucuronic acid.[3] There appears to be incomplete dominance, since liver microsomes from homozygous recessive Gunn rats (jj) did not form bilirubin glucuronide, whereas microsomes from heterozygous Gunn rats (Jj) conjugated bilirubin from 40 to 60 per cent as well as microsomes from homozygous (JJ) normal rats.[4] The homozygous recessive Gunn rat (jj) has been shown to have low activity for glucuronide conjugation with 4-methyl-7-hydroxycoumarin,[4] *o*-aminobenzoic acid,[5] *o*-aminophenol,[5] menthol,[5] and thyroxine.[6] Recent attention has been directed to substrate specificity and the apparent multiplicity of transferases for glucuronidation.[7,8]

Van Leusden *et al.*[9] reported that the UDPglucyronyltransferase-deficient Gunn rat at 3 months of age forms *p*-nitrophenol glucuronide as well as normal Wistar rats. Temple *et al.*[8] also found the sexually mature Gunn rat to be capable of *p*-nitrophenol glucuronidation, whereas White[10] and Drucker[11] reported that the mature Gunn rat did not conjugate *p*-nitrophenol. We have been examining the toxicologic significance of UDPglucuronyltransferase deficiency in the Gunn rat for a number of substrates[12] and found that there was a significant difference in the toxicity of *p*-nitrophenol when newborn homozygous Gunn rats were compared with their heterozygous littermates, but there was no difference when sexually mature rats were used (Table 1).

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TABLE 1. ACUTE TOXICITY OF *p*-NITROPHENOL IN GUNN RATS

Age group	LD_{50} (mg/kg and 95 per cent confidence limits)
Gunn jj newborn	122 (111–134)*
Gunn Jj newborn	185 (162–211)
Gunn jj adult females	178 (166–191)
Gunn Jj adult females	191 (168–216)

* Significantly different from heterozygous littermates ($P < 0.05$).

In an attempt to explain the greater toxicity of *p*-nitrophenol when homozygous newborn were compared with heterozygous littermates and in light of conflicting reports on *p*-nitrophenol glucuronidation in the Gunn rat, we examined the developmental changes in the rate of *p*-nitrophenol glucuronidation *in vitro*, which serve as the basis for this report.

EXPERIMENTAL

The Gunn rats were obtained from a closed random-bred colony of continuous monogamous matings of homozygous recessive males (jj) with heterozygous females (Jj). The newborn were identified for genotype at 2 days of age by visual inspection. Icterus is clearly apparent in the homozygous rats at this age and heterozygotes do not become icteric. Confirmation of genotype can be made by bilirubin determination; however, we have found in producing over 1000 newborn per month that icterus as observed in serum or the skin is completely reliable. The expected results of mating heterozygotes (Jj) with homozygotes (jj), that is a 50–50 outcome in offspring, has in every instance agreed with the visual selection of newborn that were saved for breeding.

Glucuronidation of *p*-nitrophenol was determined in both male and female Gunn rats of each genotype at 2, 14, 28 and 65–70 days of age. Six homogenates were analyzed in duplicates for each group and age. The mean and standard error were calculated and the results obtained from homozygous recessives of each sex were statistically compared with the results obtained from their heterozygote littermates at each age by the Student's *t*-test.

The animals were decapitated and the livers were rapidly excised and placed on ice in chilled 0·25 M sucrose. The liver specimens were blotted dry, weighed and homogenized in a motor-driven Potter–Elvehjem homogenizer with a Teflon pestle in a sufficient volume of cold sucrose to prepare a 10 per cent (w/v) homogenate. In the 2-day-old group, the livers from two or three animals were pooled to obtain a sufficient quantity of homogenate. In all other age groups, separate homogenates were prepared from the livers of individual animals.

Glucuronidation of *p*-nitrophenol was determined by a modification of the method of Isselbacher *et al.*[13] Duplicate analyses were performed on each homogenate and the incubation system consisted of: 0·2 ml of 0·5 M tris-HCl buffer, pH 7·4; 0·2 ml of 2 mM *p*-nitrophenol (recrystallized twice from ethanol and water); 0·2 ml of 1·5 mM uridine diphosphate glucuronic acid (UDPGA); and 1·0 ml of the 10 per cent liver homogenate. The total volume of the reaction mixture was 3·0 ml. The UDPGA concentration was not rate limiting, as final concentrations of 0·1, 0·33, 1·0, 3·0 and

6·7 mM were examined in both young and adult Gunn rat homogenates and yielded similar results. Three reagent blanks were employed. These included: (1) an incubation mixture in which UDPGA was omitted to serve as a standard for *p*-nitrophenol; (2) an incubation mixture in which both UDPGA and *p*-nitrophenol were omitted to serve as a tissue blank for the standard; (3) an incubation mixture in which *p*-nitrophenol was omitted to serve as a blank for the reacted homogenate. We obtained greatest activity when 0·25 M sucrose was used as a homogenizing medium rather than isotonic KCl. After incubation for 15 min at 32° in a Dubnoff metabolic shaking incubator, 3·0 ml of 0·33 N trichloroacetic acid was added to each sample to stop the reaction and precipitate proteins. The samples were centrifuged for 10 min at approximately 2000 rev/min. Three-ml aliquots of the supernatants were aspirated and, after adjusting the pH to 11·5 by addition of 2 N NaOH, were diluted to a total volume of 10 ml. The samples were read immediately with a Beckman model DU-2 spectrophotometer at 400 mμ Enzyme activity was calculated as millimicromoles of *p*-nitrophenol that disappeared per milligram of homogenate in 1 hr.

RESULTS AND DISCUSSION

The results are summarized in Table 2. The heterozygous Gunn rats conjugated *p*-nitrophenol more readily than their homozygous recessive littermates at all ages examined. The results were statistically significant for all groups ($P < 0.05$), except for the 65- to 70-day-old males. Within each genotype, the only statistically significant sex-related difference was in the 28-day-old heterozygotes, where activity was greater in males than in females. The rate of *p*-nitrophenol glucuronidation in the sexually mature (65- to 70-day-old) heterozygous Gunn rat was similar to that obtained with Sprague–Dawley rats in our laboratory. The values for males in each strain were 4·347 ± 0·292 and 4·302 ± 0·121 mμmoles/mg/hr respectively.

TABLE 2. *p*-NITROPHENOL GLUCURONIDATION IN GUNN RATS OF VARIOUS AGES

Rats	Genotype and sex	Glucuronide formed (mμmoles/mg liver/hr)			
		2 days*	14 days	28 days	65–70 days
Nonicteric	Jj males	1·433 ±0·222†	1·547 ±0·247	3·013 ±0·218	4·347 ±0·292
	Jj females	1·400 ±0·152	1·893 ±0·063	2·393 ±0·142	5·107 ±0·272
Icteric	jj males	0·500 ±0·049‡	0·840 ±0·102‡	1·827 ±0·215‡	3·333 ±0·512
	jj females	0·587 ±0·076‡	0·853 ±0·122‡	1·973 ±0·105‡	3·620 ±0·140‡

* Age of rats.
† Mean and standard error of six determinations.
‡ Significantly different from heterozygous littermates of same sex and age ($P < 0.05$).

Our observations agree with the reports[8,9] that the adult or sexually mature homozygous Gunn rat forms *p*-nitrophenol glucuronide. These data confirm the work of van Leusden *et al.*[9] that the newborn homozygous Gunn rat is deficient in UDP-glucuronyltransferase activity for *p*-nitrophenol. Conjugation of *p*-nitrophenol increases

with age in the homozygous Gunn rat; however, the developmental pattern is quite unlike normal Wistar rats, since Dutton[14] reported that 1- to 3-day-old Wistar rats conjugated *p*-nitrophenol at a greater rate than adult females. The rate of maturation of *p*-nitrophenol conjugating activity is also slower than that reported by Kato *et al.*[15] for other drug-metabolizing enzymes in the rat. They found maximum activity in rats at 30 days of age with a progressive decline in older rats. Gunn rats at 28 days of age had approximately one-half the activity of 65- to 70-day-old rats for *p*-nitrophenol glucuronidation.

The ability to demonstrate significantly different rates of UDPglucuronyltransferase activity between newborn homozygotes and newborn heterozygotes as well as the deficient activity in the newborn warrants further study with the Gunn rat as an animal model for investigations on the pharmacologic and toxicologic implications of UDPglucuronyltransferase deficiency in the neonate.

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