

LACTASE ACTIVITY: EFFECT OF AN INHIBITOR OF ADRENAL STEROIDOGENESIS

M. S. MURTHY

D.A.E. Hospital, Kalpakkam 603 102, India.

*Present address: Department of Biochemistry, Kasthurba Medical College,
Manipal 576 119, India.*

ABSTRACT

The administration of cyanotrimethylandrostenolone to pregnant rats on the 14th day of gestation produced elevated levels of activity of intestinal β -galactosidase in the offspring and delayed the normal decline in enzyme activity about the time of weaning. It would appear that the changes observed are a result of transitory pre-natal adrenal insufficiency, and that normal adrenal steroidogenesis is critical for the normal development and decline of intestinal galactosidase activity.

INTRODUCTION

MAMMALIAN small intestinal mucosa contains two β -galactosidases, which differ in several respects, including substrate specificity. One is located in the microsomal cell fraction and hydrolyses lactose primarily. The other, which comes in the supernatant fraction, shows practically no activity towards lactose but hydrolyses synthetic substrates such as 6-bromo-2-naphthyl- β -D-galactopyranoside (6-BNG)¹⁻⁴. Both enzymes are active in rat small intestine by the 18th day of gestation, increase to reach a maximum at about the time of weaning (15th to 16th day after birth), and then rapidly decline^{1,4,5}. Adrenalectomy performed on day 15 after birth has been shown to alter this pattern, causing a more gradual decline in intestinal β -galactosidase activity after weaning⁵.

In order to study the effects of pre-natal adrenocortical insufficiency on the pattern of neonatal β -galactosidase activity, cyanotrimethylandrostenolone (2 α -cyano-4,4,17 α -trimethyl-androst-5-en-17 β -ol-3-one, CTM) was administered to pregnant rats. This drug serves as a substrate analogue for 3 β -hydroxysteroid dehydrogenase, binding tightly to the enzyme and inhibiting the conversion of C19- and C21- Δ^5 -3 β -hydroxysteroids to the corresponding Δ^4 -3-ketosteroids⁶. When given to pregnant rats, it blocks corticosterone production, resulting in maternal and foetal adrenal cortical hyperplasia, hypospadias in male offspring and clitoral hypertrophy in females. These changes simulate the form of human adrenogenital syndrome characterized by deficient 3 β -hydroxysteroid dehydrogenase activity^{7,8}. Previous

studies⁹ have shown that the blockade of glucocorticoid synthesis lasts as long as six weeks after a single dose of CTM to an adult rat.

MATERIALS AND METHODS

Pregnant Holtzman rats were injected intramuscularly with a single dose of CTM (23 mg/kg) in corn oil on the 14th day of gestation. Control animals received only the vehicle. On days 18 and 20 of gestation and on days 1, 5, 10, 15, 16, 18, 20 and 30 after birth, offspring were killed with chloroform; the proximal ileum of each was isolated, rinsed with ice-cold normal saline, and rapidly frozen. β -Galactosidase activity was subsequently determined in the whole tissue, using both lactose¹⁰ and 6-BNG¹¹ as substrates. Protein was determined by the method of Lowry *et al*¹². Enzyme activities were expressed as μ mol of product formed per mg protein per hour. Plasma corticosterone levels were determined fluorometrically¹³ in pooled blood collected at the time of sacrifice.

Two additional pregnant rats were treated with CTM as above, together with intramuscular corticosterone (10 mg/kg) in propylene glycol, daily from day 14 of gestation until delivery. The intestinal β -galactosidase activity was assayed in the offspring of these rats on day 20 after birth.

RESULTS

Figures 1 and 2 show the intestinal β -galactosidase activities with lactose and 6-BNG as substrate

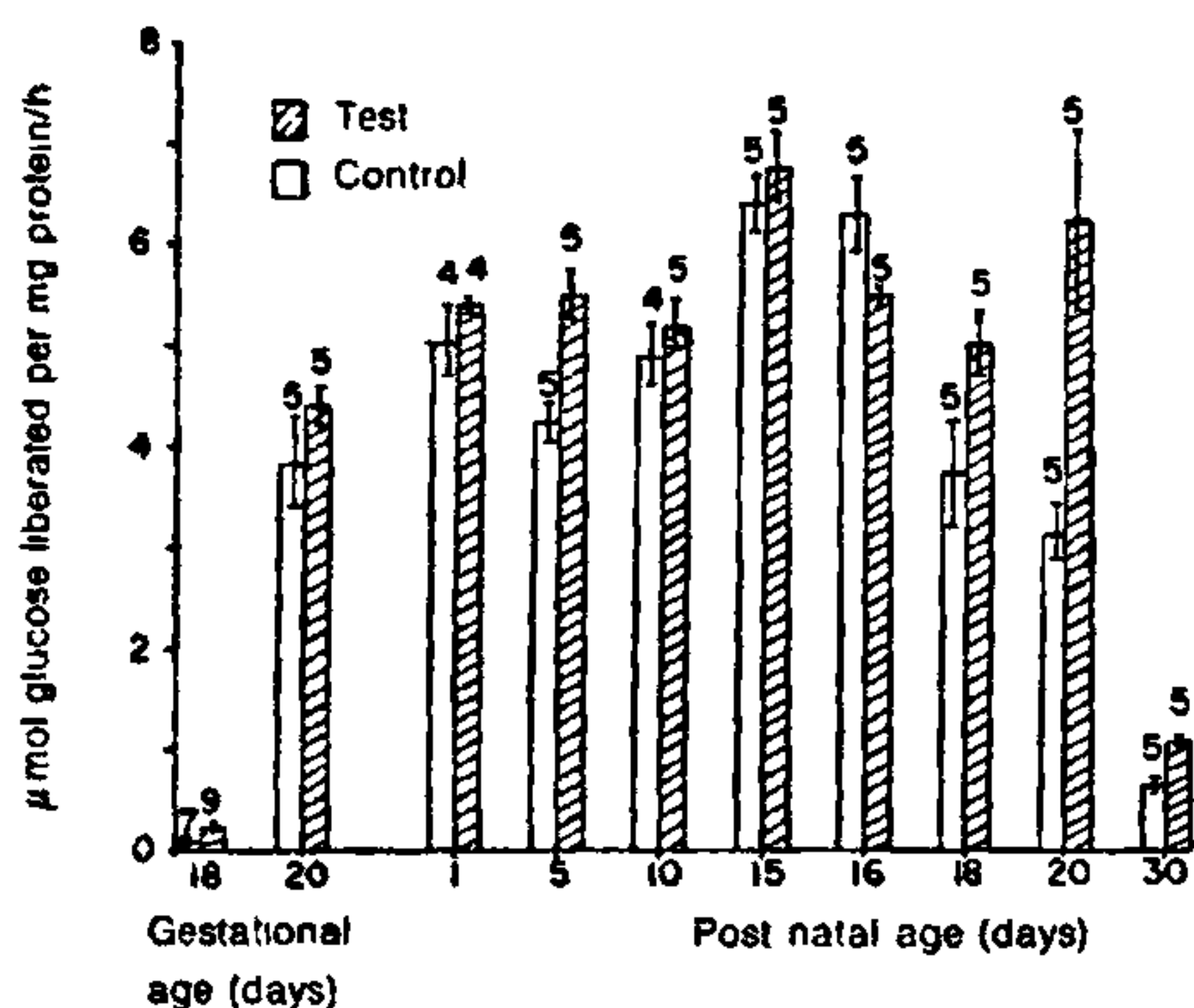


Figure 1. Mean intestinal β -galactosidase activities with lactose as substrate in offspring of control and CTM-treated rats. (Vertical bars are \pm SE of mean. The figures above the columns are numbers of observations.)

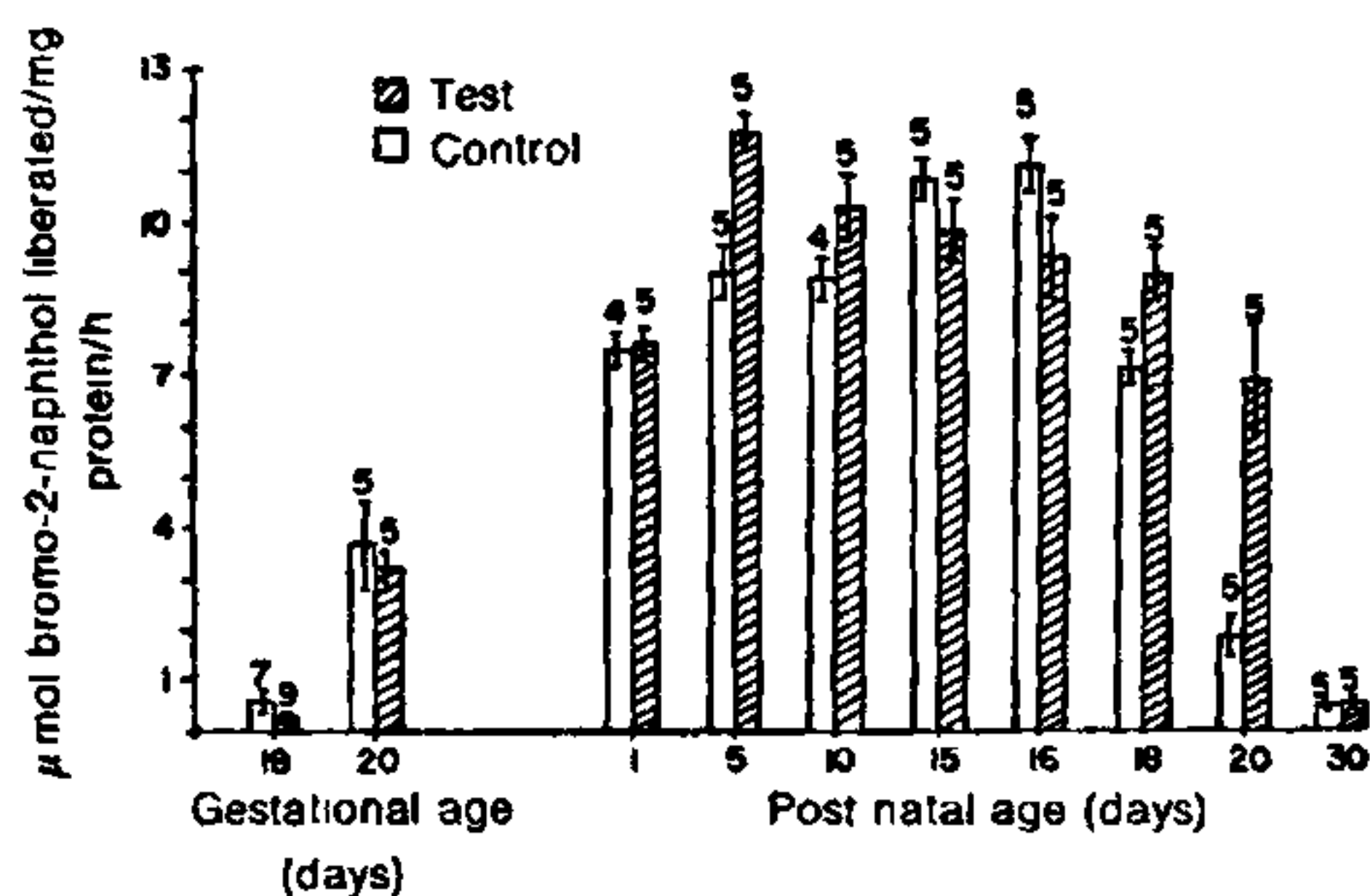


Figure 2. Mean intestinal β -galactosidase activities with 6-BNG as substrate in offspring of control and CTM-treated rats.

respectively in the test and control offspring. In control animals enzyme activity with lactose increased during pre-natal life to a peak at the time of birth; following a decline, there was a secondary rise on days 15 and 16 after birth, and then a rapid decrease in activity on days 20 and 30. The CTM-treated rats showed the same pattern of enzyme activity in pre-natal life, but after birth, levels remained relatively constant until day 20, after which a decrease in activity occurred. Enzyme levels were significantly higher (at least $P < 0.02$) in the CTM-treated rats on days 5, 20 and 30 after birth.

β -Galactosidase activity with 6-BNG in control rats, rose throughout pre-natal and post-natal life to a peak on days 15 and 16 after birth, followed by a decline. In CTM-treated rats the peak enzyme activity was reached on day 5 after birth, and the subsequent decline was much slower. Enzyme levels were significantly elevated in the CTM-treated animals (at least $P < 0.02$) on days 5, 18 and 20 after birth.

Plasma corticosterone levels in the offspring at the time of sacrifice are shown in table 1. In spite of adrenal hyperplasia which occurred in the CTM-treated rats, the plasma corticosterone levels did not differ significantly ($P > 0.05$) from control values (note that the animals were stressed with chloroform at the time of sacrifice).

The offspring of rats treated with CTM plus maintenance corticosterone showed, on day 20 after birth, mean levels of intestinal β -galactosidase activity with lactose ($2.89 \pm \text{SE } 0.55$) and 6-BNG (0.86 ± 0.13) that were not significantly different ($P > 0.10$) from those in the respective controls (3.62 ± 0.30 and 1.05 ± 0.10 μmol product per mg protein per hour).

DISCUSSION

Since total adrenalectomy on day 15 after birth is known to retard the normal decline in intestinal β -galactosidase activity that follows weaning⁵, it was to be expected that a chemical inhibitor of glucocorticoid synthesis might have a similar effect. However, the failure to document diminished plasma corticosterone levels in the CTM-treated offspring suggests that sufficient compensatory adrenal hyperplasia had occurred to permit normal corticosterone production by day 5 after birth. While previous work demonstrated persistent post-natal deficiency in specific activity of 3β -hydroxysteroid dehydrogenase by histochemical techniques¹⁴ in similarly

Table 1 Plasma corticosterone concentration in offspring of control and CTM-treated rats

Post-natal age (day)	Corticosterone ($\mu\text{g}/100$ ml)	
	Control	CTM-treated
5	34.8	34.3
10	28.6	28.6
15	17.5	26.5
18	21.5	34.3
20	20.5	28.2

treated rats, it would appear that this deficiency can be overcome by the synthesis of new adrenal protein. It is unlikely that sufficient free CTM would persist after delivery to bind all newly synthesized 3β -hydroxysteroid dehydrogenase.

It would seem that the observed post-natal changes in intestinal β -galactosidase activity are a result of transient pre-natal adrenal insufficiency. Thus the administration of supplementary corticosterone before delivery prevented the changes in galactosidase activity at 20 days of age seen after CTM treatment. This evidence also argues against a direct action of CTM upon the intestinal mucosa. Alternative explanations, that the observed changes in β -galactosidase activity were a result of exposure to increased levels of ACTH or to abnormal amounts of dehydroepiandrosterone or alternative metabolites, remain possibilities and require further investigation.

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