of some other over-aged Vth instar nymphs, without molt, up to 71 days. Azadirachtin, therefore, may have anti-hormonal effects[9,11-13], at least in addition to its anti-feedant action.

Precociously metamorphosed adultiforms of *Locusta*, obtained by surgical allatectomy or by precocene-treatment of younger instars, do exhibit male sexual behavior[19-20]. Thus, metamorphosis promoting endocrine events, or experimentally induced prothetely, exert a major effect on this behavior, allowing it to become overt, or even enhancing its ontogenetic development[19]. But adultiforms have a terminal molt, which though it does not lead to morphologically normal adult, nevertheless results in a considerable morphogenetic adult differentiation[16]. Thus, although endocrine interferences enhancing prothetelic metamorphosis do induce earlier appearance of male sexual behavior[19] (and also of some other adult behavioral patterns[21,22]), the present results show that the reverse of this conclusion is not true; delay or inhibition of the terminal molt neither prevents, nor delays, the appearance of such behavior. Moreover, exogenous JH intensifies male sexual behavior of crowded over-aged Vth instar nymphs, as does endogenous or exogenous JH in normal crowded adults[17,19,23].

Several questions remain open. It is unclear why male sexual behavior exhibited by over-aged Vth instar nymphs is less intense than that of normal adults. We know nothing about the functioning of the CA and JH metabolism in azadirachtin-treated over-aged nymphs; intensification of their male mating behavior by exogenous JH may as well reflect a low endogenous JH titre as a necessity for extra JH due to a possible lower response of the system underlying this behavior to the hormone. It is clear, however, that overt morphogenetic adult differentiation is not necessary for male sexual behavior to develop and/or to become overt in a hemimetabolous insect. Thus, overt adult behavior and overt adult morphology seem to be independent to a considerable extent in this case.

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Aldose metabolism in developing human fetal brain and liver[1]

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Summary. Aldose reductase, sorbitol dehydrogenase, and glucose-6-phosphate dehydrogenase enzyme activities were studied in human foetal brain and liver at different periods of gestation. Aldose reductase activity in liver disappears after 16 weeks of gestation whereas sorbitol dehydrogenase keeps on increasing in liver as well as in brain. In utero, some glucose metabolism may be mediated through an active sorbitol pathway in human fetuses.

Key words. Fetal development; brain, human; liver, human; aldose metabolism; sorbitol pathway.

In most mammalian species fructose is the predominant sugar in intrauterine life[3-7]. Biotransformation of glucose to fructose takes place in mammalian nerve and brain[8-11], lens[12,13], seminal vesicle[14], and sheep fetal liver[15]. Under the relatively anaerobic conditions prevailing in human intrauterine life[16,17], the permeation and metabolism of glucose in brain may follow alternate pathways. Hence it is of importance to study the conversion of glucose to fructose in human fetal tissues. We have measured the enzymatic activities of aldose reductase (alditol: $NADP^+$ 1-oxidoreductase, E.C. 1.1.1.21) and sorbitol dehydrogenase (SDH, L-iditol: NAD oxidoreductase, E.C. 1.1.1.14) in human fetal brain and liver tissues. The first enzyme catalyses the reduction of a variety of sugars to the corresponding polyols in a $NADP^+$ linked reaction:

$$\text{Aldose}+\text{NADPH}+\text{H}^+ \rightleftharpoons \text{Polyol}+\text{NADP}^+$$

The second enzyme catalyses the reaction:

$$\text{Sorbitol}+\text{NAD}^+ \rightleftharpoons \text{Fructose}+\text{NADH}+\text{H}^+$$

Table 1. Aldose reductase activity in human fetal liver and brain tissues

Period of gestation (weeks)	Specific activity (μmoles/h/g protein) Liver	Cerebrum	Cerebellum	Midbrain
9–12	1.94 ± 1.10(4)	1.95 ± 0.92(4)	2.12 ± 1.03(4)	2.61 ± 0.81(4)
13–16	4.02 ± 1.00(13)	3.44 ± 1.21(7)	3.05 ± 1.40(7)	3.02 ± 1.40(7)
17–20	0 (10)	3.67 ± 0.40(9)	3.63 ± 0.31(9)	3.71 ± 1.01(9)
21–24	0 (8)	4.10 ± 1.09(8)	4.25 ± 0.95(8)	3.84 ± 1.33(8)

The results are expressed as means ± SD, the number in the parentheses indicates the number of cases studied.

We have also measured the activities of gluco-6-phosphate dehydrogenase (G-6-PDH: E.C. 1.1.1.49) which is of great importance for the production of NADPH, required for aldose reduction.

Materials and methods. Human fetuses were obtained from normal healthy mothers undergoing hysterotomy and ligation in the S.S.K.M. Hospital attached to the Institute of Post Graduate Medical Education and Research, Calcutta, as a part of the Medical Termination of Pregnancy Clinic. The project was cleared by the Ethical Subcommittee of the Institute. The fetuses were put on ice immediately after their separation from the mothers' womb and the brain and liver were dissected within 30 min after the operation.

NAD, NADPH, NADP, D-xylose, glucose-6-phosphate, sorbitol, and bovine serum albumin were purchased from Sigma Chemical Co. (USA) and all other reagents used in this study were of analytical grade.

Aldose reductase activity was measured by the spectrofluorometric method described by Moonsammy et al.[18] using the 2000 × g supernatant from a brain or liver tissue homogenate (10% w/v) in mM tris-phosphate buffer, pH 7.3, taking D-xylose as substrate. Sorbitol dehydrogenase activity was measured according to the method of Williams-Ashman et al.[19] using the 2000 × g supernatant of brain or liver homogenates (10% w/v) in 0.1 M phosphate buffer, pH 7.8 G-6-PDH activity was measured by the method described by Kornberg et al.[20]. Protein was measured by the method of Lowry et al.[21] using bovine serum albumin as standard.

Results. It appears from the results presented in table 1 that as pregnancy advances from the 9th week to the 24th week the activity of aldose reductase gradually increases in the cerebrum, cerebellum and midbrain regions of human foetal brain. However, in liver tissue the enzyme activity increases up to the 16th week and then it diminishes to undetectable levels. Table 2 shows that sorbitol dehydrogenase activity gradually increases with the progress of gestation both in brain and liver up to the 24th week of gestation and the activity is comparable in both the tissues. Table 3 shows that G-6-PDH activity in liver is greater than that in brain tissue. In both organs the activity remains more or less the same from the 9th to 24th week of gestation.

Discussion. It is apparent from the results of the present investigation that during the development of the human fetus in intrauterine life the activities of aldose reductase and sorbitol dehydrogenase appear to increase in parallel in both brain and liver with the marked exception that in the latter tissue the activity of aldose reductase is completely abolished after the 16th week of gestation. However, glucose-6-phosphate dehydrogenase activity remains almost unaltered during this period of foetal development. It may be suggested that some glucose metabolism in intrauterine life is mediated through an active sorbitol pathway. These results also indicate that HMP shunt mechanism maintains a close coordination with the sorbitol pathway in human foetal brain and liver within the ranges of gestation period of 9–24 weeks and 9–16 weeks respectively. The reason for the unexpected disappearance of aldose reductase activity in liver after the 16th week remains to be explored.

Table 2. Sorbitol dehydrogenase activity in human fetal liver and brain tissues

Period of gestation (weeks)	Specific activitiy (μmoles/h/g protein) Liver	Cerebrum
9–12	11.70 ± 2.34 (6)	16.86 ± 4.68 (7)
13–16	25.92 ± 6.06 (15)	26.64 ± 5.82 (15)
17–20	28.80 ± 7.44 (17)	29.22 ± 6.72 (17)
21–24	29.52 ± 6.54 (6)	43.02 ± 8.76 (5)

The results are expressed as means ± SD, the number in the parentheses indicates the number of cases studied.

Table 3. Glucose-6-phosphate dehydrogenase activity of human fetal liver and brain tissues

Period of gestation (weeks)	Specific activity (μmoles/h/g protein) Liver	Cerebrum	Cerebellum	Midbrain
9–12	246.0 ± 55.2 (4)	125.4 ± 41.8 (4)	ND	ND
13–16	247.8 ± 50.1 (9)	75.0 ± 34.8 (9)	45.6 ± 18.0 (9)	65.4 ± 24.0 (9)
17–20	264.6 ± 48.6 (14)	50.0 ± 27.2 (14)	47.4 ± 20.3 (14)	45.6 ± 15.0 (14)
21–24	233.0 ± 40.2 (5)	53.4 ± 18.6 (5)	46.8 ± 16.8 (5)	53.1 ± 20.4 (5)

The values are expressed as means ± SD, the number in the parentheses indicates the number of cases studied, ND, not done.

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Hypothalamic tyrosine hydroxylase activity, plasma gonadotropin and prolactin levels after aminooxyacetic acid in ovariectomized rats[1]

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Summary. Plasma concentrations of gonadotropin, prolactin and hypothalamic tyrosine hydroxylase (TH) activity were measured in ovariectomized rats treated with aminooxyacetic acid (AOAA), a drug which elevates brain GABA levels. Hypothalamic TH activity was significantly increased with a significant decrease in prolactin (Prl) release. Plasma levels of gonadotropins were not modified by AOAA. These results support an inhibitory action of GABA on Prl release possibly mediated through hypothalamic dopamine.

Key words. Rat, ovariectomized; ovariectomy; aminooxyacetic acid; tyrosine hydroxylase, hypothalamic; gonadotropin, plasma; prolactin, plasma.

Gamma aminobutyric acid (GABA), an inhibitory synaptic transmitter in the central nervous system (CNS)[3], is involved in the control of anterior pituitary (AP) hormone release[4–6]. Both inhibitory and stimulatory actions of GABA on prolactin (Prl) release have been reported[7,8]. Aminooxyacetic acid (AOAA) elevates GABA levels in the CNS by inhibiting GABA-transaminase[9]. The present report demonstrates the role of endogenous GABA on gonadotropin and prolactin (Prl) levels after the administration of AOAA in ovariectomized (OVX) rats. Since dopamine (DA) is known to be a physiological inhibitor of pituitary Prl secretion, the activity of hypothalamic tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine biosynthesis, whose activity changes correlate well with presynaptic DA levels, was evaluated to investigate the dopaminergic mediation of GABA action.

Materials and methods. Ovariectomized adult female rats of Wistar strain were used 2–3 weeks after surgery. Aminooxyacetic acid was freshly prepared in 0.9% NaCl (pH adjusted to 7.0) and administered i.p. at a dose of 0.46 mmol/kg b.wt, in a volume equivalent to 1% of the b.wt. This dose of AOAA has been shown to produce a linear increase in GABA over the first 1-h period[9]. Controls received an equal volume of saline. Animals were decapitated after 1 h. Trunk blood was collected for gonadotropin and Prl measurement by a double antibody radioimmunoassay (RIA) using kits supplied by NIAMDD-NIH, Bethesda, USA. Results were expressed in terms of LH-RP-1, FSH-RP-1 and Prl-RP-2, respectively. The detection limits for the assay were 5 ng LH, 10 ng FSH and 0.25 ng Prl. The inter- and intra-assay co-efficients of variation were 10 and 6% for LH, 9 and 5% for FSH and 10.4 and 5.5% for Prl. Brains were removed and hypothalami (which included pre-optic area, medial basal hypothalamus and median eminence) were dissected out as described earlier[10], and TH activity was estimated according to the method of Shiman et al.[11].

Results. Saline injection did not alter gonadotropin, Prl levels or hypothalamic TH activity. Plasma gonadotropin levels were not modified by 0.46 mmol AOAA (fig. 1). The choice of this dose was based on earlier experiments where 0.46 mmol AOAA produced a maximal increase in endogenous GABA levels at 1 h[9]. Hypothalamic TH activity was significantly ($P < 0.05$) increased with a simultaneous decrease of Prl levels in plasma following the administration of AOAA (fig. 2).

Discussion. These results demonstrate that endogenous elevation of GABA by AOAA stimulates hypothalamic TH activity, resulting in the inhibition of Prl release. Earlier studies have

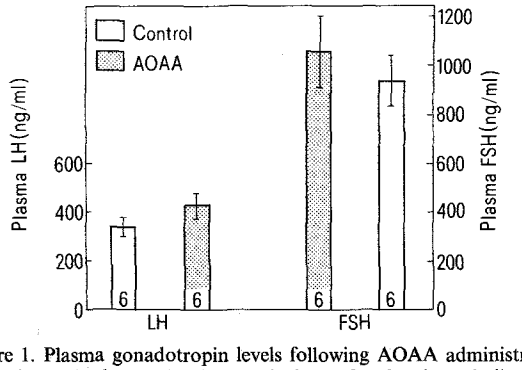

Figure 1. Plasma gonadotropin levels following AOAA administration in ovariectomized rats. Numbers at the base of each column indicate the number of animals in each group. Vertical lines represent SEM.

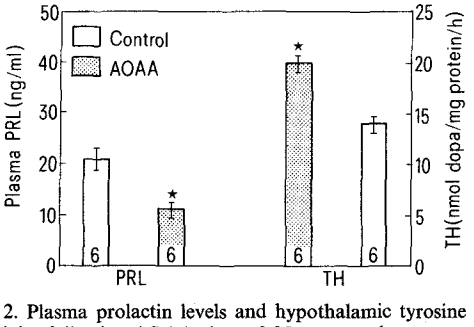

Figure 2. Plasma prolactin levels and hypothalamic tyrosine hydroxylase activity following AOAA. *$p < 0.05$ vs control.