

# Serum Prolactin Levels in Humans from Birth to Adult Life

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## *Extract*

Serum prolactin (HPr) and serum growth hormone (HGH) were determined by double antibody radioimmunoassay methods. Markedly elevated levels of serum prolactin with considerable variation were observed in the neonatal period. No significant difference was observed in six matched arteriovenous cord blood samples. No sex difference was noted in the full term infants, whereas the mean value for 24 premature male infants in the 1st week of life ( $190 \pm 17$  ng/ml SEM) was significantly higher ( $P < 0.001$ ) than mean values for 34 premature female infants ( $104 \pm 10$  ng/ml SEM). During the first year of life, the mean prolactin value for both boys and girls was approximately 10 ng/ml. Mean prolactin levels for both male and female children, aged 2-12 years, were approximately 5 ng/ml. Mean levels for the adolescent female were not increased significantly over those for adolescent males. However, the mean prolactin level of all values determined for adult females ( $7.9 \pm 0.40$  ng/ml SEM) was significantly increased ( $P < 0.001$ ) over the mean level for adult males ( $5.2 \pm 0.55$  ng/ml, SEM). Daily serum prolactin throughout the menstrual cycle in six normal female subjects was compared with daily serum HLH levels. Considerable fluctuation was evident, particularly in the luteal phase, where the mean prolactin level was observed to be statistically higher ( $P < 0.005$ ) than the mean follicular phase level.

Insulin hypoglycemia did not produce a significant increase in serum prolactin in 10 normal subjects, whereas arginine infusion produced a twofold increase in mean serum prolactin at 30 min with a return to basal values by 60 min. Glucagon administered intravenously did not produce any significant change in the already elevated levels of prolactin observed on *days 1* and *3* of life. Serum prolactin was uniformly and completely suppressed by L-dopa in six subjects for 1-4 hr following a single oral dose of 250 mg. In normal children, the maximal increases in both prolactin and thyroid-stimulating hormone (TSH) were observed at 15-30 min after the intravenous injection of thyrotropin-releasing hormone (TRH) and values were still slightly elevated at 120 min after injection.

These studies document the pattern of secretion of serum prolactin from birth to adulthood. The physiologic basis for the increased levels of serum prolactin in the neonate has not been clarified by our studies. Significantly increased levels of prolactin are observed at a time when maximum increases in neonatal breast hypertrophy are observed clinically. Significant mean differences are observed in the adult female population compared with adult males. L-Dopa acts at the hypothalamic level to alter pituitary secretion via alterations in releasing and inhibiting hypothalamic

hormones, whereas TRH acts directly on the pituitary. Our studies indicate that responses to these agents in the prepubertal child are qualitatively similar to those in adults. The response of the neonatal hypothalamic pituitary axis to these agents remains to be studied.

#### *Speculation*

These studies provide a basis for interpretation of disturbances in hypothalamic-pituitary regulation of prolactin secretion. Additional studies are required to define the physiologic roles for prolactin in the human during both intrauterine and extrauterine life as well as the normal ontogeny of control mechanisms for prolactin secretion in the neonate and infant. The availability of sufficient purified human prolactin for metabolic balance studies will also be required to enhance our knowledge of this recently isolated human hormone.

#### *Introduction*

The existence of a pituitary prolactin distinct from growth hormone in the human has been firmly established [7, 13, 17, 21, 23, 27]. As previously reported [16], we have developed a specific and sensitive radioimmunoassay for the determination of prolactin in serum. We have applied this assay to the study of serum prolactin in the normal infant, child, and adolescent, since at present only limited data are available concerning physiologic levels of prolactin during these various periods of life. In addition, known modifiers of growth hormone and prolactin secretion in adults have been utilized to investigate factors regulating normal prolactin secretion in childhood, as a prelude to studies of abnormal secretion of this hormone.

#### *Patient Material*

The subjects studied in this report were derived from a variety of sources, but all were considered to be endocrinologically normal unless otherwise stated.

##### *Newborn*

One hundred four samples, including cord blood, were obtained from full term infants from birth through 6 weeks of life. Data for 65 of these samples have been published previously [16], and are incorporated for comparative purposes. Samples from 82 gestationally premature infants (38 male and 44 female) were also studied from birth through 6 weeks of life. Multiple samples were obtained serially from many of these infants and only infants without significant metabolic or respiratory disease were selected for study. Mode of delivery was not known to the authors. Both

of these groups of subjects were obtained through the courtesy of Dr. Robert Usher, Royal Victoria Hospital, Montreal.

##### *Infancy*

Fifty-one samples were serially obtained at 3-month intervals from infants 3-12 months of age. These infants were being followed regularly because of difficulties with carbohydrate regulation in the neonatal period because of spontaneous hypoglycemia or because they were infants of diabetic mothers. At the time of the sampling they all appeared normal upon physical examination, and had normal glucose, cortisol, and HGH concentrations. However the infants of diabetic mothers group had elevated serum insulin levels which persisted at the sampling periods at 3 and 6 months.

##### *Childhood and Adolescence*

Samples were obtained from 19 normal children, aged 2-12 years and from 54 adolescent patients, aged 13-16 years. These subjects consisted of siblings of patients seen in the endocrine clinic and hospitalized or outpatient subjects seen at the Montreal Children's Hospital.

##### *Adult*

Twenty-seven normal adult males consisting of laboratory and hospital personnel and electively hospitalized patients, aged 24-72 years, were studied. Daily samples were obtained throughout a 30-day period from six normal adult females, aged 20-26 years.

##### *Special Study Groups*

Groups of 10 normal children, aged 2-16 years, being evaluated for shortness of stature and with no

other significant findings, were given intravenous insulin (0.1 i.u./kg) with samples obtained at 0, 20, 40, 60, 90, and 120 min and arginine-HCl infusions (0.5 g/kg) with samples obtained at 0, 30, and 60 min. Six subjects in these groups were studied with both tests. In addition, a group of 10 hypopituitary subjects, aged 2-16 years, submitted for study as part of a requisite for inclusion in the MRC Therapeutic Trial of Growth Hormone in Canada, were similarly studied for comparison. Seven of these subjects are now receiving growth hormone but none had received GHG prior to these studies. Intravenous glucagon (300  $\mu$ g/kg) was administered to 7 normal newborns on *day 1* or *day 3* of life, and samples were obtained at 0, 5, 15, 30, 60, 90, and 120 min.

Six subjects received oral L-dopa (250 mg) and specimens were obtained at 0, 30, 60, 90, 120, 240, and 360 min. Four of these were normal children and two were idiopathic hypopituitary patients before the onset of any growth hormone therapy. Thyrotropin-releasing hormone was administered intravenously (500  $\mu$ g) to 13 normal children. Determination of serum prolactin in all 13 subjects and serum TSH in 11 of these subjects was performed at 0, 5, 10, 20, 30, 45, 60, and 120 min. The latter samples were obtained through the courtesy of Drs. S. Kaplan and M. Grumbach, University of California, and the serum TSH responses have been reported [5].

### Methods

Serum prolactin and serum growth hormone were determined by double antibody radioimmunoassay methods, details of which have been previously reported [16]. Recent modifications in the prolactin assay have included the use of a purified human pro-

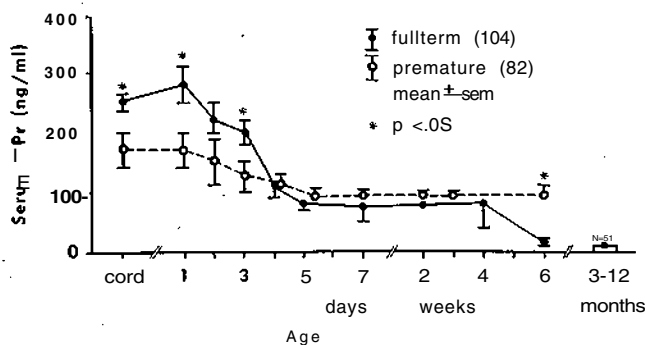


Fig. 1. Serum prolactin in the neonate. Age-dependent changes in serum prolactin (HP<sub>r</sub>) in full term and premature infants. Results are shown as mean  $\pm$  SEM, with significant mean differences ( $P < 0.05$ ) indicated by an asterisk (\*).

Table I. Serum prolactin in the neonate

Age	Full term, ng/ml				Premature, ng/ml			
	n	Mean	SD	SEM	n	Mean	SD	SEM
Cord <sup>2</sup>	31	246	88	16	8	172	88	31 <sup>2</sup>
Day								
P	18	278	118	28	13	169	106	30 <sup>2</sup>
2	13	226	75	22	9	152	107	36
3 <sup>2</sup>	10	204	66	20	9	124	73	24 <sup>2</sup>
4	7	114	61	23	7	110	52	24
5	9	82	36	12	5	92	31	14
7	4	76	36	18	7	92	20	8
Week								
2	1	80			11	94	25	8
3					7	94	10	4
4	5	87	58	26				
6 <sup>2</sup>	3	17	6	3	6	96	34	142

<sup>1</sup> n: Number.

<sup>2</sup>  $P < 0.05$ .

lactin standard with a biologic potency of 30 i.u./mg [17] and antisera produced against this purified human prolactin. Values determined using these new assay materials yield mean prolactin levels identical with those we have reported previously [9, 10, 16]. The values reported here were determined utilizing the original human prolactin antisera [16] and the purified prolactin as reference standard [17]. Sensitivity of the human prolactin assay permits detection of values as low as 2 ng/ml when a 100- $\mu$ l sample size is employed. All values were determined in duplicate utilizing 100  $\mu$ l samples. Whenever possible, individual clinical groups had determination of prolactin levels performed in the same assay to avoid interassay variability. Intra- and interassay variations did not exceed 10% and 20%, respectively.

Serum HLH was determined by double antibody radioimmunoassay [14] with values quoted in terms of human pituitary reference preparation LER 907 [1].

### Results

#### Neonatal Period, Infancy, and Childhood

Values for serum prolactin in the neonate are illustrated in Fig. 1 and Table I. Markedly elevated levels of serum prolactin with considerable variation were observed in the neonatal period. No significant difference was observed in six matched arteriovenous cord blood samples. No sex differences were noted in the full term infants. The mean value for 34 premature female infants in the 1st week of life ( $104 \pm 10$  ng/ml SEM) was significantly lower ( $P < 0.001$ ) than the

mean value for 24 premature male infants ( $190 \pm 17$  ng/ml SEM) (Fig. 2). The male premature mean value did not differ significantly from the mean value for all full term infants in the 1st week. When compared irrespective of sex, significant mean differences were observed between full term and premature infants in cord blood and on *days 1* and *3* of life, at which time mean prolactin values in the full term infants were higher (Fig. 1). Subsequent mean prolactin values were similar in the two groups until 6 weeks of life, at which time a decrease in the mean value for full term infants led to another significant difference.

During the remainder of the 1st year of life, the mean prolactin value in both boys and girls was approximately 10 ng/ml (Figs. 1 and 3). Mean prolactin levels in both male and female children, aged 2-12 years, were approximately 5 ng/ml (Fig. 3). In the adolescent female mean levels were increased, but not significantly, over those seen in adolescent males. Males with adolescent gynecomastia did not have elevated serum prolactin levels [29]. The sex difference in mean prolactin levels became significant in the adult population studied, in which the mean prolactin level of all values determined for adult females ( $7.9 \pm 0.40$  ng/ml SEM) was significantly increased ( $P < 0.001$ ) over the mean level for adult males ( $5.2 \pm 0.55$  ng/ml SEM). Figure 4 illustrates daily serum prolactin compared with daily serum HLH levels throughout the menstrual cycle in six normal female subjects. Considerable fluctuation was evident particularly in the luteal phase, where the mean prolactin level was observed to be statistically higher ( $P < 0.005$ ) than the mean follicular phase level. Additional data on 15 normal menstrual cycles are similar to those presented

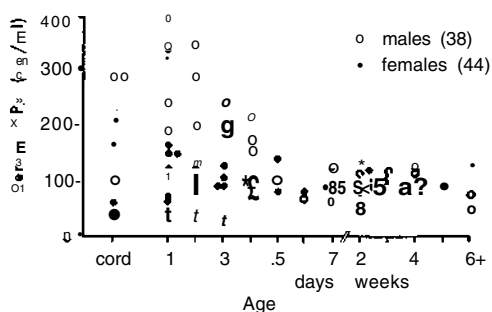


Fig. 2. Serum prolactin in the premature infant. Age and sex-dependent changes in serum prolactin (HPr) in gestationally premature infants. The mean HPr level for female premature infants in the 1st week of life was significantly lower than the mean for male premature infants (see text).

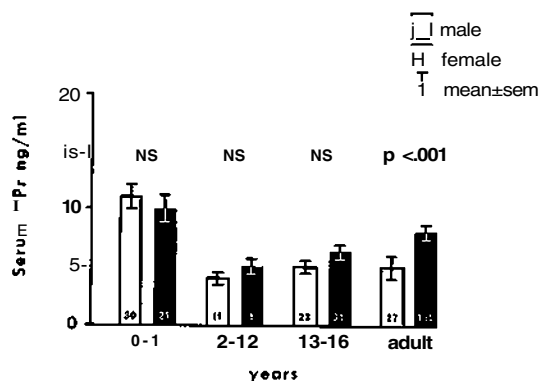


Fig. 3. Serum prolactin (HPr) in childhood and adolescence. Age and sex-dependent changes in serum prolactin in children and adults. Only adult females showed a significant difference over adult males. TVS: Not significant.

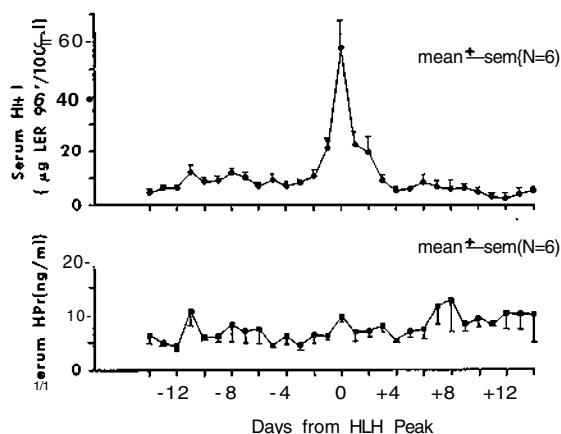


Fig. 4. Serum prolactin and the menstrual cycle. Daily serum prolactin (HPr) and luteinizing hormone (HLH) during the menstrual cycle in six normal adult females. The mean of all luteal phase levels ( $9.0 \pm 6.1$  ng/ml SD,  $n = 84$ ) was increased ( $P < 0.005$ ) over the mean of all follicular phase levels ( $6.7 \pm 3.9$  ng/ml SD,  $n = 68$ ).

here. These data were determined with different assay standards and are therefore not included here.

#### Effect of Insulin Hypoglycemia, Arginine, and Glucagon

Insulin hypoglycemia did not produce a significant increase in serum prolactin (Fig. 5A), although the expected increase in serum growth hormone was observed in all normal subjects. No significant increases in serum prolactin or HGH were observed in the hypopituitary subjects (Fig. 5B), although basal prolactin values were in the normal range, as we have reported [20]. Arginine produced a twofold increase in mean serum prolactin ( $P < 0.025$ ) at 30 min in 7 of 10

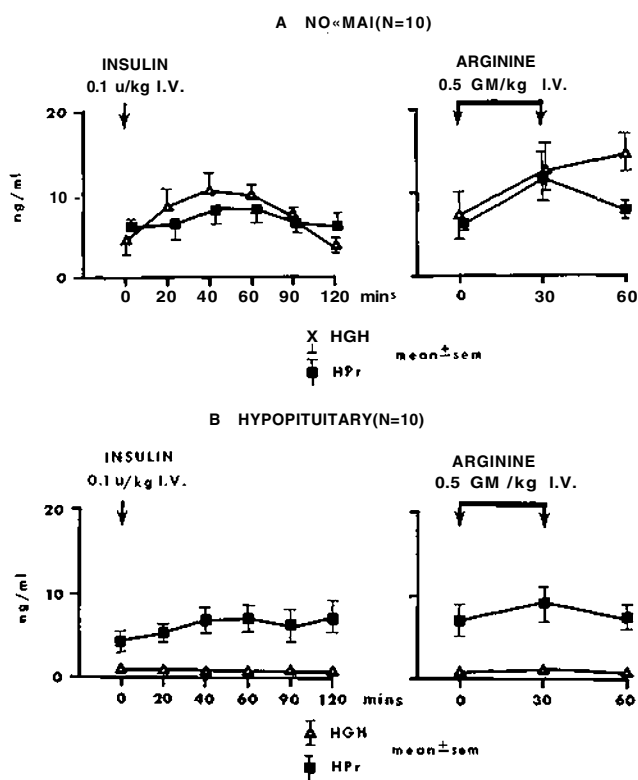


Fig. 5. A: Serum growth hormone (GHG) and prolactin (HPr) responses to insulin and arginine in 10 normal children. Serum HPr was significantly increased ( $P < 0.025$ ) in the 30-min arginine samples. B: Serum GHG and HPr responses to insulin and arginine in 10 hypopituitary children. No significant increases were noted.

normal subjects (Fig. 5 A) with a return to basal values by 60 min. This was not observed in the hypopituitary subjects (Fig. 5 B) and considerable variation in response was observed with both tests. Glucagon administered intravenously did not produce any significant change in the already elevated levels of prolactin observed on days 1 and 3 of life (Fig. 6). However, additional growth hormone increases in response to the injected glucagon were observed in all cases. One 3-year-old boy also did not show any prolactin response to i.v. glucagon but did have a normal increase in serum GHG.

#### Hypothalamic-Pituitary Axis Studies

L-Dopa produced an increase in serum growth hormone in three of four normal subjects with no increase observed in the two hypopituitary subjects [15] (Fig. 7). Serum prolactin was uniformly and completely suppressed by L-Dopa in all six subjects for 1-4 hr following a single oral dose of 250 mg, a response identical

with that observed in adults [11, 22]. A rebound increase in serum prolactin was usually observed at 6 hr. As recently reported and for reasons not yet fully understood, the synthetic hypothalamic hormone TRH, acting directly on the pituitary, increases not only serum TSH but also increases serum prolactin [2, 11, 19]. In normal children, the maximal increases in both prolactin and TSH were observed at 15-30 min after the i.v. injection of TRH (Fig. 8), and values were still slightly elevated at 120 min after injection. Similar responses have been observed in idiopathic hypopituitary children [6].

#### Discussion

These studies document the pattern of secretion of serum prolactin from birth to adulthood. Markedly increased levels of serum prolactin were observed in

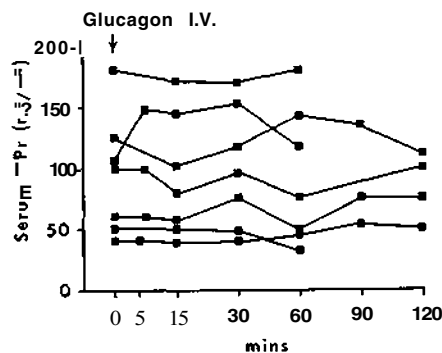


Fig. 6. Serum prolactin (HPr) response to i.v. glucagon (300 iug/kg) in seven neonates on day 1 or 3 of life. No significant changes were noted.

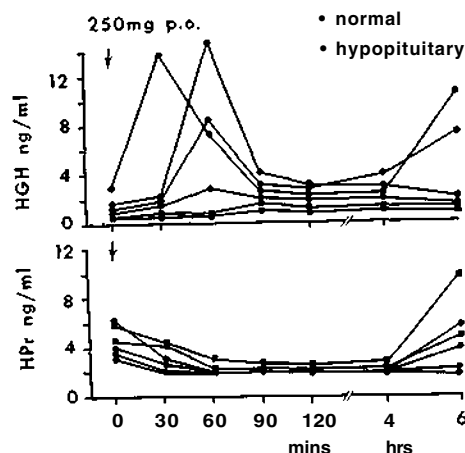


Fig. 7. Serum growth hormone (GHG) and prolactin (HPr) responses to oral L-dopa (250 mg) in four normal and two hypopituitary children. Serum GHG increased in only the three normal children, whereas serum HPr was suppressed in all six children.

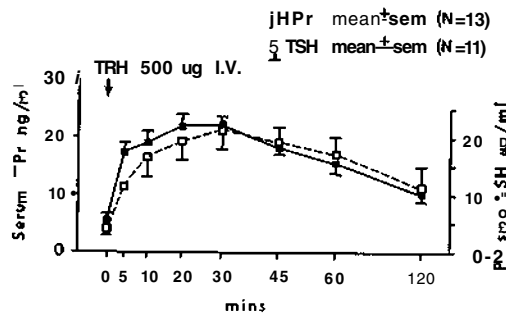


Fig. 8. Serum prolactin (HPr) and thyroid-stimulating hormone (TSH) response to thyrotropin-releasing hormone (TRH) (500 µg i.v.) in normal children.

the neonate and persisted for at least 6 weeks of life. These increased levels appear to be due to increased secretion by the fetal and neonatal pituitary, inasmuch as the biologic half-life of prolactin in adults is approximately 15 min [28], which indicates that continued secretion of pituitary prolactin is required to maintain the observed levels. There are no data to suggest decreased metabolic turnover for prolactin in the neonate, and Cornblath *et al.* [4] have even suggested an increased turnover rate for the elevated levels of HGH in the neonate. The physiologic basis for the increased levels of serum prolactin in the neonate has not been clarified. Since stress or anesthesia is a potent stimulator of prolactin release in animals [3] and man [10], a potential relationship to the stress of delivery and extrauterine adaptation could be postulated. Such correlations were not possible in our study group. The relative importance of these elevated neonatal levels of serum prolactin in association with increased levels of estrogen from a maternal source in the production of neonatal breast hypertrophy and secretion remains to be elucidated. However, it is significant that increased levels of prolactin are observed at a time when maximum increases in neonatal breast hypertrophy are observed clinically. Whether prolactin has any growth-promoting potential during the rapid growth of the newborn, particularly when considered in the light of similarly increased HGH secretion [4], is highly speculative at present. However, animal prolactins have been shown to produce metabolic effects similar to HGH in hypopituitary humans [25].

After elevated levels of serum prolactin are observed during the first 6 weeks of life, values decrease until mean values near 5 ng/ml are observed throughout most of childhood. During and after adolescence a progressive increase in mean serum prolactin levels for females is observed, until significant mean differences

are observed in the adult female population compared with adult males. Similar sex differences in adults have recently been reported by Jacobs *et al.* [18], who used a mixed heterologous radioimmunoassay system. This mean increase is mainly attributable to the increased mean level observed during the luteal phase as compared with the follicular phase in these normal subjects. No significant peak increases in serum prolactin were observed in conjunction with the ovulatory mid-cycle HLH peak. These luteal phase increases are perhaps related to increased estrogen secretion during this part of the cycle, inasmuch as estrogen has been shown to produce prolactin surges in animals [26]. In adult subjects, insulin-induced hypoglycemia with 0.2 t/kg, with its greater degree of hypoglycemia and attendant stress [8, 24], has been shown to increase serum prolactin levels in contrast to the lack of significant response we observed in normal children when 0.1 x/kg of insulin was used. In addition, arginine infusions did not increase serum prolactin in adults [10], whereas we observed a significant increase at 30 min after arginine infusion in 7 of 10 normal children but not in hypopituitary children. Prolactin responses to glucagon have not been previously recorded and we observed no increase in the neonatal period. However, responses later in life require additional study.

L-Dopa is thought to act at the hypothalamic levels to alter pituitary secretion via alterations in releasing and inhibiting hypothalamic hormones, whereas TRH acts directly on the pituitary [12]. Our studies indicate that responses to these agents in the prepubertal child are qualitatively similar to those observed in adults. The responses of the neonatal hypothalamic-pituitary axis to these agents remain to be studied. It should be possible to define the normal maturation of this axis with the use of techniques similar to those we have reported.

Our studies do not clarify the various physiologic roles for prolactin in man. However, we have documented the existence of significant levels of prolactin at various periods of life and the alterations of these levels by various therapeutic agents. It is hoped that such studies will provide a base line for additional study and interpretation of data in pediatric patients with disordered hypothalamic-pituitary function.

### Summary

Prolactin was demonstrated in the sera of all subjects studied, with elevations observed particularly in the neonatal period. Significant differences were observed

between adult male and female subjects; however, there was considerable overlap. Agents known to modify hypothalamic pituitary activities in adults have now been shown to alter prolactin secretion in children in a qualitatively similar fashion. L-Dopa consistently produced decreases in serum prolactin in both normal and hypopituitary subjects, whereas TRH produced increases in serum prolactin. The significance of these latter findings in relationship to the day-to-day regulatory mechanisms for prolactin secretion remains to be investigated.

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