Relationship between plasma unbound-bilirubin concentration and cerebellar bilirubin content in homozygous Gunn rat sucklings

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Summary. Suckling increases and starvation (16 h) decreases plasma unbound-bilirubin concentration in 15-day-old homozygous Gunn rats. The effect of suckling-induced increse of plasma unbound-bilirubin concentration on cerebellar bilirubin content was examined. Cerebellar bilirubin content of the suckled rats did not increase, but rather decreased, in spite of the elevated concentration of plasma unbound-bilirubin. There were significant negative correlations between cerebellar bilirubin content and b. wt both in the suckled and starved groups.

Plasma unbound-bilirubin, a dissociated form of plasma albumin-bound bilirubin, is considered to be transferred from plasma to tissues and to develop its toxic effect on cellular functions[2-5]. Many experiments suggest that plasma unbound-bilirubin concentration is closely related to the risk of kernicterus[3,6,7]. However, no direct relationship between plasma unbound-bilirubin concentration and kernicterus has been established[8,9]. In 15-day-old rats, suckling induces the increase of plasma unbound-bilirubin concentration[10]. It is also reported that in experimentally induced kernicterus the incidence of yellow staining in brain was highest in cerebellar cortex from 15- to 22-day-old rats[11]. These observations tempted us to investigate a possible correlation between plasma unbound-bilirubin concentration and cerebellar bilirubin content, as an indicator of the development of kernicterus. The results showed that the elevation of plasma unbound-bilirubin by suckling did not induce the increase of cerebellar bilirubin content. Significant correlations were observed between cerebellar bilirubin content and body weight both in the suckled and starved groups.

Materials and methods. Animals: Gunn's strain of jaundiced rats (j/j; 14-day-old) were used throughout the study. Littermates diagnosed by yellow skin colour as j/j were equally allotted to suckled and starved groups. Dams were maintained on laboratory diet (NMF, Oriental Yeast Co.) and tap water.

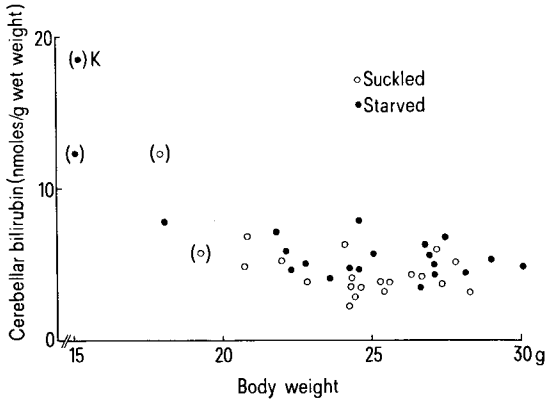
Treatment of animals: Starvation: rats were isolated from mothers from 17.00 h till 09.00 h of the next day for starvation (16 h). Suckling: rats were left with their mothers and nursed ad libitum through the same period. Blood was sampled at 10.00 h. Detailed experimental conditions are referred to in the previous report[10].

Chemical analysis. Cerebellar bilirubin content was determined by the method of Katoh et al.[4]. Plasma total bilirubin concentration was measured according to the method of Malloy and Evelyn[12]. Unbound-bilirubin concentration was determined as described previously[10].

Results. The table shows plasma unbound- and total bilirubin concentrations, cerebellar bilirubin content and body weight of the suckled and starved groups. Plasma unbound-bilirubin concentration of the suckled group was 4.9 times higher than that of the starved. Cerebellar bilirubin content, however, was 33% lower in the suckled group. Plasma total bilirubin concentration and cerebellar bilirubin content were 35 and 33% higher in the starved

	Suckled	Starved	
Plasma unbound-bilirubin (mg/dl)	0.44±0.26* (19)	0.09±0.02 (19)	$p < 0.01$
Cerebellar bilirubin (nmoles/g wet weight)	4.71±1.97 (19)	6.28±3.30 (19)	$p < 0.01$
Plasma total bilirubin (mg/dl)	9.1±1.7 (19)	12.3±1.7 (19)	$p < 0.01$
Body weight (g)	24.9±2.2 (19)	25.2±2.9 (19)	**

*Each value represents the mean ± SD.**Not statistically significant. Number of experiments in parentheses.



Relationship between b. wt and cerebellar bilirubin content. Starved: Rats were isolated from their mothers for 16 h. Suckled: Rats were left with their mothers and nursed ad libitum through the same period. ( ): Littermates not represented in the table, since their plasma volumes were too small for the assay of unbound-bilirubin. *K:* Kernicterus occurred spontaneously. The correlations of the suckled and starved groups between b. wt and cerebellar bilirubin content were −0.426 ($n = 21$, $p < 0.05$) and −0.626 ($n = 21$, $p < 0.01$), respectively.

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group than in the suckled, respectively. The figure shows the relationship between body weight and cerebellar bilirubin content. Statistically significant negative correlations were obtained both in the suckled and the starved groups.

Discussion. The marked increase of plasma unbound-bilirubin concentration by suckling is shown in the table. The cerebellar bilirubin content of the suckled group was, however, lower than that of the starved, in spite of the increase of plasma unbound-bilirubin concentration. Our earlier study showed that plasma free fatty acid concentration of the suckled group was 4 times higher than that of the starved, and the molar ratio of free fatty acids/albumin of the suckled rats reached 7[10]. Since plasma unbound-bilirubin concentration increases when the free fatty acids/albumin molar ratio exceeds 3–5[13–15], the increased concentration of plasma unbound-bilirubin (table) may be accounted for by the elevated level of plasma fatty acids. However, the present findings are not necessarily in agreement with the generally accepted concept that in newborns an increased concentration of plasma unbound-bilirubin, which is easily transferred across the blood-brain-barrier[6], may result in accumulation of the pigment in the brain tissue with an enhanced risk of kernicterus[15,16]. The reason why cerebellar bilirubin content and plasma unbound-bilirubin concentration did not show parallel changes remains a problem for further investigation.

There were significant negative correlations both in the suckled ($r = -0.462$, $n = 21$, $p < 0.05$) and starved ($r = -0.626$, $n = 21$, $p < 0.01$) groups between body weight and cerebellar bilirubin content (figure). The results are consistent with the finding that in humans kernicterus is rife among small-for-date babies[9,17].

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Cataract induced by administration of a single dose of sodium selenite to suckling rats

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Summary. A single dose of sodium selenite to male suckling rats causes permanent or intermittent cataracts. The resistance to the lethal effect of selenite in suckling rats is significantly higher in comparison with adult animals.

In previous papers from our laboratory, effects of selenium compounds in rats of different age were reported[1]. In the experiments, in which selenite was administered to suckling rats, a new, till unknown and unexpected effect of selenite upon the eyes was investigated.

Materials and methods. For the experiments male Wistar rats (substrain Konárovice) were used. On the 2nd day of life, the sucklings were divided in such a way that 8 males were kept with one mother. On the 10th day of life (i.e. when they are in absolute nutritive dependence on the mother[2]) a single dose of 0.02 M solution of Na_2SeO_3 was administered s.c. to 5 experimental groups receiving 5, 10, 20, 40 and 60 μmoles/kg b.wt, respectively. The first control group received s.c. 0.02 M solution of a sulphur compound (Na_2SO_3, i.e. an element homologous with selenium), in a dose of 60 μmoles/kg b.wt. The second control group was without any treatment. As reference group the 2-month-old male rats were used to which 0.02 M solution of Na_2SeO_3 was administered s.c. in amount of 20 μmoles/kg b.wt. Mothers of the sucklings, as well as the group of adult males, were fed on a standard laboratory diet with water ad libitum. All animals were checked daily and the experiment was terminated 20 days after the treatment.

Results and discussion. The survival of experimental animals and the occurrence of cataracts are summarized in the table. Except for the cataracts, all surviving animals had no other signs of disease. Cataracts in the sucklings were visible by the naked eye after the opening of their eyes, i.e. on the 14–16th day of life. The opacity

The lethal and cataractogenic effect of sodium selenite on adult and suckling male rats (20 days after treatment)

Age	Group of rats	Dose (μmoles/kg b.wt)	No. of rats	Mortality 24 h	Mortality 7th day	Cataract Permanent	Cataract Intermittent	Without evident damage
Sucklings (10-day-old)	Na_2SeO_3	60	24	24	0	0	0	0
		40	36	0	8	22	6	0
		20	31	0	1	20	5	5
		10	28	0	0	5	6	17
		5	40	0	0	0	0	40
	Without treatment	0	16	0	0	0	0	16
	Na_2SO_3	60	16	0	0	0	0	16
Adults (2-month-old)	Na_2SeO_3	20	15	11	0	0	0	4