compared to non-vanadium-exposed erythrocytes and those erythrocytes in which some vanadium compound was less effective in creating the membrane effect.

The reduction of the number of erythrocytes in V(IV)-treated mice was statistically significant on days 2, 4, and 8 compared to controls. It has been reported that vanadyl sulfate induced an increase in erythrocyte production as reflected by percentage of radioiron incorporation that occurred eight days after vanadium treatment [10]. Those data suggested that the production effect followed in the wake of an earlier hemolytic effect. This would, of course, lower the oxygen-carrying capacity of the blood and, in response to such a reduction, the homeostatic response of accelerated erythrocyte production would be expected. The results reported here agree quite well with the earlier reports. The depression of the peripheral erythrocyte count temporarily coincides with the maximum hemolytic effect for all three test vanadium compounds. Even though there seems to be variable potency concerning alternation of erythrocytic membranes via hemolysis and reduction in the number of circulating erythrocytes, it appears that all test vanadium compounds were most effective four days following exposure. In addition, there is a clear temporal correlation between the magnitude of the hemolytic index and the observed reduction in the number of peripheral erythrocytes obtained from vanadium-treated mice.

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Critical period for induction of congenital hydrocephalus and dysplasia of subcommissural organ by prenatal X-irradiation in rats

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Summary. A single whole-body X-irradiation of pregnant Wistar rats at a dose of 1.05 Gy at 10.30, 12.30 and 14.30 h respectively, of gestational day 10 resulted in significantly high incidences of hydrocephalic offspring. No hydrocephalic offspring resulted from X-irradiation of pregnant rats with 1.05 Gy at 16.30 h, whereas a dose of 1.22 Gy at 16.30 h resulted in a low but statistically significant incidence of hydrocephalus. Neither 1.05 Gy nor 1.22 Gy X-irradiation of pregnant rats at 18.30 h resulted in any hydrocephalic offspring. Dysplasia of the subcommissural organ was noticed in all the hydrocephalic brains histologically examined.
Key words. Congenital hydrocephalus; subcommissural organ; prenatal X-irradiation; critical period; rat.

Congenital hydrocephalus has been shown to occur spontaneously or in inherited form in many mammalian species, including human beings. A number of investigators have already expressed several different ideas on the pathogenetic mechanism of congenital hydrocephalus [1]. Recently, we investigated the histological abnormalities in the congenital hydrocephalus spontaneously occurring in the MT/HokIdr mouse and the CWS/Idr rat, and suggested that the dysmorphogenesis of the subcommissural organ (SCO) may be involved in the causation of congenital hydrocephalus [2, 3]. Dysplasia of the SCO has also been reported by other authors in the inherited hydrocephalus occurring in rats [4, 5].

Congenital hydrocephalus has experimentally been induced in viable offspring in laboratory animals by treatment of the mothers with several teratogens [6, 7]. We have reported that the treatment of the mothers with 100 R (0.87 Gy) X-irradiation, or the administration of 5 mg/kg methylnitrosourea to pregnant rats on gestational day 9.5, results in high incidences of viable hydrocephalic offspring, and that the SCO of these hydrocephalic brains is reduced in size and developed only at the caudal roof of the third ventricle [7, 8].

The purposes of this study were to delineate the precise period of susceptibility of the embryo to X-irradiation which produces congenital hydrocephalus, and to deter-

mine whether the dysplasia of the SCO is always associated with this brain anomaly.

Materials and methods

Virgin females of albino Wistar rats were housed with males between 17.00 h and 9.00 h the next morning. The females with sperm in vaginal smears following mating were taken as being at gestational day 0. On gestational day 10, pregnant rats were given a single whole body X-irradiation with a dosage of 1.05 Gy at one of the following times; 10.30 h, 12.30 h, 14.30 h, 16.30 h and 18.30 h. Other groups of pregnant rats were X-irradiated with a dosage of 1.22 Gy at 16.30 h or 18.30 h of the same gestational day. The conditions of X-irradiation were 200 kV, 15 mA, filter: 0.5 mm + 0.5 mm Cu, average target distance: 70 cm (rotating irradiation), exposure rate: 0.22 Gy/min, HVL = 12.7 mm Al.

On the day of delivery, designated postnatal day 0, the number and the sex of neonates were recorded. On postnatal day 10, the pups were weighed and examined for external malformation. Control offspring from untreated mothers were also counted on postnatal day 0, and weighed and examined in the same way on postnatal day 10. Pups were killed by cardiac perfusion with 10% formalin after ethyl ether-anesthetization, then the eyes were enucleated and their size examined. Eyes smaller than about two-thirds of the normal eye were regarded as showing microphthalmia. The brains were removed and examined for ventricular dilation by a coronal bisection with razor blades. Statistical analysis was performed using the Mann-Whitney U test with the litter as the experimental unit.

For histological examination, five normal and five hydrocephalic brains were taken from each X-irradiated group, fixed for 24 h with Bodian II solution, and embedded in paraffin. Five normal brains of 10-day-old pups from the untreated group were also prepared by the same method. Sagittal sections were stained with hematoxylin and eosin before light microscopic examination of the SCO.

Results

Teratological study. No significant differences were observed in the mean litter sizes among all the X-irradiated groups and the untreated control, but significant numbers of offspring had died by postnatal day 10 in all the X-irradiated groups. The mean body weights on postnatal day 10 were not different among all the X-irradiated groups and the untreated control (table).

In the 1.05 Gy X-irradiated groups, about 20–25% of hydrocephalic offspring were obtained by the irradiation at 10.30 h, 12.30 h, and 14.30 h (table), but the variations in the incidences were relatively high among the litters; that is, 10–50% in the group irradiated at 10.30 h, 0–50% in the group irradiated at 12.30 h, and 0–44.4% in the group irradiated at 14.30 h. No such anomaly was observed in the offspring of the mothers irradiated at 16.30 h and 18.30 h. In the 1.22 Gy X-irradiated groups, a low but statistically significant incidence of hydrocephalus occurred in the offspring of the mothers irradiated at 16.30 h (the variation was 0–23.1%), but no offspring with such anomalies were found in the group irradiated at 18.30 h (table).

Types and incidences of malformations in offspring of untreated and X-irradiated pregnant rats

Group	No. of dams	Mean litter size (± SD)	No. of pups dying by postnatal day 10* (% ± SD)	No. of offspring on postnatal day 10	Mean body weight (g) (± SD)	No. of malformed pups on postnatal day 10 (% ± SD)			
						Hydrocephalus	Micro- or anophthalmia	Club foot	Oligodactyly of hind foot
Untreated	10	11.8 (± 2.4)	0	118	16.7 (± 1.7)	0	0	0	0
X-irradiated 1.05 Gy									
10.30 h	7	13.6 (± 1.8)	17 (17 ± 9.5)[2]	78	18.1 (± 2.4)	20 (25.1 ± 13.3)[2]	53 (67.5 ± 14.3)[2]	0	0
12.30 h	7	12.0 (± 3.3)	13 (17.6 ± 14.2)[2]	71	15.6 (± 2.2)	16 (20.3 ± 18.0)[2]	32 (49.8 ± 31.5)[2]	2 (2.1 ± 3.6)[1]	1 (1.1 ± 2.9)
14.30 h	7	14.7 (± 1.7)	19 (18.4 ± 13.1)[2]	84	17.2 (± 2.4)	21 (26.8 ± 18.7)[2]	51 (63.1 ± 21.1)[2]	6 (7.8 ± 14.6)[1]	3 (3.5 ± 4.5)[1]
16.30 h	7	13.1 (± 3.4)	7 (6.8 ± 6.9)[2]	85	17.5 (± 2.1)	0	41 (46.2 ± 20.4)[2]	9 (8.1 ± 18.6)[1]	7 (6.3 ± 14.0)[1]
18.30 h	7	13.9 (± 1.5)	6 (6.4 ± 8.9)[1]	91	16.3 (± 1.2)	0	40 (43.8 ± 15.6)[2]	3 (3.4 ± 4.3)[1]	2 (2.1 ± 3.6)[1]
1.22 Gy									
16.30 h	10	12.7 (± 1.3)	9 (7.6 ± 9.1)[2]	118	17.7 (± 2.4)	5 (4.0 ± 7.6)[1]	89 76.3 ± 12.6)[2]	9 (7.2 ± 8.5)[2]	5 (4.1 ± 5.9)[1]
18.30 h	10	13.3 (± 1.4)	15 (11.6 ± 10.1)[2]	118	18.3 (± 2.4)	0	77 (66.2 ± 32.6)[2]	17 (14.5 ± 17.7)[2]	16 (13.6 ± 17.8)[2]

*including stillbirths; [1] $p < 0.05$ compared with untreated group; [2] $p < 0.01$ compared with untreated group.

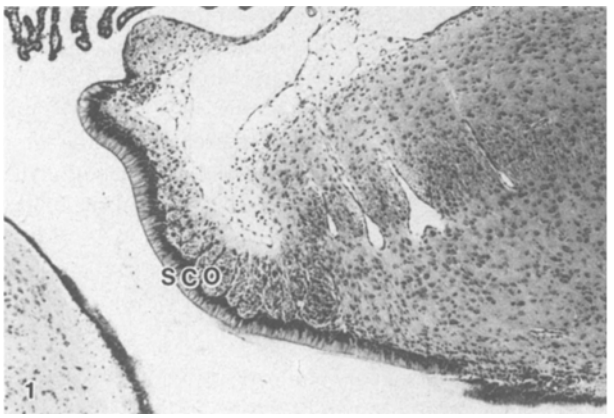

Figure 1. Sagittal section of the subcommissural organ (SCO) developing so as to extend from the caudal roof of the third ventricle to the roof of the anterior end of the cerebral aqueduct in the normal brain of a 10-day-old rat. ×80.

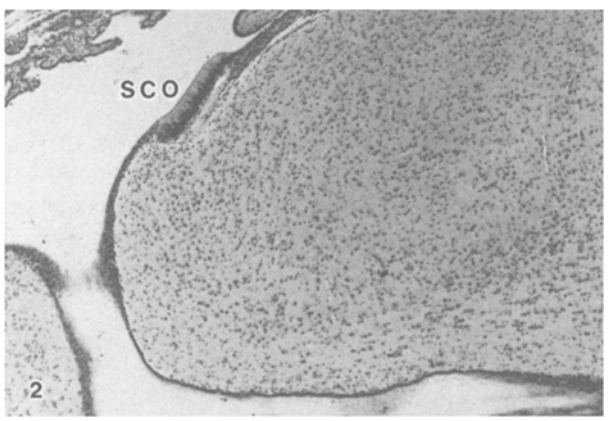

Figure 2. Hydrocephalic brain of a 10-day-old rat born from a mother given 1.05 Gy X-irradiation at 14.30 h on gestational day 10. Dysplastic subcommissural organ develops only at the caudal roof of the third ventricle (SCO). ×60.

A large number of offspring in all the X-irradiated groups displayed micro- or anophthalmia. There was a low but significant occurrence of club foot in the groups X-irradiated after 12.30 h, and also oligodactyly of the hind feet in the groups X-irradiated after 14.30 h (table).

Histological study. In the normal brains from normal pups of the untreated group and also from all the X-irradiated groups, the SCO was developed so as to extend from the roof of the caudal end of the third ventricle to the roof of the anterior end of the cerebral aqueduct (fig. 1). In 18 of a total of 20 hydrocephalic brains of the X-irradiated groups examined, the SCO was greatly reduced in size and developed only at the caudal roof of the third ventricle, at some distance from the anterior end of the cerebral aqueduct (fig. 2). In the remaining two cases, which were found in the group given 1.22 Gy X-irradiation at 16.30 h, in addition to the reduced SCO at the caudal roof of the third ventricle, a small-sized SCO was formed at the roof of the cerebral aqueduct, somewhat apart from the entrance of the cerebral aqueduct and

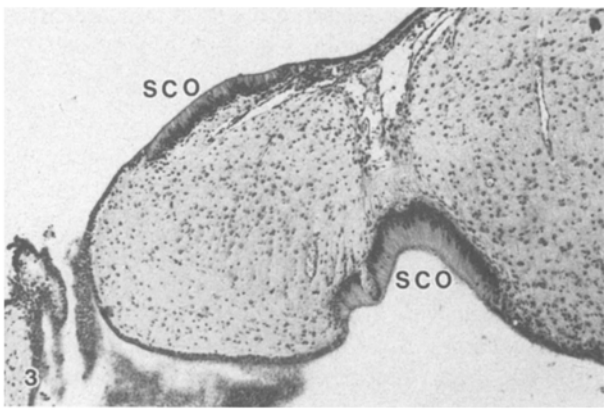

Figure 3. Hydrocephalic brain of a 10-day-old rat born from a mother given 1.22 Gy X-irradiation at 16.30 h on gestational day 10. Dysplastic subcommissural organ develops at the caudal roof of the third ventricle and also at the roof of the cerebral aqueduct somewhat apart from the entrance of the cerebral aqueduct (SCO). ×80.

corresponding to the caudal end of the normal SCO, whereas no SCO developed at the entrance to the cerebral aqueduct (fig. 3).

Discussion

In our previous study, we showed that the X-irradiation of pregnant rats with 0.87 Gy on gestational day 9.5 (about 13.30 h on gestational day 9) resulted in a significantly high incidence of hydrocephalic offspring, whereas no such anomaly was found in the offspring after maternal 0.87 Gy X-irradiation on gestational day 10.5 [7]. The present study showed that significantly high incidences of hydrocephalus are produced by maternal X-irradiation on gestational day 10 when the dose is higher than 1.05 Gy. The incidence of this anomaly showed considerable variation between litters, probably because the mating time used in this study was long (between 17.00 h and 9.00 h on the next morning), so that the developmental stage of the embryos might have been somewhat varied between litters. Nevertheless, no offspring displaying hydrocephalus were obtained by the X-irradiation at 18.30 h of gestational day 10. The present study also suggests that this critical period for the induction of congenital hydrocephalus is closely related to the sensitive period of the SCO precursor cells to X-irradiation, since all the hydrocephalic brains histologically examined in the present study displayed dysplasia of the SCO.

It has been shown that the onset of the specific morphological appearance of SCO in the rat embryo is observed on gestational day 13.5; before this time the dorsal neuroectoderm of the cerebral aqueduct of the rat embryo was homogeneous, consisting of pseudo-stratified columnar cells [7]. In the mouse embryo, Rakic and Sidman [9] have reported that the birthday (time of final replication of nuclear DNA) of SCO precursor cells occurs between gestational days 11 and 15. From these facts, it may be suggested that the target cells in rat embryos damaged by

X-irradiation before 18.30 h on gestational day 10, defects in which result in congenital hydrocephalus, may be the proliferating precursor cells of the SCO but not those engaged in the specific morphogenetic differentiation. The present study strongly suggests that the dysmorphogenesis of the SCO is primarily involved in the pathogenesis of congenital hydrocephalus induced by maternal X-irradiation. A number of investigators have discussed the functional significance of the SCO in many vertebrate species, but none of the suggestions have been confirmed by direct evidence[2]. Thus, the precise mechanism by which the dysmorphogenesis of the SCO could induce congenital hydrocephalus awaits further elucidation.

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Mineral composition of pigeon milk

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Summary. Mineral analysis of pigeon milk indicates its major elements to be P > Ca > K > Na > Mg (in that order) and trace elements Fe > Zn > Mn > Cu. Whereas the levels of Ca, K, Mg, Na and Mn remain fairly constant in the first week, those of P, Fe, Zn and Cu fall significantly during this period. Compared to cow's and human milk, pigeon milk has definitely higher levels of trace elements. Similarly, the trace mineral content of pigeon milk exceeds that of pigeon egg albumen or yolk. Except for Fe, Mn and Cu, pigeon milk is richer than adult pigeon feed in its mineral content. The Ca:P ratio of pigeon milk increases from 0.3 to 1.1 in the first five days. It appears that the high trace mineral content of pigeon milk is one of the factors contributing to the phenomenal postnatal growth of squabs.
Key words. Pigeon milk; major elements; trace minerals; nutrition; postnatal growth; squabs.

One of the spectacular avian adaptations is the synthesis and secretion by pigeons and doves (Columbidae) of a nutritive material in their crop, popularly known as pigeon milk[1]. The process has close parallelism to mammalian lactation, in that both pigeon milk and mammalian milk are holocrine products controlled by prolactin, and both constitute the most wholesome food for their respective neonates. Unlike mammalian milk, however, pigeon milk is produced by both male and female parent pigeons. In physical consistency the pigeon milk is semi-solid and cheese-like; it is formed by the clumping of sloughed-off crop squamous epithelial cells. The altricial pigeon young (squabs) fed exclusively on this milk show a phenomenal rate of postnatal growth[2,3]. The chemical composition of this unique avian juvenile diet reveals that it is a rich source of proteins and lipids but not of vitamins and carbohydrates[4]. However, except for a few preliminary analyses of proteins and lipids[5–10], practically nothing is known of the growth-promoting properties of pigeon milk. In this paper we report its mineral composition and compare it with that of cow's milk, human milk, pigeon egg albumen and yolk, and adult pigeon feed.

Materials and methods

Domestic pigeons (*Columba livia*) were maintained on a mixed-grain diet of cereals and pulses (supplemented with minerals and vitamins) and bred in the aviary of the Department. Pigeon milk was collected from the crops of 1–5-day-old squabs as described earlier[11]. It must be mentioned that parent pigeons do not store the milk in their crops at any time, so collection from the adult would not be feasible. Any fragments of grains found mixed with the milk were carefully removed, and samples collected on different days were separately pooled and stored at – 20 °C until used. The mineral composition (Ca, Mg, Fe, Cu, Mn and Zn) of pigeon milk, pigeon egg yolk and albumen, and pigeon feed was analysed, after digesting the dry samples with a perchloric acid:nitric acid mixture (1:2 v/v), by atomic absorption spectrophotometer (Varian AAS-975). Na and K content was estimated by flame photometry. Phosphorus was determined by the vanadomolybdophosphoric yellow color method. Published data on the minerals of cow's milk and human milk[12] were taken for comparison.