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Final number of Purkinje and other large cells in the chick cerebellum increased by oxygen and by glucose

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Cerebral cell numbers at birth (mammals) or at hatching (birds) tend to remain uniform[19] within the species and the strain; however, occasionally, they may undergo considerable deviations, presumably due to variations in the environmental conditions during the sensitive proliferation period of these cells. The deviations may be spontaneous or induced[19].

In recent work[17] we have obtained evidence in support of the above hypothesis. We have devised the following system. (1) The environmental conditions (temperature) were changed only during a short period (days 5–7 of embryonal life) which coincides with the proliferation of essentially only three types of cerebellar neurons: Purkinje, Golgi II and large neurons of the central cerebellar nuclei[2–4]. (2) The cell types chosen permit their unequivocal quantitative identification due to their large size. (3) These neurons were counted when their number becomes essentially final (functionally mature cerebellum which is already present right after hatching in the precocial birds such as chick). Using this system we have found[17] that the final number of Purkinje, Golgi II and large neurons of the central cerebellar nuclei is influenced by the temperature of incubation during this period: temperatures higher than optimal increased this number and lower than optimal decreased it.

The present work concerns the search for other factors that may also influence proliferation of these neurons during their period of division (days 5–7) and thus change their final number. Two such factors were identified: oxygen content and glucose concentration in the egg.

Cells in tissue culture or explanted chick embryos (in vitro) are known to grow faster at elevated oxygen or glucose concentration[6,7,10,12]; there are also a few reports on the effects of these agents on embryonal growth in vivo. The most rapid growth of embryos and the highest hatchability was observed when the eggs were incubated at elevated oxygen content[1,5,13,14]. Eighty per cent of free egg glucose is catabolized during the first 7 days of incubation[9,11], and injection of additional glucose into eggs reduced mortality of the embryos[8]. None of these reports dealt with brain growth.

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In the present work, the eggs and their handling were essentially as described in our previous work[15–18]; the experimental and the control groups were matched for egg weight and incubated at 37.5 ± 0.1 °C ('control' incubator). In experiment A, at days 5–7 the experimental group was transferred to another 37.5 °C incubator provided with a plastic box, continuously supplied with oxygen, to maintain around the eggs an oxygen content of 45–50%, as measured by Beckman Oxygen Analyzer Model D2, connected to the box. After day 7, the eggs were returned to the 'control' incubator maintained at normal atmospheric oxygen content (21%). In experiment B, all eggs were incubated in the 'control' incubator, but after 4 days of incubation each experimental egg was punched, injected (into albumen) with 0.2 ml of 75% w/v aqueous glucose solution, the holes sealed with paraffin, and the incubation continued. Since in previous work[15–18] it was established that injection of eggs with 0.2 ml saline has no effect on embryonal development, such control was not used in the present work.

Direct cerebellar cell enumeration at hatching was performed as described previously[15–17]. The cerebella were dissected out, weighed and preserved at room temperature in 10% buffered formalin for a period of 10 days to 1 month. They were then disintegrated in 2 ml water in a conical tube, using a glass rod tipped with a rubber policeman. The coarse suspension was then subjected to ultrasonication for 4 min at 55 W, using a microprobe of 3.5 mm diameter tip. The uniform fine suspension was then diluted 1:2 and stained with 0.005 ml of 1% thionine solution; the Purkinje,

TABLE I

Cerebella of chick embryos incubated at elevated oxygen or glucose concentration

Different batches of eggs were used in experiments A and B. All values ± standard deviation. Large neurons: P, Purkinje; G, Golgi II; ccn, central cerebellar nuclei.

Experiment	*Number of animals*	*Treatment**	*Body weight (g)*	*Cerebellum*		
				Weight (mg)	*Cell number** × 10^{-5}*	
					P + G + ccn	*Other*
A	34	Control	44.3±2.6	108.6±12.7	3.0±0.46	903±138
	31	Experimental (oxygen)	42.9±2.9	109.3±13.5	3.6±0.52	960±180
		Δ***	−3.1	+0.6	+20%	+6.3
		Probability****	n.s.	n.s.	<0.0005	n.s.
B	39	Control	43.0±3.2	109.2±9.5	2.78±0.36	958±88
	37	Experimental (glucose)	41.1±3.8	105.4±7.4	3.45+0.51	1043±86
		Δ***	−4.4	−3.4	+24	+9
		Probability****	n.s.	n.s.	<0.0005	<0.0005

* Oxygen treatment, days 5–7; glucose injection, day 5; body weight and cerebellar parameters, at hatching. See text for other details.

** Average of 6 counts/cerebellum.

*** Δ, difference to control, in per cent of control.

**** Student's *t*-test; n.s., not significant.

Golgi II and central cerebellar nuclei cells (together), easily identified by their large size, and separately all the other cells were then counted in a 0.1 mm deep Spencer–Neubauer hemacytometer, using a magnification of 430 in the microscope. All countings were made in a 'blind' fashion, without knowing to which group the sample belonged. The erythrocytes (approximately 1.5%), epithelial cells and blood vessel cells, easily identified under the microscope, were not counted.

The results are represented in Table I. As expected, the oxygen treatment (exp. A) during days 5–7 only did not significantly affect body weight, cerebellar weight or the number of those cerebellar cells whose period of proliferation occurs after day 7. In contrast, the number of Purkinje, Golgi II and large neurons of the cerebellar nuclei, all of which proliferate mainly during days 5–7, was increased by the incubation in a higher oxygen content; the increase was statistically highly significant. Thus, the effect of elevated oxygen content was similar to previously reported[17] effect of elevated temperature of incubation during this period.

As can be further seen from Table I, (exp. B) introduction of glucose on day 5 of incubation only did not significantly affect body weight and cerebellar weight; in this respect the effect was similar to that of oxygen (exp. A); however, glucose produced a small but significant increase in the number of cerebellar cells that proliferate after day 7. Possibly, excess glucose remained in the egg throughout incubation, which is in contrast to higher temperature or oxygen treatment which disappear immediately after the conclusion of the treatment (day 7). As in the case of higher temperature and oxygen, glucose also produced a considerable increase in the number of Purkinje, Golgi and large neurons of the cerebellar nuclei, and this increase was statistically highly significant.

The conclusion emerging from this and previous[15–17] studies is that, at least in the case of types of neurons studied, the induced increases in their numbers are not (or not fully) eliminated until the maturity of the organ studied, in this case cerebellum. Thus, the *final* number of such neurons may be increased by a variety of conditions (temperature, oxygen content, glucose concentration) prevailing during the sensitive period of neuronal proliferation.

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