A Study of Mitotic Index in Differentiating Central Nervous System of Chick

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Received December 6, 1967

The development of the nervous system of chick is associated with the emergence of the cytoarchitectural features that form its scaffolding. The nervous system of the bird passes through some basic epigenetic processes as cellular proliferation, differentiation and migration when two major cell types viz. the neurons (the functional cells of the nervous system) and supporting elements (ependymal, neuroglial, sheath and capsular cells) are established (Romanoff 1960). Thus in the several strata of the neural tube initially composed of a single layer of the columnar epithelial cells (Duncan 1957), three zones can be identified:

- (a) an innermost ependymal zone of the neural germinal epithelium surrounded by
- (b) the mantle zone composed of undifferentiated cells and (c) the peripheral or marginal zone within which the white substance develops.

Though adult nerve cells seldom divide (Weiss 1958, Bertalanffy and Lau 1963) cell proliferation occurs at the early stage of neural differentiation (Kallen 1965) and the ventricular neural epithelial cells in the active mitotic phase are regarded as 'germinal' ones (Hamburger 1948, Corliss and Robertson 1963, Watterson 1965) at least after the establishment of the mantle zone. In recent years, a number of studies on avian and mammalian embryos has indicated that mitotic figures in the neural tube always occur near the lumen (McKeehan 1966) though there is a migration of the epithelial nuclei to and from the lumen during a complete mitotic cycle (Watterson *et al.* 1956, Sauer and Walker 1959, Fujita 1963, Martin and Langman 1965). The present investigation concerns with the survey with the mitotic index in the following four principal zones; the fore-, mid-, hind-brain and the spinal cord of chick, at different stages of development. Quantitative data are obtained on the potency of cell proliferation in these four sectors of the central nervous system of chick which may be useful to understand the problem of differentiation of the neurons.

Material and methods

The study is based on white leghorn chick embryos incubated at a constant temperature of 38°C. The central nervous system of the embryos at different ages were dissected out and fixed in alcoholic Bouin's or San Falice fixatives. After routine process of embedding in paraffin, serial sections were made at 10 μ and stained in Heidenhain's haematoxylin. Mitotic indices were ascertained by considering the dividing and the non-dividing cells in the tissues (Bertalanffy and Lau 1963). Countings of cells were followed by the method of McKeehan (1951) by taking nine areas, 0.105 mm² each, in each of the three zones viz. ependyma, mantle and periphery around the neurocoele (Fig. 1). In the early stage of development, when these three zones remain very thin, the entire cell population was taken into consideration. Alternate sections were followed in order to avoid duplication in countings. Mitotic indices were recorded in the cerebrum (fore-brain), optic lobes (mid-brain), cerebellum (hind-brain) and the spinal cord of the same embryo. At least five embryos of every stage of development were studied. As it is known that, contrary to the results found in the amphibian larvae (Shell 1961), treatment of embryos with colchicine

Table 1. Mitotic index in the ependymal, mantle and peripheral regions in the fore-, mid- and hind-brain and the spinal cord of differentiating CNS of chick embryos

	2 Days						4 Days						6 Days						8 Days						10 Days											
	F			M			H			S			F			M			H			S			F			M			H			S		
	F			M			H			S			F			M			H			S			F			M			H			S		
Ependymal layer	Total cells						1263	—	—	—	1204	2879	2535	2905	2144	3906	5554	2482	2541	4424	4140	2186	3282	4500	2374	2237	4856									
	No. of div. cells						46	—	—	—	31	146	116	71	61	146	75	25	56	151	49	5	58	98	11	13	51									
	No. of non-div. cells						1217	—	—	—	1173	2733	2419	2834	2083	3760	5279	2457	2485	4273	4091	2181	3224	4402	2363	2224	4805									
	Mitotic index						3.64	—	—	—	3.55	5.00	4.57	2.44	2.84	3.73	1.42	1.0	2.26	3.41	1.18	.22	1.76	2.17	.46	.62	1.05									
Mantle layer	Total cells						—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—									
	No. of div. cells						—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—									
	No. of non-div. cells						—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—									
	Mitotic index						—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—									
Peripheral layer	Total cells						—	—	—	—	—	—	—	—	—	2437	2649	460	—	2425	2408	—	—	2141	1566	2465	2242									
	No. of div. cells						—	—	—	—	—	—	—	—	—	4	3	0	—	6	0	—	—	9	1	38	11									
	No. of non-div. cells						—	—	—	—	—	—	—	—	—	2433	2646	460	—	2419	2408	—	—	2132	1565	2427	2231									
	Mitotic index						—	—	—	—	—	—	—	—	—	.16	.11	0	—	.24	0	—	—	.42	.06	1.54	.49									
Grand total	Total cells						1263	—	—	—	1204	2879	2535	2905	2144	6343	8003	2942	2541	11142	6548	2186	3282	10874	6074	6128	7098									
	No. of div. cells						46	—	—	—	31	146	116	71	61	150	78	25	56	209	49	5	58	117	16	58	62									
	No. of non-div. cells						1217	—	—	—	1173	2733	2419	2834	2083	6193	7925	2917	2485	10933	6499	2181	3224	10757	6058	6070	7036									
	Mitotic index						3.64	—	—	—	3.55	5.00	4.57	2.44	2.84	2.36	.97	.84	2.26	1.87	.74	.22	1.76	1.07	.26	.94	.87									

Table 1 (contd.)

	12 days				15 days				18 days				20 days				
	F	M	H	S	F	M	H	S	F	M	H	S	F	M	H	S	
Ependymal layer	Total cells	5235	2110	1993	2883	3357	1912	2358	1797	1702	1668	1707	1902	1092	2248	1563	2094
	No. of div. cells	83	8	14	28	35	6	18	10	16	4	6	7	11	2	1	6
	No. of non-div. cells	5152	2102	1979	2855	3322	1906	2340	1787	1684	1664	1703	1895	1081	2246	1562	2088
	Mitotic index	1.58	.37	.70	.97	1.05	.31	.76	.55	.94	.23	.35	.36	.52	.08	.06	.28
Mantle layer	Total cells	2632	1084	1355	—	1544	2978	1908	1005	1971	2989	1848	1185	1022	1168	2862	1306
	No. of div. cells	4	3	5	—	5	6	4	5	2	3	4	3	2	2	6	3
	No. of non-div. cells	2628	1081	1350	—	1539	2972	1904	1000	969	986	1844	1182	1020	1166	2856	1303
	Mitotic index	.15	.27	.36	—	.32	.20	.21	.5	.20	.30	.21	.25	.19	.17	.20	.22
Peripheral layer	Total cells	1804	2153	2510	1347	1334	2030	3979	1465	970	1423	3256	1877	1154	1243	2926	1712
	No. of div. cells	4	1	49	5	2	2	101	1	1	1	48	4	1	3	9	2
	No. of non-div. cells	1800	2152	2461	1342	1332	2028	3878	1464	969	1422	3208	1873	1153	1240	2917	1710
	Mitotic index	.22	.04	1.95	.37	.15	.09	1.53	.06	.10	.07	1.47	.21	.08	.24	.30	.11
Grand total	Total cells	9671	5347	5858	4230	6235	6920	8245	4267	3643	4080	6811	4964	3268	4659	7351	5112
	No. of div. cells	91	12	68	33	42	14	123	16	19	8	58	14	41	7	16	11
	No. of non-div. cells	9580	5335	5790	4197	6193	6906	8122	4251	3624	4072	6753	4950	3254	4652	7335	5101
	Mitotic index	.94	.22	1.16	.78	.67	.20	1.49	.37	.52	.19	.85	.28	.42	.15	.21	.21

of varied concentrations for different periods develop some abnormalities (Overton 1958, Watterson 1965), normal untreated embryos have been considered in this study.

Observations

The different aspects of the study viz. cell number, dividing cells, non-dividing cells, mitotic index at different stages of the embryos have been shown in Table 1.

It may be observed that mitotic indices in the brain and in the spinal cord of the 48 hours' embryos are nearly equal viz. 3.64 in the brain and 3.55 in the spinal cord region. As the central nervous system is small and not properly differentiated, the border areas of the mid- and the hind-brain can not be distinguished. Moreover, owing to the slender nature, the three zones round the neurocoele can not be demarcated.

In the 4-day embryos, different sectors of the nervous system are formed though the three zones round the neurocoele can not be distinguished properly. At this stage, it may be observed that the mitotic index at the fore brain area increases to 5.00 while that of the spinal cord decreases to 2.84. In the mid and in the hind brain regions these figures are 4.57 and 2.44 respectively. In the 6- and in the 8-day embryos, the mitotic indices in all the four sectors fall from the corresponding figures of 4-day embryos. Thus in the cerebrum mitotic indices are 3.73 and 3.41 while in the spinal cord, the related values are 2.26 and 1.76 respectively. Similarly in the mid and in the hind brain regions of the 6-day embryos, mitotic indices are 1.42 and 1.00 respectively and the corresponding figures in the 8-day embryos are 1.18 and 0.22

Fig. 1. Diagram of the field used in making cell counts in a cross section of the central nervous system. The rectangles show the approximate positions of samples for cell counts in the three zones of a section in the study.

From 10-day onwards, the three zones viz. ependymal, mantle and peripheral in almost all the sectors become evident. In 10-day embryos, it may be observed that the mitotic index in the cerebral areas become further diminished to 2.17. In the mantle and in the peripheral areas the figures are 0.23 and 0.42—the mitotic index in the peripheral area being slightly greater than that of the mantle area. In the optic lobes the mitotic index in the ependymal layer is 0.46 while those of the mantle and in the peripheral areas are 0.18 and 0.06 respectively. In the cerebellum, mitotic index is highest in the peripheral zone i.e. 1.54 as against 0.62 in the ependymal or 0.49 in the mantle area. The peripheral zone of the spinal cord shows a mitotic index of 0.49 against 1.05 of the ependymal layer. Here, the mantle area can not be demarcated properly.

Mitotic index becomes gradually decreased in the subsequent phases of development in the ependymal layer of cerebrum e.g. it is 1.58 and in 12-day embryos the value becomes 0.52 in 20-day embryos. In the mantle areas of the cerebrum there is a slight increase in the mitotic index in the 15-day embryos after which it is decreased while in the peripheral zone mitotic index shows gradual downward trend.

A more or less similar picture is observed in the ependymal and mantle layer of the midbrain except in the 18-day embryos where a slight increase in the mitotic index in the mantle area is recorded. But at 20 days' incubation it goes down again. It may be seen in the peripheral area that the mitotic index does not follow a straight line curve and at 20 days' incubation there is much increase in the magnitude viz. 0.24 from 0.07 of 18-day embryos. The spinal cord shows a similar decline in the mitotic index as that of the cerebral area, the slight alteration of the curve being at the peripheral zone of 18-day embryos when there is a small increase in the mitotic index. In the cerebellum, the curve for the mitotic index in the ependymal layer shows a decline upto 8-days of incubation but subsequently it increases upto 15 days. In the mantle zone, the mitotic index gradually decreases while there is remarkable increase in its magnitude in the peripheral zone of the 10 to 15-day embryos. There is again a drop in the mitotic index in the subsequent days of development.

Discussion

In chick, the central nervous system is laid down as a results of induction by the primitive streak. It is known that the anterior part of the streak which is a strong organizer induces the brain (archencephalic induction) while the posterior part of the streak gives rise the spinal cord (spino caudal induction) (Waddington 1956). The cell types incorporated in the different sectors of the central nervous system are not physiologically equipotent (Brachet 1960). From the present survey of the mitotic indices it is revealed that the cells do not divide at equal proportion through the nervous system.

Thus it may be observed that in the embryos of 48 hours of incubation i.e. immediately after the termination of the primary organizer action, the mitotic index of the brain and that of the spinal cord are nearly equal. But later in the forebrain, the mitotic index exceeds that of the spinal cord area. In

case of chick, the formative phase of the brain begins with differentiation of the medullary plate and extends through the fourth and fifth day of incubation (Romanoff 1960) when mitotic index as seen from the study is the highest in all the sectors of the brain and in spinal cord region (Fig. 2). The second

Fig. 2. Mitotic index in the ependyma in the differentiating central nervous system of chick.

phase of the development of the brain starting on the 5th or 6th day concerns with the structural modifications with differentiation of the nervous elements along with nerve centres and tracts. The definite cytoarchitecture of the central nervous system is attained by about 12th day of incubation. This process as revealed from the present survey is associated with a gradual fall in the mitotic indices and in the proliferating phase of the germ cells from the ependyma. But this decline is not uniform in all the sectors as the cells in the mid- and hind-brain lose the proliferating property on the 6th day but those in the cerebral area retain this potency upto the 12th day and in the spinal cord upto the 8th day of incubation. A tissue, according to Price (1958), should be regarded as growing when its mitotic index (i.e. percentage of dividing to nondividing cells at the same period) exceeds 1.5. Hence, it may be said that growth and differentiation of the chick brain nearly reaching completion at 12 days of incubation are always associated with varied proliferating phase of cells at different stages of incubation indicating an unequal differentiating kinetics in the central nervous system. So far as the mitotic indices of the mantle and the peripheral zones are concerned it is observed that the mantle zone with a mitotic index always below 1.5 level, never shows a proliferating property. The cells of this region migrating from the germinal layer (Hamburger 1948) remain undifferentiated initially to be transformed into neural elements (Romanoff 1960). But the occasional increase in mitotic indices in this zone may be due to the mesenchyme cells that migrate

in this area (McKeehan 1966).

In connection with the mitotic index in the peripheral area, it may be noted that the cells which intrude from outside into these portions of the central nervous system undergo mitosis at a quite different rate; it never attains 1.5% mark in the fore-, mid-brain and in the spinal cord area while in the hind brain particularly at 10 to 15 days' development the mitotic index exceeds 1.5% mark and these undifferentiated cells are believed to be associated with the development of 'folia' in the cerebellum (Romanoff 1960). This process of mitotic activity in the peripheral region of the cerebellum, is accompanied with a simultaneous increase in the mitotic index of the ependymal zone. Considering the three areas together, it may be observed that the proliferating phase of the mid- and the hind-brain area is active upto

Table 2. Details of the calculation of Regression Coefficient and its test of significance

	S.S.(Z)	S.S.(X)	S.P.(ZX)	b	Regression mean square	Error mean square	Calculated f value	Remark
Fore-brain	4.7277	310.1429	-37.9161	-0.1223	4.6371	0.0129	359.46 for 1 & 7 d.f.	highly significant
Mid-brain	10.9548	227.875	-47.7501	-0.2086	9.9606	0.1657	60.11 for 1 & 6 d.f.	do
Hind-brain	8.6378	227.875	-31.9672	-0.1402	4.4818	0.6927	6.47 for 1 & 6 d.f.	do
Spinal cord	6.6295	310.1429	-45.1982	-0.1456	6.5808	0.0070	940.11 for 1 & 7 d.f.	do

S.S.(Z)=Sum of square of Z when $Z = \log_e Y$ and Y=measurement of mitotic index in the ependymal layer.

S.S.(X)=Sum of square of X where X is the number of days.

S.P.(ZX)=Sum of product of Z and X.

b=the regression coefficient.

4 days of incubation while in case of the forebrain and the spinal cord, it may extend upto 8 days. It may be argued that the proliferating processes in the central nervous system in chick considered together is not governed by a simple cephalocaudal gradient and the mitotic behaviour in each region is different from the other as stated by Frieova and Jelinek (1963) that each part of the central nervous system of chick exhibits its own characteristics in mitotic behaviour. But if the ependymal zone is considered as the 'germinal' area in all the sectors of the central nervous system (Hamburger 1948, Corliss and Robertson 1963, Watterson 1965), it may be observed that measuring mitotic index in the log scale, the regression of log (mitotic index) is linear. The estimated regression coefficients in the ependymal area (Table 2) are (a) -0.1223 (fore-brain) (b) -0.2086 (mid-brain) (c) -0.1402 (hind-brain) and (d) -0.1456 (spinal cord). From analysis variance it is found that all regres-

sions are highly significant.

Summary

1. Mitotic indices in the four principal sectors of the developing central nervous system in chick have been studied.
2. It is found that initially the brain and the spinal cord have more or less similar mitotic indices. Later varied mitotic indices are observed in the different sectors of the central nervous system.
3. The ependymal layer shows the proliferating property with a mitotic index reaching 1.5 level, but the mantle and the peripheral zones never show the proliferating nature, except the peripheral zone of the hind-brain.
4. The proliferating property of the ependymal layer gradually becomes diminished with a highly significant regression value.
5. The implications of the observations have been discussed.

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