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Title: The Histogenesis and Mode of Growth of the Choroid Plexuses of the Hamster (Mesocricetus auratus).

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The patterns of cell proliferation in the choroid plexuses of embryonic and post-natal hamsters were studied by means of autoradiography (H^3-thymidine). In addition, the PAS technique was used to determine the times of first appearance, relative amounts, location, and times of disappearance of glycogen from the plexuses.

Many mitotic figures and nuclei labeled with H^3-thymidine are present in the epithelium and stroma of all three plexuses during embryonic and early post-natal life. A very low level of DNA synthesis also occurs in both of these tissues in the adult animal.

Growth and extension of the choroidal epithelium appears to occur in the stalk regions of the telencephalic and myelencephalic plexuses and the basal halves of the folds of the diencephalic and myelencephalic plexuses. Some growth, but at a much slower pace, also occurs in the distal portions of the plexuses. Labeled connective tissue cells were scattered throughout the stroma of the plexuses. A comparison of the mitotic indices of the stromal cells with those of the epithelial cells in the distal portions

of the plexuses suggests that the actively dividing stromal cells may be pushing the relatively less active epithelium into the brain ventricles, and during this process the epithelial cells in these regions become stretched and consequently flattened.

The Histogenesis and Mode of Growth of the Choroid Plexuses of the Hamster (*Mesocricetus auratus*)

by

John Jerome Dropp

A DISSERTATION

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INTRODUCTION

Several hypotheses have been proposed since 1958 to explain the mode of growth of the mammalian choroid plexuses. In referring to the human plexuses Boyd (1958) remarked: "I myself have never seen mitoses, even in embryonic choroid plexuses and this absence of the signs of normal cell division presents a general biological problem." Similarly Kappers (1958), being unable to find mitotic figures in the developing human telencephalic plexuses, stated: "Certainly, the way in which the enormous surface growth of the epithelium occurs has still to be studied more thoroughly." Several investigators reported only a few mitotic figures in the epithelium of some but not all of the developing mammalian plexuses (Zand, 1930; Tennyson and Pappas, 1964; Shuangshoti and Netsky, 1966). As a result many authors of books have been led to consider the formation of the epithelial choroid plexuses to be passive in the sense that the simple and polyinvaginating vascular elements of the pia mater thrust a mitotically inactive epithelium into the several brain ventricles (Patton, 1948, 1953; Voetmann, 1949; Kuntz, 1950; House and Pansky, 1960; Netter, 1962; Torrey, 1962; Klosovski, 1963; Ham, 1957; Langman, 1964; Manner, 1964). However, Birge (1962) and Shuangshoti and Netsky (1966) do not concur with this "passive" hypothesis. Although no details were provided, Birge remarked:

> "....in tracing choroid plexus development, it becomes evident that plexus formation does not involve solely an inpushing of the choroidal epithelium. Rather the epithelial infolding which occurs is accomplished by a phase of active growth and extension of the choroidal

epithelium accounting for the substantial increase in the volume of the epithelium during plexus formation" (p. 93).

A second "passive" hypothesis is that of Kappers (1958), who suggested that the telencephalic plexus epithelium grows by a "pushing out" of cells from the pseudostratified epithelium of the stalk region. In substantiation he cited the absence of mitotic figures in the distal portion of the plexus. This "pushing out" of cells as he says, causes a gradual reduction in the height of the stalk epithelium to form a single-layered "villus". And noting further that these cells contained large quantities of glycogen, he suggested that perhaps such cells were incapable of mitotic divisions. Kappers says: "....mitosis is not easily practicable in cells which are crowded with glycogen and in which the nucleus is located in the extreme apical part" (p. 19).

A third and more plausible hypothesis is that of Tennyson and Pappas (1964), who in an electronmicroscopic study of the developing telencephalic and myelencephalic plexuses of the rabbit, reported mitotic figures in the epithelial cells in the stalk regions alone. They stated that "....growth and extension of the choroidal epithelium probably occurs in the stalk. Vascular and interstitial elements, however, may proliferate throughout the stromal core...." (p. 386).

A fourth hypothesis, but akin to that of Tennyson and Pappas (1964). Using Colcemid Knudsen (1964) found many mitotic figures in the epithelium lining the plexuses in mouse embryos of various ages. These figures were most commonly seen in the stalk regions of the

telencephalic and myelencephalic plexuses, as well as in the proximal portions of the folds of the diencephalic and myelencephalic plexuses. In conclusion he stated:

> "Apparently the epithelial cells are not formed diffusedly, but in certain zones, a condition resembling the formation of epithelium elsewhere, for example in the ventricle and intestine. On the other hand, the location of mitoses in connective tissue points to a diffuse interstitial growth of this tissue...." (p. 181).

In a fifth and final proposition to explain the mode of growth of the plexuses, Shuangshoti and Netsky (1966), in a study of the histogenesis of the human choroid plexuses, remarked:

> "In spite of the absence of mitoses except in Stage I, we suggest that slow proliferation of choroidal epithelium occurs and is characterized by stratification and desquamation of superficial epithelial cells, followed by replacement from adjacent underlying cells" (p. 290).

Even though many authors have reported the absence of mitotic activity in the choroid plexuses, these plexuses nevertheless become active under abnormal conditions. Volzhima (1963) has observed a compensatory growth of the diencephalic plexus to twice its normal size in puppies from which the plexuses of all the other ventricles had been removed. He also noted that regeneration of the telencephalic plexus occurs if the stalk region of the plexus is left intact. Both of these phenomena occurred by ".... mitotic division of the epithelial cells...." (p. 243).

The day-to-day proliferative activities of the various tissues of the developing choroid plexuses have not been presented for any vertebrate. Miale and Sidman (1961) were specifically interested in the histogenesis of the mouse cerebellum, and although they also

noted many cells of the myelencephalic plexus labeled with thymidine-H^3, they failed to indicate precisely which cell types carried the label. Additionally, some authors have noted labeled epithelial cells in the adult choroid plexuses of man (Johnson et al., 1960), of mice (Shuangshoti and Netsky, 1966), and of both mice and rats (Messier and Leblond, 1960). The present study describes and compares the patterns of cell proliferation in the epithelial and connective tissue cells of the embryonic, early post-natal, and adult choroid plexuses of the golden hamster (Mesocricetus auratus Waterhouse). Presented also, is a correlated account on the histogenesis of these plexuses.

A total of 16 embryonic, 28 neo-natal and 5 adult hamsters was used. Seven of the embryos were obtained from adults which had received a single intra-peritoneal injection (luc/g of body weight) of H^3-thymidine (specific activity of 6.7 curies/millimole) 8 hours prior to sacrifice. The other embryos were obtained from females which had received similar injections of H^3-thymidine 24, 48, and 72 hours previous to sacrifice. Fourteen juveniles, ranging in age from one day to 56-days, also received a single injection and were decapitated 8 hours later. Fourteen litter mates which were not given H^3-thymidine, were similarly sacrificed at the same time for glycogen studies. All animals were injected at 8:00 a.m.

The brains of the animals were fixed in Carnoy's fluid for 6 to 8 hours, dehydrated through a series of graded alcohols (ethyl), cleared in xylol, embedded in paraffin, and cut at 6 to 8 microns.

The sections for autoradiography were mounted on nitric acid-cleaned slides coated with gelatin-chrome-alum solution, deparaffinized (xylol), and hydrated in a graded alcohol series to distilled water. In a darkroom provided with a safelamp (Wratten No. 1 red filter) the sections were covered with Kodak AR. 10 Stripping Film, dried for approximately 2 hours in a light-tight dust-free box (Hillemann and Ritschard, 1964), exposed for 30 days at 4°C in light-tight slide boxes containing Drierite and carbon dioxide to minimize fogging by background radiation, developed (Kodak D. 19) for 6 minutes, dipped in a stop bath (distilled water containing 6 to 8 drops of glacial acetic acid) for 1 minute, fixed (Kodak acid fixer) for 15 minutes,

washed in two 15-minute changes of distilled water, stained with hematoxylin and eosin, placed in xylol-cedarwood oil (1:1) overnight to minimize air aspiration, and mounted (Permount). All of the solutions used for development of the emulsion and staining of the sections were kept at 17-18°C in a constant temperature bath (Hillemann et al., 1966).

All slides were examined under oil immersion (1000 X). Only nuclei which possessed at least 5 grains were considered labeled and therefore counted. Mitotic indices (the quotient obtained by dividing the number of labeled cells in a population by the total count of cells) were determined for each of the tissues in the various areas investigated. In most instances the minimal number of cells counted was 1000. The highest sample count in epithelial tissue was 2000 and in connective tissue, 1890. However, due to the unavoidably low total cell count of the plexuses during their initial stages of development, the total cell counts were as low as 600-700 in a few instances. The total number of epithelial cells counted was 201,203 and the total number of connective tissue cells counted was 163,270.

The periodic-acid-Schiff procedure (P.A.S.) was used to locate glycogen. Three spaced sections of each of the three choroid plexuses of each of the animals were deparaffinized with xylol and hydrated with graded ethyl alcohols to distilled water, treated with periodic acid (0.6% aq.) for 7 minutes, washed in running water for 5 minutes, stained with Schiff's reagent for 30 minutes, washed in running water for 10 minutes, dehydrated in a graded series of alcohols, cleared in

xylol and mounted (Permount). In order to remove glycogen from the tissues and thus distinguish it from mucopolysaccarides which also react with P.A.S. three additional adjacent sections were treated with 1.0% diastase for 1 hour at 37°C prior to being placed in periodic acid.

OBSERVATIONS

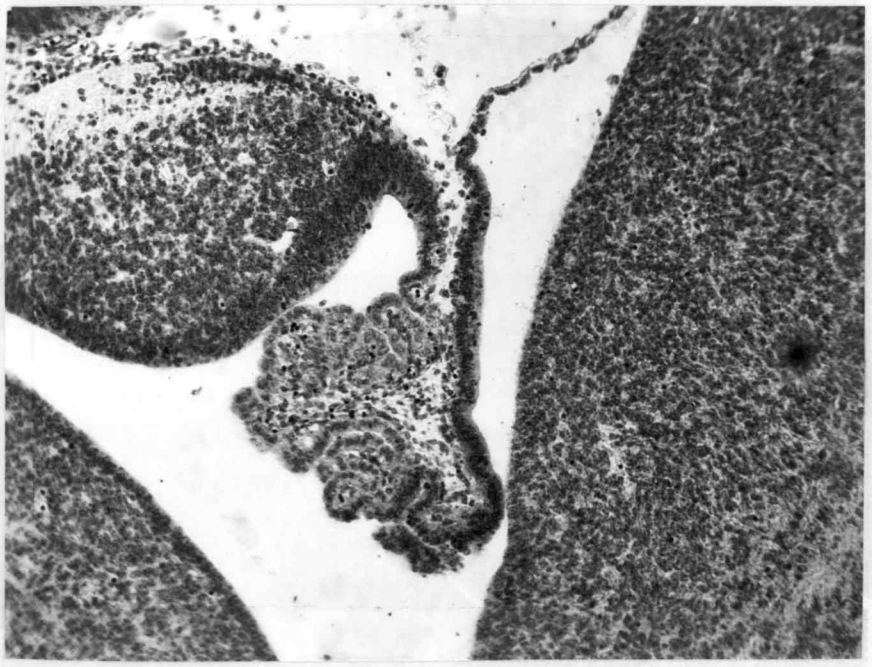
A. Histogenesis of the choroid plexuses.

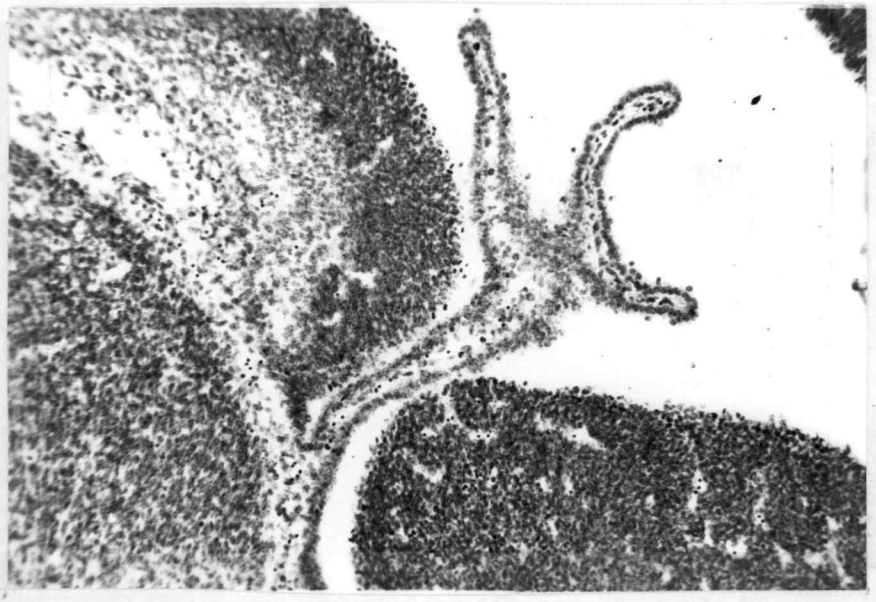
1. MORPHOLOGY OF THE DEVELOPING AND ADULT PLEXUSES: The development of the choroid plexus of the fourth ventricle begins on the tenth day of gestation. The primordium of the plexus may be divided into two regions, the stalk, and folded area (Figure 1). The stalk consists of a pseudostratified columnar epithelium along with its lining vascular stroma. This epithelium is composed of an anterior portion in continuity with both the neural epithelium proper (cephalic), and the folded area, along with a posterior portion which joins this folded region to the thin roof (posterior membraneous area) of the fourth ventricle. The numerous folds are individually composed of a single-layered epithelium (lamina choroidea epithelialis) covered by a highly vascular stroma (Figure 1). The myelencephalic plexus in the adult hamster is morphologically similar to that which is found in its embryonic and neo-natal periods, except for the fact that the pseudostratified epithelium of the stalk region is replaced by a single layer of low columnar cells.

The anlagen of the choroid plexuses of the lateral ventricles appear initially on the eleventh day of gestation, and as seen in section are digitiform and completely covered by a pseudostratified columnar epithelium. Although these rudiments in the 12-day embryo remain predominantly covered by a pseudostratified epithelium, there are nevertheless distinct and widely separated areas at the distal ends of the primordia where this epithelium is being transformed into a

Figure 1. The myelencephalic choroid plexus in the 12-day embryo. (x100).

Figure 2. The telencephalic choroid plexus in the lateral ventricle of the 13-day embryo. (x100).





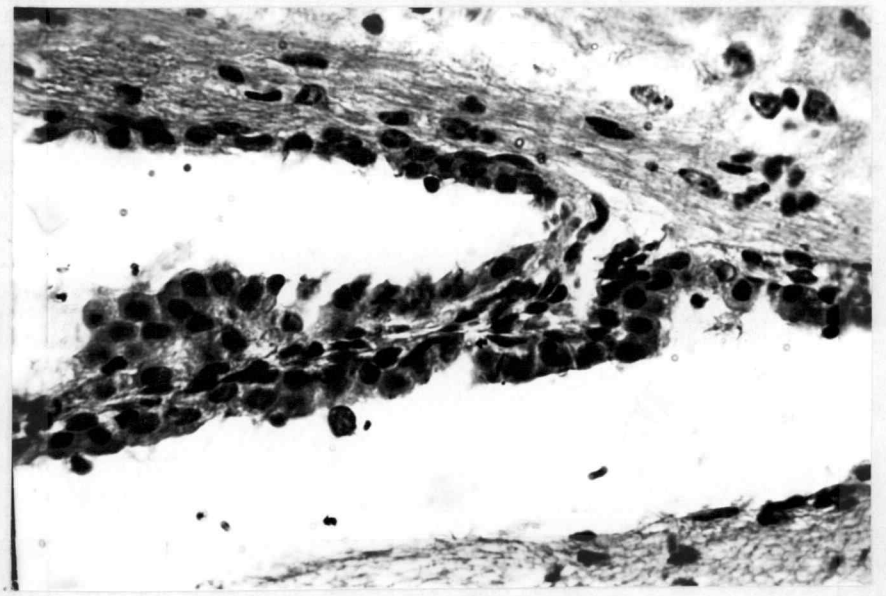
single layer of tall columnar cells. After day 13 of pregnancy each of the paired anlagen may be described as a three-dimensional unbranched fold (digitiform in section) covered distally by a simple epithelium and proximally by a pseudostratified columnar epithelium (Figure 2). The stalk region in each of the two adult telencephalic plexuses, as in the myelencephalon, is lined by a single layer of low columnar cells (Figure 3).

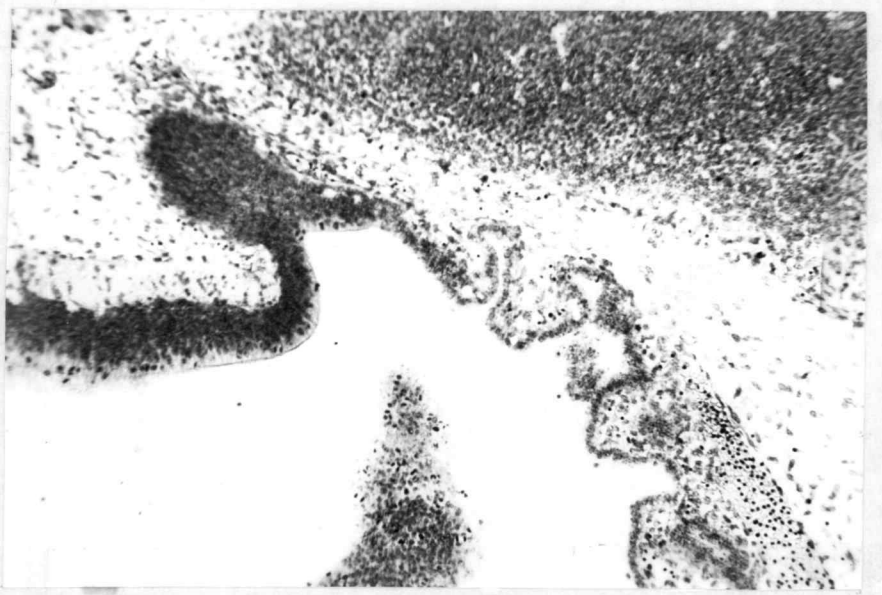
The choroid plexus of the third ventricle is the last of the three to make its appearance, and does so on day 13 of gestation. Early, its rudiment consists of numerous shallow folds of the thin roof of the third ventricle (Figures 4, 5). Later, these folds deepen to slender sacculations (Figure 5). In contrast to the paired plexuses of the telencephalon, and the plexus of the myelencephalon, that of the diencephalon lacks a pseudostratified columnar epithelium and is covered instead by a simple epithelium.

2. CHANGES OF THE EPITHELIAL CELLS DURING DEVELOPMENT: Throughout gestation and early post natal life the pseudostratified columnar epithelium, which lines the stalk region of the myelencephalic plexus, steadily decreases in both thickness and extent; so that at 56 days post partum this epithelium comes to occupy only that very small region where it merges with the neural epithelium proper. At some undetermined time between day 56 post partum and one year, the stalk region epithelium is transformed from a pseudostratified columnar into a simple low columnar type of epithelium whose component cells measure

Figure 3. The stalk region of the telencephalic choroid plexus of the lateral ventricle in the adult hamster. (x450).

Figure 4. The anlage of the diencephalic choroid plexus in the 13-day embryo. (x100).





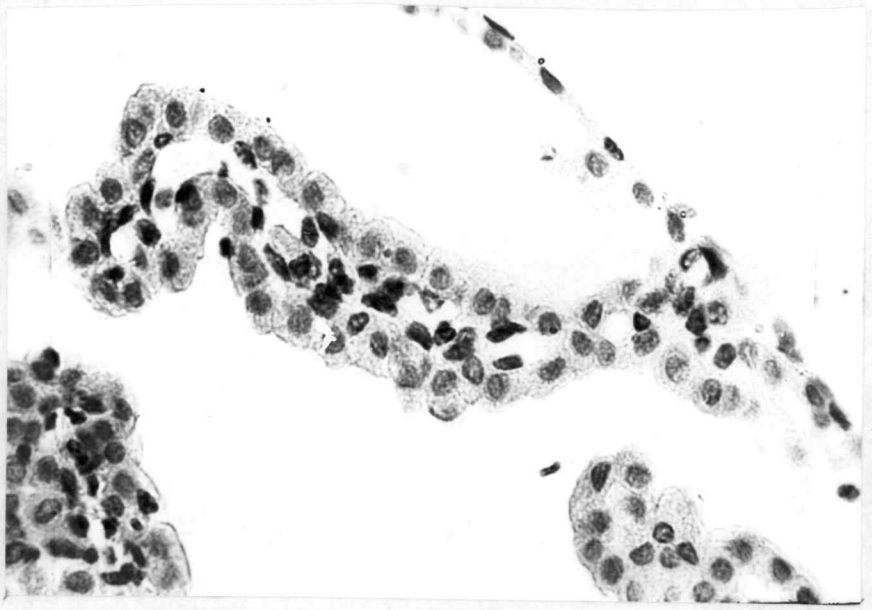
about 11u in height and 6u in width as seen in sectioned material.

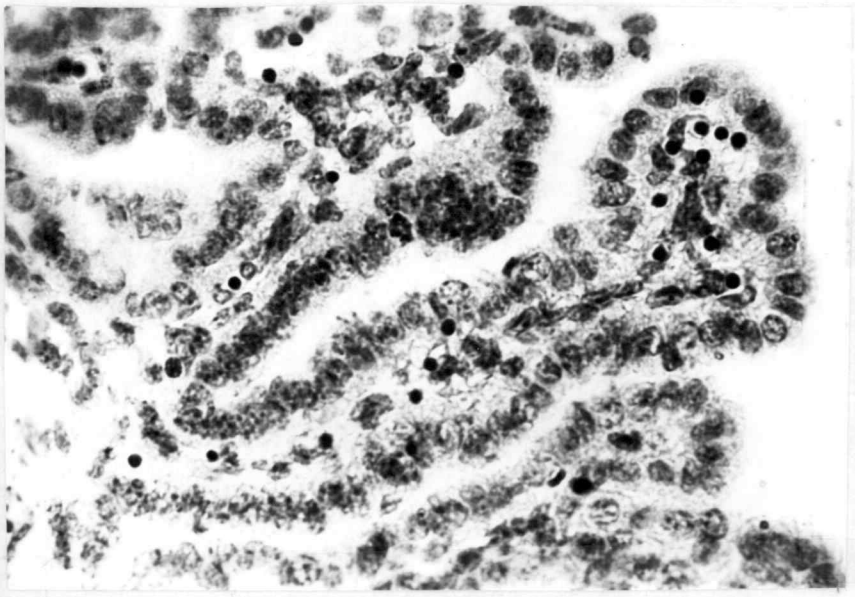
The lining epithelial cells of the folded area undergo shape changes during development from the tall columnar, to the cuboidal type. The epithelial cells covering the proximal halves of the folds are low columnar in the early portion of gestation (13u x 7u), and then gradually decrease in height to become cuboidal at the end of the embryonic period (11u x 11u). See Figure 6. In embryos of 11 through 13 days of gestation the component epithelial cells of the distal portions of the folds are high columnar (18u x 7u), but thereafter these cells become low columnar (12u x 7u) and remain thus to term. In both the neonatal and adult hamster, the epithelial cells of the proximal halves of the folds are cuboidal (8u x 8u to 7u x 10u) in shape, but those cells lining the distal portions are low columnar (10u x 8u) until approximately 28 days post partum when they become cuboidal (7u x 10u to 10u x 10u).

The epithelial portion of the telencephalic choroid plexuses in the 11-day embryo consists entirely of a pseudostratified columnar epithelium varying from 20u to 40u in thickness. Although in the 12-day embryo these primordia remain covered predominantly by a pseudostratified epithelium, yet in scattered areas in the distal portions of these anlagen, the epithelium makes a transition to a simple tall columnar type (18u x 6u). See Figure 7. This simple tall columnar condition of these restricted areas of the epithelium persists on day 13, but beginning on day 14 of conception, the distal portions of the primordia are covered completely by this simple columnar

Figure 5. A fold of the diencephalic choroid plexus in the two-week old hamster. (x450)

Figure 6. Folds of the myelencephalic choroid plexus in the 13-day embryo. A mitotic figure is seen in an epithelial cell of the basal half of one of the folds. (x450).





epithelium. This transformation from a pseudostratified to a simple epithelium which proceeds disto-proximal, also occurs in the developing telencephalic choroid plexuses of the human (Kappers, 1958; Shuangshoti and Netsky, 1966), of the rabbit (Tennyson and Papas, 1964), and of the chick (Smith, 1966). Throughout the remainder of gestation and during neo-natal life, the stalk region epithelium, like that of the myelencephalic plexus, decreases progressively in both extent and thickness. Also, as in the myelencephalic plexus, the pseudostratified columnar epithelium of the stalk region transforms into a simple low columnar epithelium (11u x 6u) sometime between the 56th day post partum and one year.

During the embryonic and neo-natal periods the epithelial cells of the distal portions of the telencephalic choroid plexuses undergo a sequential change in shape from pseudostratified columnar (20u - 40u in thickness) on day 11, to tall columnar (18u x 6u) on days 12 and 13, to low columnar (10u x 8u) on day 14 of gestation to day 12 postnataly and finally to cuboidal (8u x 8u; 7u x 10u; 10u x 10u; 9u x 12u) thereafter.

The epithelial portions of the dienecephalic choroid plexus undergo similar sequential changes in shape as those of the folded area of the myelencephalic plexus. The epithelial cells of both proximal and distal portions of the folds change in shape from high columnar (13u x 7u and 17u x 6u respectively) on days 13 and 14, to cuboidal (8u x 8u) and low columnar (12u x 8u) respectively on day 7 post partum, and finally to cuboidal (8u x 8u; 7u x 10u) thereafter

(Figures 4, 5).

3. "VESICULAR" CELLS OF THE DEVELOPING TELENCEPHALIC PLEXUS: Klosovski (1963), in an investigation of the choroid plexuses of human embryos, described what he termed "vesicular" cells in the epithelium of the telencephalic plexuses. These cells were characterized as having a "....well defined, thin layer of cytoplasm, resembling a cell membrane, and the presence of a huge mass of cytoplasm...., not staining by the usual methods" (p. 9). These vesicular cells were found only in the telencephalic plexuses, and not at any time during the ontogenesis of the diencephalic and myelencephalic plexuses. Similar cell types have been described in the cat (Purin, 1963) and human (Shuangshoti and Netsky, 1966) telencephalic plexuses.

Cells similar to those described above are seen in the distal portions of the telencephalic plexuses of the hamster on days three through five post partum. The cytoplasm of these cells either fails to stain at all, or at most very lightly with hematoxylin and eosin. The cytoplasm of these cells appears very foamlike. Also, a great deal of "secretion material" was noted in the ventricle near the apical surfaces of these cells. Klosovski (1963) also noted what he termed "....the products of the secretory activity of the cells" (p. 11) on their luminal surfaces. With reference to the hamster, similar "vesicular" cells were found but restricted solely to the telencephalic plexuses.

4. GLYCOGEN IN THE EPITHELIUM OF THE EMBRYONIC AND NEO-NATAL PLEXUSES: Table I summarizes the first appearance, persistence, relative amounts, location, and times of disappearance of glycogen in the epithelial cells of the developing, neo-natal and adult choroid plexuses of the hamster. Definitions of the several terms used to designate the relative amounts of glycogen are presented in Appendix I. It is of interest to note that at no time in the embryonic, neo-natal, or adult animal was glycogen detectable in the neural epithelial cells proper which line the first, second, third, and fourth ventricles. This observation is in keeping with those of Askanazy (1914), Goldman (1913), and Kappers (1958) for man. Returning to the hamster, glycogen was present in the epithelial cells of the posterior membranaceous area of the fourth ventricle throughout gestation and in neo-natal life to day 11.

B. Mitotic activity of the epithelial and connective tissue cells.

1. MYELENCEPHALIC CHOROID PLEXUS (EPITHELIAL CELLS): Table II and Figure 9 demonstrate that the proliferative activity of the stalk region epithelial cells is high (18-36%) throughout gestation as well as during the first few days following parturition, and that it gradually decreases until 35 days post partum when it reaches a low of 0 - 0.5%, a level retained in the adult.

Beginning on day 13 (two days after the appearance of an anlage of the folded area), the mitotic index of the epithelium of the proximal halves of the individual component convolutions was

Table I

Glycogen in the epithelial cells of the embryonic, neo-natal, and adult choroid plexuses of the hamster.

AGE	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 1pp	Day 2pp	Day 3pp	Day 5pp	Day 8pp	Day 11pp	Day 13pp	Day 15pp	Day 17pp	Day 19pp	Day 28pp	Adult
MYELENCEPHALIC PLEXUS																			
Stalk area	NP	M(b)	M(b)	L(b)	L(b)	L(b)	NP	NP	NP	NP	NP	NP	NP	NS	NS	NS	NS	NS	NP
Folded area																			
Proximal half of fold	NP	L(a)	L(a)	L(a)	L(a)	L(a)	L(a)	L(a)	L(a)	L(a)	L(a)	L(a)	L(a)	NP	NP	NP	NP	NP	NP
Distal half of fold	NP	L(b)	M(b)	M(b)	A(t)	L(a)	L(b)	L(b)	L(b)	L(b)	L(b)	L(b)	L(b)	Mn(b)	NP	NP	NP	NP	NP
TELENCEPHALIC PLEXUS																			
Stalk area	NA	Mn(b)	Mn(b)	Mn(b)	Mn(b)	Mn(b)	Mn(b)	NP	NP	NP	NP	NP	NS	NS	NS	NS	NS	NS	NP
Distal fold	NA	NA	Mn(b)	M(b)	A(t)	L(b)	L(b)	L(b)	M(b)	L(b)	L(b)	L(b)	L(b)	Mn(b)	Mn(b)	Mn(b)	Mn(b)	NP	NP
DIENCEPHALIC PLEXUS																			
Proximal half of fold	NA	NA	NA	L(t)	L(b)	L(b)	L(b)	Mn(b)	Mn(b)	Mn(b)	Mn(b)	NP	Mn(b)	NP	NP	NP	NP	NP	NP
Distal half of fold	NA	NA	NA	L(t)	L(b)	L(b)	L(b)	Mn(b)	Mn(b)	Mn(b)	Mn(b)	NP	Mn(b)	NP	NP	NP	NP	NP	NP

Code: A, Abundant; M, Moderate; L, Low; Mn, Minimal
NP, Not present; NA, Not applicable; NS, Not studied
(a), apical portion of cell; (b), basal portion of cell; (t), throughout cell; pp, post partum

Table II

Mitotic indices of the epithelial cells of the developing, neo-natal and adult choroid plexuses of the hamster.

	MYELENCEPHALIC			TELENCEPHALIC		DIENCEPHALIC	
Age	Stalk	Basal half of fold	Distal half of fold	Stalk	Fold	Basal half of fold	Distal half of fold
10	NC	NC	NC	NA	NA	NA	NA
11	36.03	37.50	40.00	40.33	33.31	NA	NA
12	32.20	30.55	34.26	28.22	25.13	NA	NA
13	31.43	45.71	5.26	19.56	3.80	33.61	7.69
14	18.82	19.44	6.30	27.41	3.21	46.83	19.40
15	34.93	32.41	7.77	20.31	2.48	20.00	12.07
16	20.79	20.37	10.00	20.64	6.76	15.75	9.92
1pp	28.36	13.97	6.10	25.65	0.21	13.97	0.66
2pp	22.22	5.62	0.22	17.50	0.22	10.03	0.89
3pp	14.74	6.48	1.53	7.49	1.61	9.42	1.96
5pp	9.71	2.02	0.37	10.82	0.99	0.49	0.28
8pp	12.31	0.84	0.38	5.82	1.66	0.47	0.98
11pp	4.45	0.93	0.19	10.29	0.99	0.27	0.19
13pp	10.00	0.10	0.09	3.28	0.45	NC	NC
15pp	6.30	0.29	0.09	0.19	0.00	0.19	0.19
17pp	5.40	0.19	0.93	0.00	0.49	0.67	0.18
19pp	NC	0.42	0.38	0.09	0.00	NC	NC
27pp	1.27	0.29	0.49	0.39	1.72	0.99	0.83
35pp	0.55	0.49	0.00	NC	0.19	0.00	0.00
39pp	0.09	0.09	0.00	0.00	0.00	0.94	0.04
56pp	NC	0.00	0.00	NC	0.00	0.00	0.00
adult	0.00	0.11	0.00	0.00	0.00	0.22	0.00
adult	0.00	0.00	0.17	0.14	0.09	0.00	0.00
adult	0.20	0.14	0.00	0.00	0.00	0.31	0.11

NA, not applicable
NC, not counted
pp, post partum

Table III

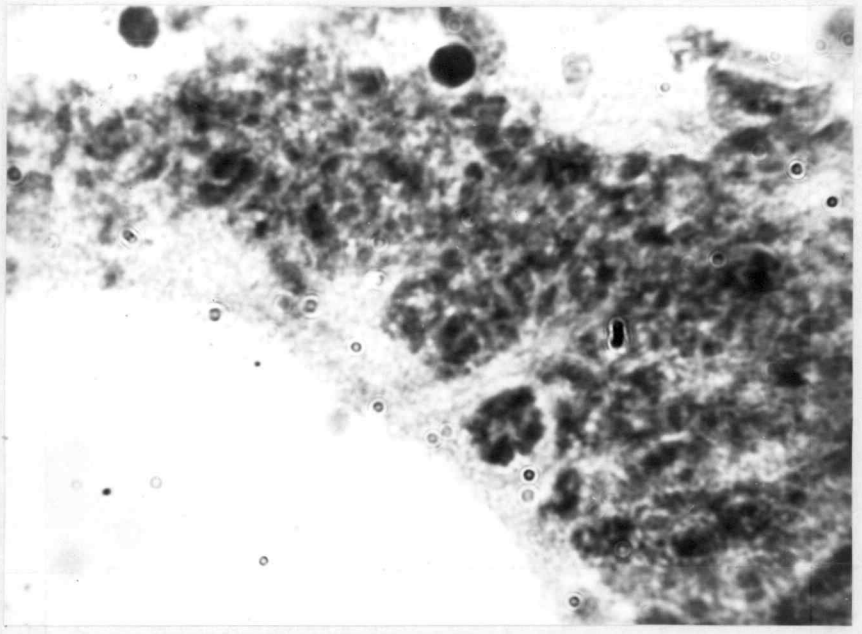
Mitotic Indices of the connective tissue cells of the developing, neo-natal and adult choroid plexuses of the hamster.

	MYELENCEPHALIC		TELENCEPHALIC		DIENCEPHALIC	
Age	Stalk Region	Folded Area	Stalk Region	Fold	Proximal half of fold	Distal half of fold
10	NC	NC	NA	NA	NA	NA
11	35.44	34.37	48.31	32.22	NA	NA
12	32.27	14.74	35.61	45.28	NA	NA
13	19.16	38.76	23.28	20.79	28.57*	28.57*
14	22.22	19.21	27.31	29.22	30.07*	30.07*
15	22.80	19.29	18.11	16.21	26.72	24.37
16	19.55	21.94	9.03	16.41	12.56	17.46
1pp	22.78	18.57	22.28	18.28	12.01	20.63
2pp	24.39	11.26	13.05	14.81	14.19	20.03
3pp	14.83	7.46	20.23	10.40	11.66	10.31
5pp	7.75	3.85	8.29	9.77	8.67	4.31
8pp	6.11	3.00	6.29	7.54	7.10	8.66
11pp	2.03	7.92	0.44	6.17	1.01	2.33
13pp	2.91	5.00	0.91	1.66	NC	NC
15pp	0.86	1.98	0.00	0.29	0.99	1.01
17pp	0.72	0.19	0.09	0.00	1.21	0.66
19pp	NC	1.01	0.00	0.13	NC	NC
27pp	0.11	0.98	2.83	0.38	0.56	0.69
35pp	0.41	0.88	NC	0.22	0.33	0.09
39pp	0.08	0.37	0.00	0.74	0.11	0.31
56pp	NC	0.72	NC	0.53	0.05	0.13
adult	0.00	0.00	0.17	0.00	0.00	0.22
adult	0.17	0.30	0.00	0.00	0.33	0.00
adult	0.09	0.08	0.00	0.17	0.00	0.24

NC, not counted
NA, not applicable
pp, post partum
*, mitotic index of connective tissue cells in entire fold.

Figure 7. A portion of the distal half of the telencephalic choroid plexus of the right lateral ventricle in the 13-day embryo. Tritium-labeled cells are present in both the epithelium and stroma. (x970).

Figure 8. The anterior portion of the stalk region of the myelencephalic choroid plexus in the 13-day embryo. A mitotic figure is present near the luminal surface of this epithelium. (x970).



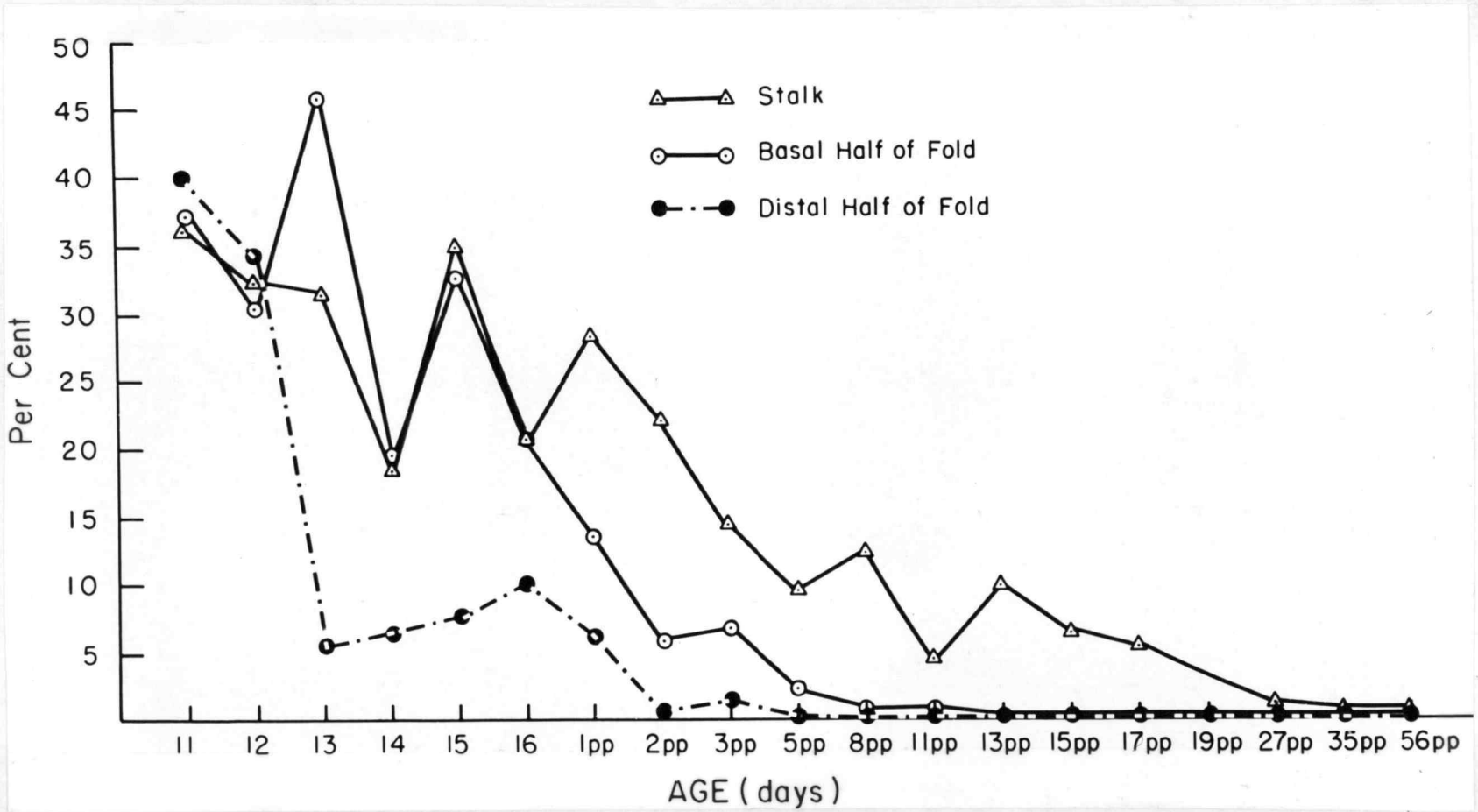


Figure 9. Mitotic indices of the epithelial cells of the myelencephalic choroid plexus in embryonic (days 11-15) and postpartum (pp) hamsters.

consistently higher than that obtaining in the distal halves, until day 13 post partum. Thereafter these indices become about equal and persist as such in the adult animal (Table II, Figure 9).

The mitotic activity of the epithelial cells within the proximal portions of the folds is variable but high (19-45%) during embryonic life (Figure 15). Following parturition it steadily decreases until five days post partum whereafter it remains very low (0-.9%). The proliferative activity of the epithelial cells of the distal halves of the folds decreases sharply on the thirteenth day of gestation and then remains at a low level (5-10%) until two days post partum. At this time it falls to a new low of .09-1.5% where it remains. In the adult plexus, mitotic activity of the epithelial cells of both the proximal and distal halves of the folds is very low (0-.3%).

2. TELENCEPHALIC CHOROID PLEXUS (EPITHELIAL CELLS): Mitotic activity in stalk region epithelial cells is irregular and high (17-40%) until two days post partum (Table II, Figure 10), when it falls progressively until the fifteenth day after birth when it drops to a new and permanent low of 0.19%.

Clusters of epithelial cells containing nuclei labeled with H^3-thymidine were scattered throughout the epithelium lining the distal portion of this plexus, but no mitotic figures were evident. Proliferative activity is high (25%) in these cells on day 12 of gestation but falls sharply on day 13 to 3.8% and persists at a low level to term (Table II; Figures 7, 11). In both the neo-natal and

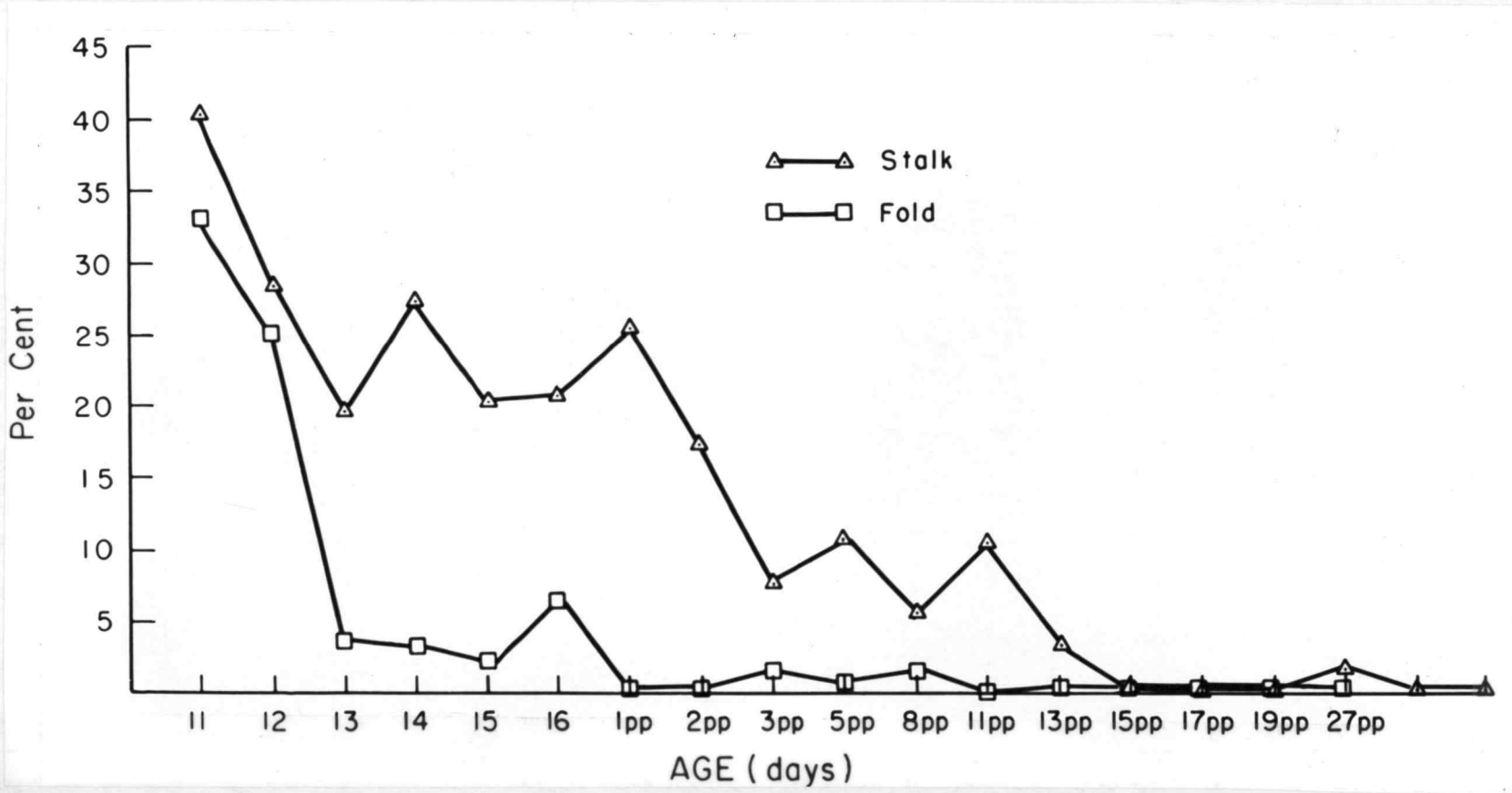


Figure 10. Mitotic indices of the epithelial cells of the telencephalic choroid plexus in embryonic (days 11-15) and postpartum (pp) hamsters.

adult plexus mitotic activity of these cells is very low (0 - 1.7%). See Table II and Figure 11.

3. DIENCEPHALIC CHOROID PLEXUS (EPITHELIAL CELLS): Throughout pregnancy and neo-natal life mitotic activity of the epithelial cells of the proximal halves of the folds is consistently higher than that of the epithelial cells of the distal portions of the folds (Table II, Figure 11). Mitotic activity in the epithelium of the proximal and distal portions of the folds stands at 33.6 and 7.6% respectively on day 12 of gestation. By day 13, it has risen to 46.8 and 19.4% respectively. During the remainder of gestation as well as during the first few postnatal days, it falls steadily until it reaches a low of 0.6% in the distal epithelium (day 1 post partum) and in the proximal epithelium a low of 0.5% (day 5 post partum). From day 5 into adulthood the index for both epithelial portions remains within a range of 0 - 0.9%.

4. CONNECTIVE TISSUE CELLS OF THE PLEXUSES: In all of the plexuses the highly vascularized stroma is loose in appearance throughout gestation (Figures 1, 2, 4, 7, 12, 15). Numerous mitotic figures were noted in the mesenchymal cells of the stroma, and mitotic indices were correspondingly high (16 - 48%) (Tables III, Figures 12, 13, 14). At the beginning of the neo-natal period the stroma is still loosely organized, but with further development, it becomes progressively more compact. Proliferative activity of the stromal mesenchymal cells in all of the plexuses is greatest on the first few days

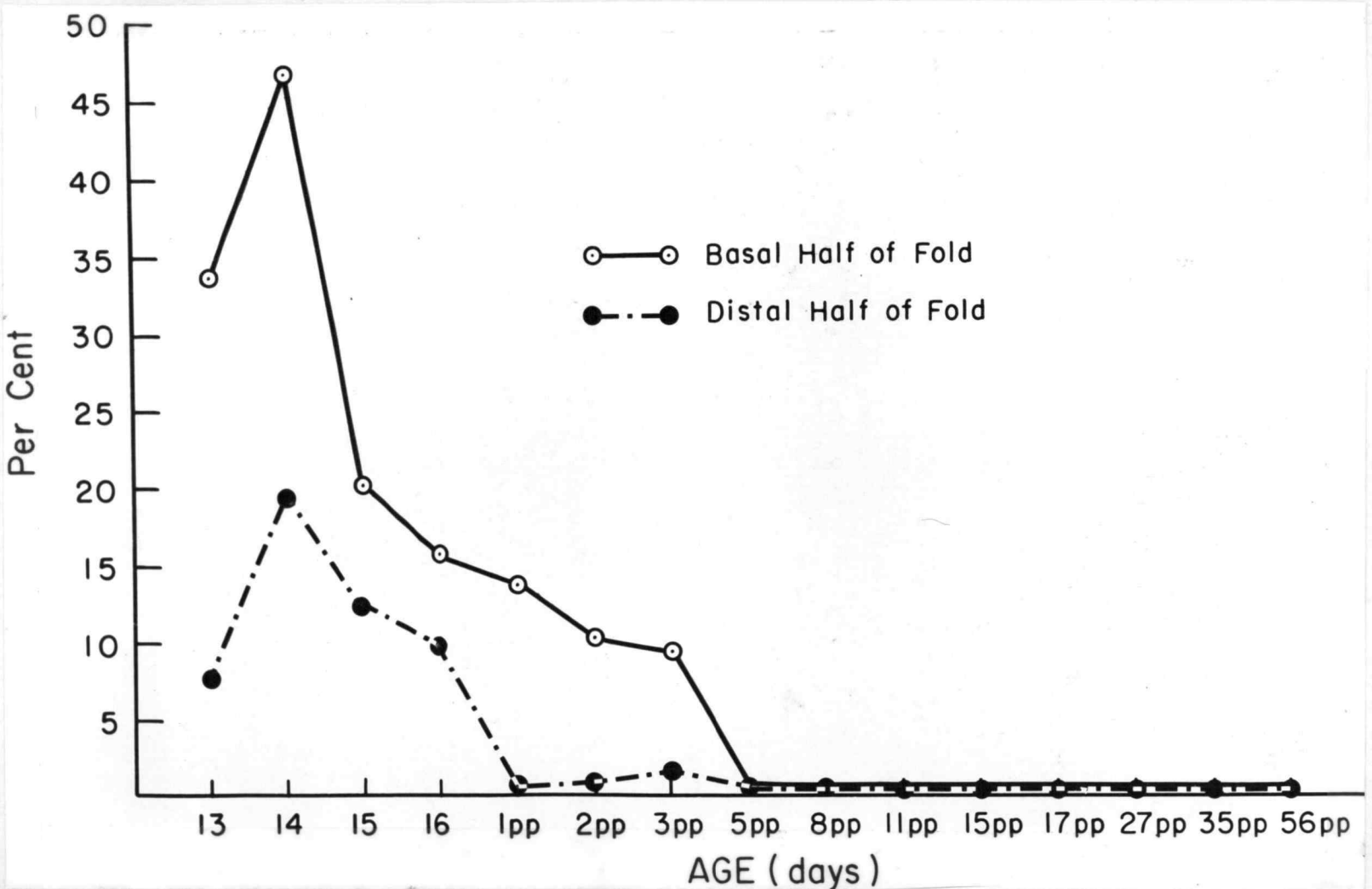


Figure 11. Mitotic indices of the epithelial cells of the diencephalic choroid plexus in embryonic (days 13-15) and postpartum (pp) hamsters.

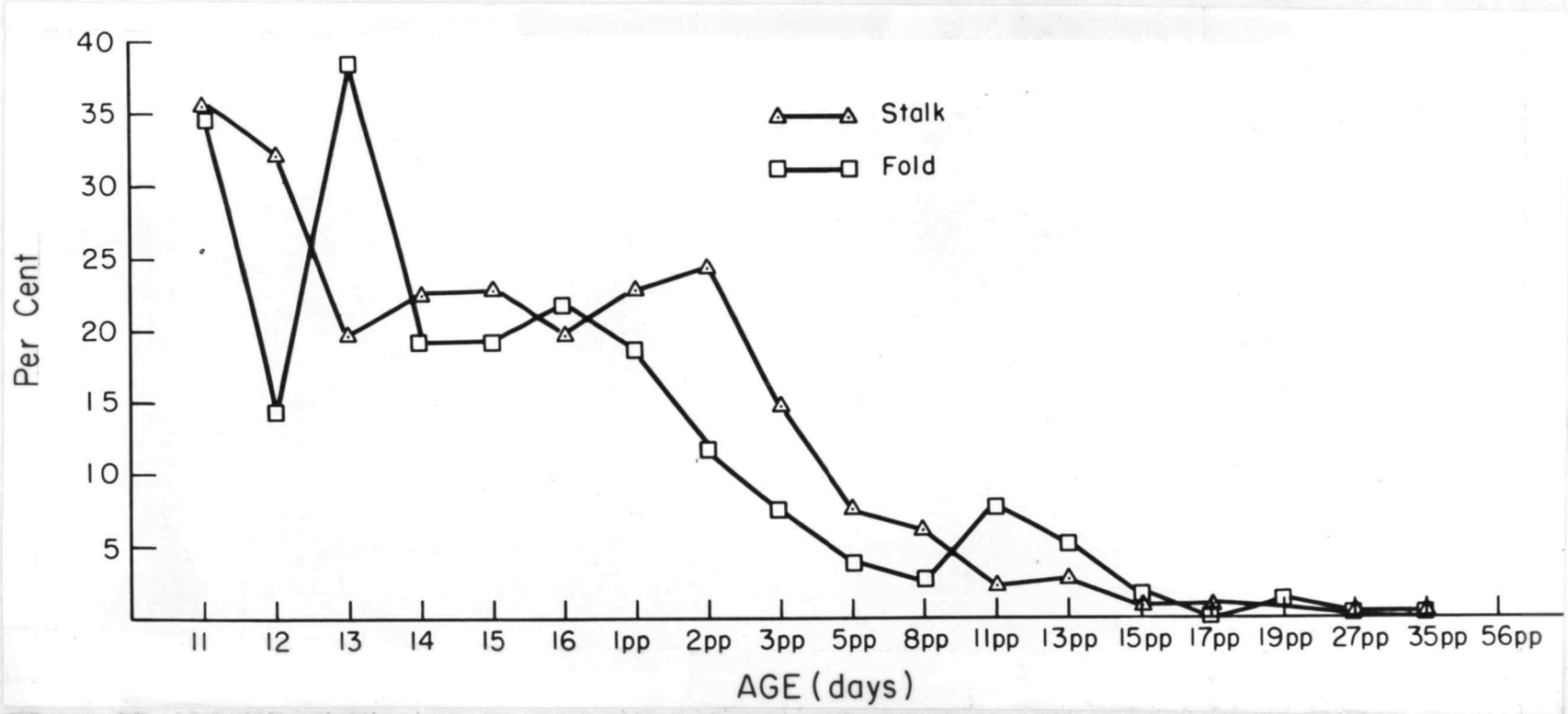


Figure 12. Mitotic indices of the connective tissue cells of the myelencephalic choroid plexus in embryonic (days 11-15) and postpartum (pp) hamsters.

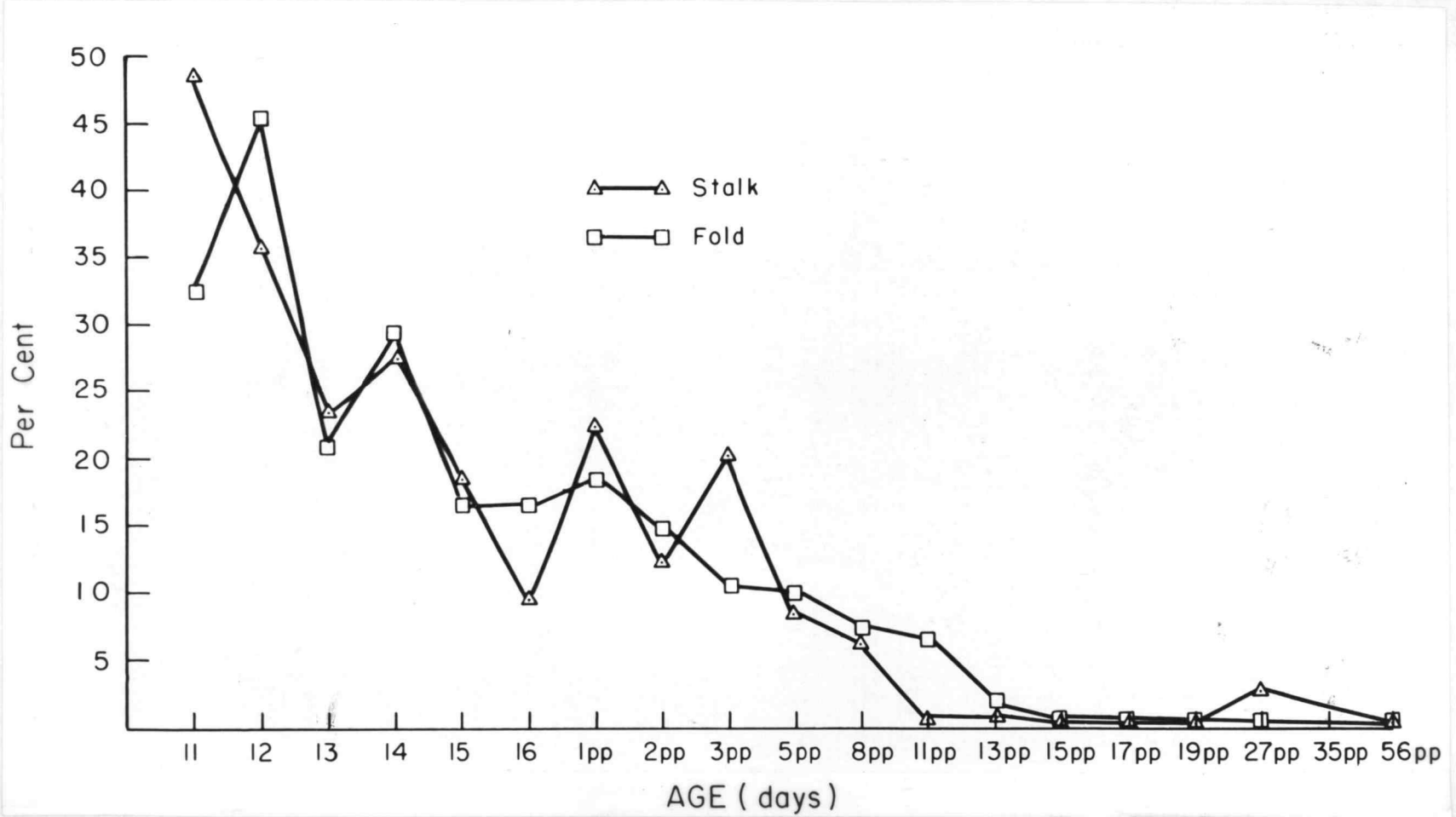


Figure 13. Mitotic indices of the connective tissue cells of the telencephalic choroid plexus in embryonic (days 11-15) and postpartum (pp) hamsters.

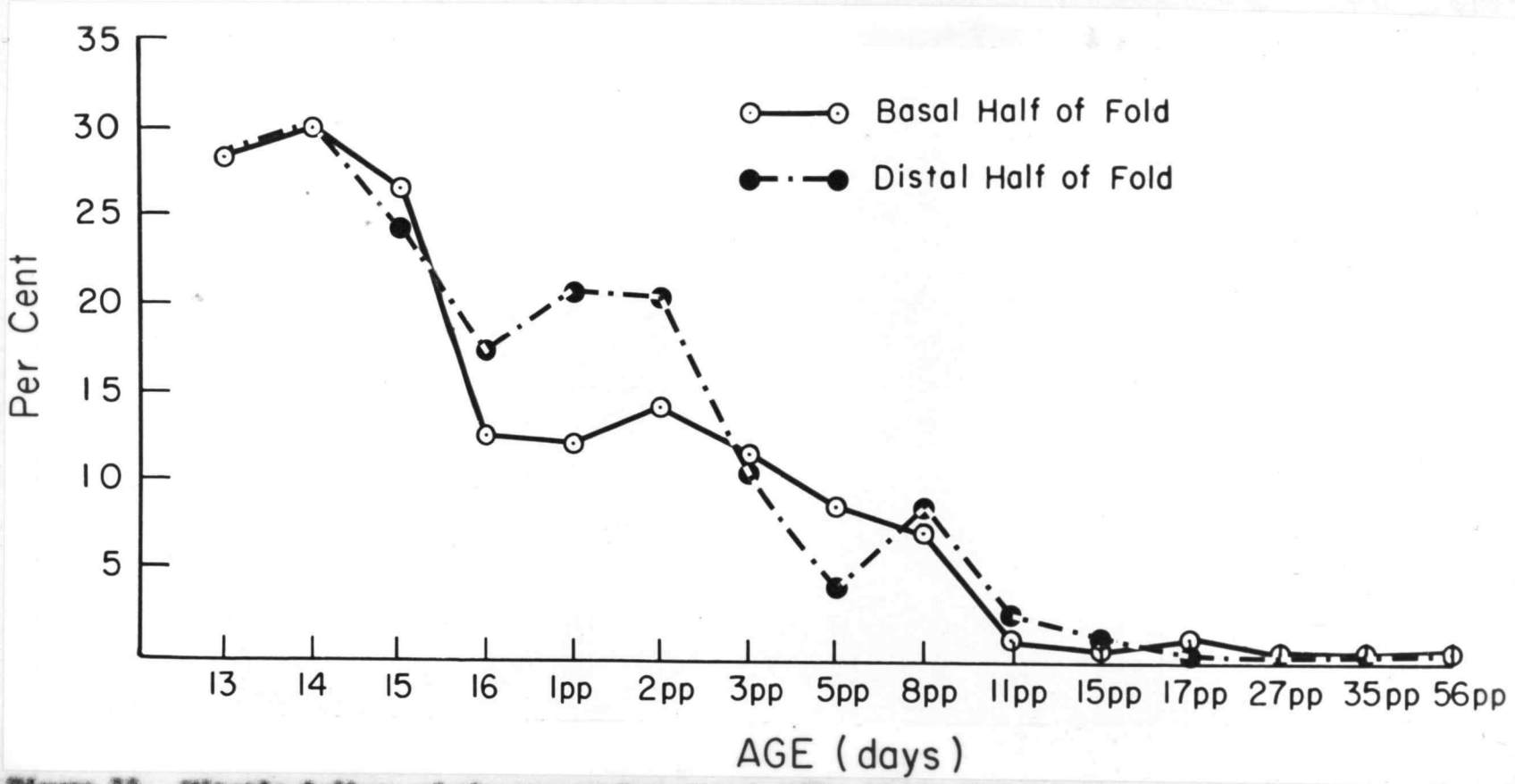
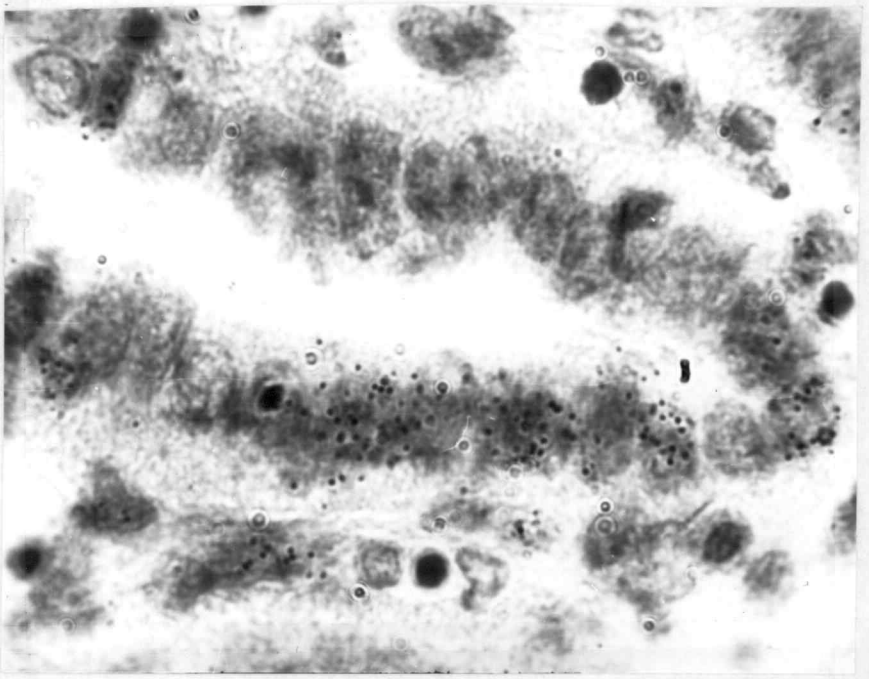


Figure 14. Mitotic indices of the connective tissue cells of the dienecephalic choroid plexus in embryonic (days 13-15) and postpartum (pp) hamsters.

Figure 15. Epithelial cells labeled with H^3-thymidine in the basal half of one of the folds of the myelencephalic choroid plexus in the 13-day embryo. (x970).



following birth (7 - 24%) after which it steadily falls until approximately two weeks post partum to a low of 0 - 1.0% (Tables III, Figures 12, 13, 14).

Very few connective tissue cells are present in the adult plexuses. A few very flat, and darkly staining nuclei were scattered throughout the very compact stroma. Although no mitotic figures were observed, autoradiographic procedures detected a low mitotic index of 0.3% (Table III).

5. NUCLEI OF THE EPITHELIAL CELLS OF THE DEVELOPING PLEXUSES: Throughout embryonic and early post natal life the nuclei of the epithelial cells of the stalk regions (pseudostratified) of both the telencephalic and myelencephalic plexuses are elongate and the bulk of them are confined to the basal two-thirds of this epithelium (Figures 1, 2, 8). All mitotic figures are limited to those nuclei adjacent to the luminal surface of this epithelium, and have a random distribution (Figures 1 and 8). This epithelium (simple columnar) in the adult plexuses contains round, either basally or centrally located nuclei. Throughout gestation and early post-natal life the nuclei of the epithelial cells of the distal portions of these plexuses and of those of the diencephalic plexus are large, round, basally or centrally located, and contain a loose network of chromatin. The nuclei thereafter remain either round or oval and either basally or centrally located but become progressively smaller and darker staining.

A few mitotic figures were seen in the epithelium lining the distal portions of the diencephalic and myelencephalic plexuses, but

they were restricted to those cells in the proximal halves of the folds (Figure 6). Mitotic figures were never observed in the single-layered epithelium covering the distal portions of the telencephalic plexus.

DISCUSSION

It is of interest to note that, as based on the literature, a great deal of both intra- and interspecific variation must exist in regard to the times of appearance of the anlagen of the choroid plexuses in various mammals (Table IV). In the Tamandua, mouse, rabbit, man, skunk, pig, cow, and deer the primordium of the myelencephalon plexus is the first to appear, followed by that of telencephalic, and this in turn by that of the diencephalon (Table Part A). The same sequence occurs in the hamster.

The developing telencephalic plexus of the rat and guinea pig (Cohen and Davis, 1938), of man (Kappers, 1958; Klosovski, 1963; Shuangshoti and Netsky, 1966), of the cat (Purin, 1963), and of the rabbit (Tennyson and Pappas, 1964), has been described and illustrated as being greatly lobulated and containing an abundant connective tissue stroma. By comparison, in the hamster this plexus presents the configuration of a slender unbranched fold with a scant connective tissue stroma. The telencephalic plexus of the hamster may be morphologically similar to that of the mouse in view of the following comment of Knudsen (1964):

> "....the choroid plexus in the lateral ventricles of the mouse is well suited to growth studies on account of its regular form and modest size....whilst the condition of growth in more complex plexuses of larger animals or humans are perhaps less clear on account of the irregular surface" (p. 179).

Tennyson and Pappas (1964) working on the rabbit, and Shuangshoti and Netsky (1966) working on man, have described the stalk regions of the telencephalic and myelencephalic plexuses as being lined by a

Table IV

Sequence of the times of appearance of the anlage of the choroid plexuses of mammals (from the literature).

A. Myelencephalic (1st); telencephalic (2nd); diencephalic (3rd).
1. Tamandua (Krabbe, 1939a)
2. skunk (Krabbe, 1939c)
3. cow (Krabbe, 1939c)
4. deer (Sakurai, 1906)
5. pig (Weed, 1917)
6. rabbit (Cohen and Davies, 1938)
7. mouse (Rugh, 1964)
8. man (Kappers, 1958; Shuangshoti & Netsky, 1966).

B. Telencephalic (1st); myelencephalic (2nd); diencephalic (3rd).
1. Norway rat (Henneberg, 1937)
2. rabbit (Strong, 1956; Tennyson & Pappas, 1964)
3. man (Kollmann, 1861)

C. Telencephalic (1st); diencephalic (2nd); myelencephalic (3rd).
1. Galeopithecus volans (Krabbe, 1939a)

D. Telencephalic and myelencephalic (1st - at same time); diencephalic (2nd).
1. rat (Cohen & Davies, 1938)
2. mouse (Krabbe, 1939b)
3. guinea pig (Cohen & Davies, 1938)
4. rabbit (Minot & Taylor, 1905)
5. Tarsius (Hubrecht & Keibel, 1907)

E. Myelencephalic (1st); telencephalic and diencephalic (2nd - at same time).
1. Lobodon (Krabbe, 1939c)

F. Telencephalic and myelencephalic before diencephalic (the sequence of the former plexuses was not indicated).
1. Echidna (Krabbe, 1939a)
2. Spermophilus (Krabbe, 1939c)
3. Microcebus (Krabbe, 1939c)
4. Bradypus (Krabbe, 1939c)
5. Dasypus (Krabbe, 1939c)

G. Telencephalic before myelencephalic and diencephalic (the sequence of the latter plexuses was not indicated).
1. Talpa (Krabbe, 1939a)
2. Ericulus (Krabbe, 1939a)
3. Vespertilio (Krabbe, 1939a)

H. Telencephalic (1st); myelencephalic (2nd); diencephalic not mentioned.
1. Spermophilus (Volker, 1922)

pseudostratified columnar epithelium in the embryo, the newborn, and the adult. Opalski (1930) who studied the adult human telencephalic plexus, stated that its stalk region was lined by a "multilayered" epithelium. In the hamster, a pseudostratified columnar epithelium does indeed line the stalk regions of the telencephalic and myelencephalic plexuses during intrauterine and neo-natal life, but in the adult animal, they are lined instead by a simple columnar epithelium (Figure 3). This transformation occurs sometime between postnatal day 56 and one year.

Although Shuangshoti and Netsky (1966) could always find areas of stratified or pseudostratified epithelium in the distal portions of all developing and post-natal plexuses of man, no such areas were noted in the hamster diencephalic or myelencephalic plexuses during comparable stages. But such areas were present in the distal portion of the telencephalic plexus on days 12 and 13 of gestation, and beginning with day 14 it becomes completely lined by a simple epithelium. These observations on the hamster are consistent with those of Kappers (1958) and Klosovski (1963) for man, of Tennyson and Pappas (1964) for the rabbit, of Purin (1963) for the cat, and of Smith (1966) for the chick.

In 1958 Kappers remarked:

> "....although no special staining methods, usually employed in studies of blood morphology, were used the cytological pictures highly suggests that during the first phase of its histogenetic development the stroma of the telencephalic plexus gives origin to very different kinds of blood cells...." (p. 6).

However, Tennyson and Pappas (1964) in an electron miscroscopic study of the developing telencephalic and myelencephalic plexuses of the rabbit, found almost all the immature blood cells to be intravascular and therefore did not consider the plexus to be a haemopoetic organ. The results of the present investigation on the hamster relative to the location of immature blood cells (both labeled and non-labeled), are similar to those reported by Tennyson and Pappas (1964) for the rabbit.

Many authors have observed glycogen in the epithelium of the developing mammalian choroid plexuses. In man (Loeper, 1904; Klestadt, 1912; Askanazy, 1914; Gage, 1917; Weed, 1917; Sundberg, 1924; Kappers, 1958; Klosovski, 1963; Shuangshoti and Netsky, 1966), and pig (Weed, 1917), it is present only during intrauterine life. This epithelium however contains glycogen during both embryonic and early post-natal life in the mouse (Goldmann, 1913; Kappers, 1958), in the rat (Goldmann, 1913; Cancilla et al., 1966; Schachenmayr, 1967), in the cat (Marinesco, 1928; Purin, 1963), and in the rabbit (Tennyson and Pappas, 1961, 1964). The latter observation is similar to that made in the hamster.

In the hamster these glycogen stores disappear first from the epithelium lining the diencephalic plexus (day 13 pp), then from that of the myelencephalic plexus (day 15 pp), and finally from that of the telencephalic plexus (day 28 pp). This sequence is similar to that reported by Purin (1963) for the cat, and by Klosovski (1963), and Shuangshoti and Netsky (1966), for man, although in man glycogen

is lost before term. These same authors also observed that glycogen was most abundant (visual assessment) in the epithelium of the telencephalic plexus, and least plentiful in that of the diencephalic plexus. The same applies to the hamster.

Although the epithelial cells of the pig (Gage, 1917), of man (Kappers, 1958; Klosovski, 1963), and of the cat (Purin, 1963), have been observed to be filled almost completely with glycogen at certain times, the same cannot be said for the hamster. At those times of greatest abundance, glycogen filled approximately one-half of the cells of the hamster epithelium. The glycogen was commonly basally located within individual cells. Perhaps the comparatively shorter length of gestation of the hamster (16 days) may account for the smaller glycogen stores.

Many investigators (Meek, 1907; Weed, 1917; Kappers, 1958; Klosovski, 1963; Tennyson and Pappas, 1964) have noted sequential changes in the position of the nucleus of the epithelial cell during ontogenesis (central, to apical, to central or apical, to basal or central, and finally to basal) and related this phenomenon in turn to changes in the glycogen content of the cell. In the hamster whose plexuses appear to contain comparatively less glycogen, most of the nuclei of the epithelial cells are always either basal or central. However, apically located nuclei were noted in the few cells that contained an abundant amount of glycogen. Whether or not these changes in nuclear position are related to the glycogen content of the cells is still questionable in view of the observations of

Smith (1966). Although she did not discuss this topic, she did describe similar changes in the position of the nuclei in the epithelial cells of the chick telencephalic plexus, even though these cells contain no glycogen during development. Perhaps these changes in nuclear position may be related to the secretory activities of the developing plexuses. Lastly, it may be added that although several different hypotheses (Kappers, 1958; Purin, 1963; Shuangshoti and Netsky, 1966) have been advanced in an attempt to explain the role of these glycogen stores during embryonic and early post-natal life, their true functional significance remains unknown.

Although glycogen has been detected in the epithelial cells of the adult plexuses of man (Jacob, 1924), of the mouse, rat, and rabbit (Shimizu and Kumamoto, 1952), of the cat (Purin, 1963), and of hibernating mammals such as the hedgehog, Erinaceus europaeus and the bat, Myotis sips (Okache, 1958), no glycogen was noted in the epithelium lining the adult hamster plexuses. This observation in the hamster is in keeping with that of Goldmann (1913) for the rat, of Wislocki and Dempsey (1948) for the rhesus monkey, of Kappers (1958), and Shuangshoti and Netsky (1966) for man, of Tennyson and Pappas (1961) for the rabbit, and of Shantha et al., (1967) for the squirrel monkey.

Many authors have reported no or at most, only a very few mitotic figures in the epithelium of developing mammalian choroid plexuses. Neither Boyd (1958) nor Kappers (1958) were able to locate any mitotic figures in the epithelium lining the developing human

telencephalic plexuses. Zand (1930) was able to detect mitotic figures only in the epithelial cells at the region of junction of the choroid plexus and the neural epithelium proper in human embryos. Shuangshoti and Netsky (1966) noted only a few figures in the telencephalic plexuses of very young human embryos (7th week); in these the plexus is lined completely by a pseudostratified epithelium. Tennyson and Pappas (1964) also reported mitotic figures in only the stalk regions (pseudostratified) of the telencephalic and myelencephalic plexuses in rabbit embryos. Such figures were never observed in the "....more differentiated tip of the choroidal fold" (p. 386).

However Knudsen (1964), using Colcemid, observed numerous mitotic figures in the stalk regions of the telencephalic and myelencephalic plexuses, as well as in the epithelium lining the proximal portions of the folds of the myelencephalic and diencephalic plexuses of the mouse. In the hamster also, numerous mitotic figures were seen in the stalk region epithelium of the myelencephalic and telencephalic plexuses (Figure 8). However, they were seen only rarely in the epithelium lining the proximal halves of the folds of the diencephalic and myelencephalic plexuses (Figure 6). Both Knudsen (1964), and Tennyson and Pappas (1964), reported in the mouse and rabbit respectively, that connective tissue cells containing mitotic figures were found throughout the stroma. The same holds for the hamster.

In 1961 Miale and Sidman, using autoradiographic methods (thymidine-H^3), remarked that the developing mouse myelencephalic plexus

> ".... contained many labeled cells and mitotic figures in 11-, 13-, and 15-day embryos. The concentration of newly formed cells was diminished by 17 days and continued to decrease postnatally" (p. 291).

These authors however, did not indicate which cell types were labeled (epithelial or connective tissue cells), or the cell location (stalk, folded area, et cetera) in the plexus, being concerned specifically with the histogenesis of the cerebellum. In the present investigation epithelial cells labeled with tritiated thymidine were most commonly found in the stalk regions (pseudostratified) of the telencephalic plexuses, in the proximal portions of the folds of the diencephalic plexus, and in both the stalk region (pseudostratified) and proximal halves of the folds of the myelencephalic plexus. Labeled connective tissue cells were scattered throughout the stroma of the plexuses.

Both Kappers (1958) and Tennyson and Pappas (1964), being unable to find mitotic figures in the choroidal epithelium lining the distal portions of the telencephalic plexuses in the human and rabbit respectively, suggested that growth and extension of this epithelium occurs in the stalk region (pseudostratified). In 1964 Knudsen, using Colcemid, noted that in the mouse telencephalic plexus

> "....a broad peripheral zone of the surface nearest the free border is always completely without epithelial mitoses (on the average, 40 - 50% of the surface area) .....thus the epithelial cells seem to be formed in certain zones near the root and not diffusedly...." (p. 181).

However in the hamster, growth and extension of this epithelium appears to occur in both the stalk (pseudostratified) and distal portion (simple) of the plexus, although at a much slower pace in the latter. Proliferative activity was greatest in the stalk region both throughout

gestation and during the first few days post partum. Thereafter it progressively decreases until day 15, when it becomes very low and remains so. In the distal portion of the plexus, mitotic activity becomes minimal on day one post partum, and remains so. It is of interest to note that in the distal portion of the plexus the number of labeled cells, which occur in scattered groups, increases in those embryos taken from females injected with tritiated thymidine 24 and 48 hours prior to sacrifice (unpublished data). Since tritiated thymidine was no longer available in the circulation approximately three to four hours after its injection, the new labeled cells appearing between eight hours and two days and three days after injection must have arisen from division of those which had been labeled earlier. In the latter embryos, the number of grains per nucleus also decreased slightly.

Since the telencephalic plexuses of the mouse and hamster are structurally very similar, one wonders why the mode of growth of the epithelium should differ in these closely related animals. Among other possibilities, perhaps the different treatments (Colcemid versus thymidine-H^3) and time intervals (1-3 hours versus 8 hours) used in these two studies may account for the differences found.

The epithelial cells lining the distal portions of the telencephalic plexus undergo a considerable decrease in size (high columnar to cuboidal or low cuboidal) during development in the pig (Flexner and Stiehler, 1938), in man (Kappers, 1958; Klosovski, 1963; Shuangshoti and Netsky, 1966), in the cat (Purin, 1963), in the

rabbit (Tennyson and Pappas, 1964), and in the chick (Smith, 1966). A similar change occurs in the hamster. In an investigation of the developing chick telencephalic plexus, Smith (1966) noted that this change in shape of the epithelial cells might be related to either the anatomical displacements undergone by the plexus or to "....some internal rearrangements of cell constituents...." (mitochondria) (p. 386). However, Shuangshoti and Netsky (1966), noting large accumulations of mucin in the stroma of the distal portion of the developing human telencephalic plexus, remarked that

> "....such physiologic enlargement spreads the thick pseudostratified surface epithelium to become the simple cuboidal type. The stroma of the stalk is scant, hence this epithelium remains pseudostratified throughout the course of development and even in post-natal life" (p. 290).

Although hematoxylin was used, there was no large accumulation of mucin in the developing telencephalic plexus of the hamster. This observation is in keeping with that of Kappers (1958) for the mouse, whose telencephalic plexus is morphologically very similar to that of the hamster. This change in shape of the epithelial cells (pseudostratified to cuboidal or low cuboidal) of the hamster telencephalic plexus may be explained perhaps by the different growth rates of the connective tissue and epithelial cells. If one compares the mean mitotic indices (Appendix II) of the connective tissue cells with the epithelial cells in the stalk and distal portions of the plexus, one finds that in the former they are approximately the same (13.11 and 13.22% respectively), whereas in the latter they differ markedly (11.05 and 4.11% respectively). This latter difference is

even more pronounced during both gestation and early post-natal life (Tables II, III). Hence, it appears that the actively proliferating connective tissue cells may be pushing the relatively less active epithelium of the distal portion of the plexus into the brain ventricle, and that during this process the epithelial cells in this region become stretched and consequently flattened. In the stalk region the epithelium remains unchanged since its mitotic index is very similar to that of the stroma. A similar phenomenon appears to be occurring also in the diencephalic and myelencephalic plexuses.

In the diencephalic plexus, which possesses no stalk region (pseudostratified), growth and extension of the choroidal epithelium occurs mainly in the proximal portions of the folds, whereas in the myelencephalic plexus, which possesses both a stalk and folded area, growth and extension of the lining epithelium occurs in both the stalk and proximal halves of the folds. Some growth of the epithelium, albeit at a much lower rate, occurs also in the distal portions of the folds of both of these plexuses. These observations are in keeping with those of Knudsen (1964) for Colcemid-treated mouse embryos. Knudsen (1964) however compared the growth of the plexus epithelium to that of the epithelium lining the intestine. Presumably, he meant that, like the intestinal epithelial cells, those of the plexuses are formed in mitotically active zones at the bases of the folds, and then leaving these zones, they glide up the sides to the fold tips, where they are extruded into the ventricular

lumen. Nothing of this nature was observed in the hamster. In embryos obtained from females injected with tritiated thymidine at 24, 48, and 72 hours prior to sacrifice most of the labeled cells remained in the same locations where they were in the embryos from females injected 8 hours prior to sacrifice. In the former, the number of labeled cells in these areas increased while the number of grains per nucleus decreased. No evidence was found to indicate that these labeled cells moved to the tips of the folds (unpublished data).

A few authors, using autoradiographic methods (thymidine-H^3), have reported finding labeled choroidal epithelial cells in adult mammals. Schultze and Oehlert (1960) reported finding some labeled nuclei in the choroid plexuses of adult rats and mice. In an autoradiographic investigation of neuroglia proliferation, Altman and Altman (1962) remarked that, "....except for an occasional cell, the cells of the choroid plexus showed no labeling" (p. 316). In both of these studies neither the plexuses examined, nor the cell types labeled, and their locations in the plexuses were noted. Johnson et al., (1960) observed in man "....a moderate degree of DNA synthesis in the epithelial cells of the choroid plexus...." (p. 638) of the fourth ventricle. Shuangshoti and Netsky (1966) reported finding labeled epithelial cells in the choroid plexuses of adult mice. Although no actual counts were made, Messier and Leblond (1960) estimated the epithelial cells of the choroid plexuses of adult male mice and rats to have a mitotic index between 0.4 - 1.0 per

cent. However, neither the plexuses examined (telencephalic, diencephalic, myelencephalic) nor the locations of the labeled cells (stalk, folded area, et cetera) were indicated in these last three studies.

In the adult hamster, mitotic activity of the epithelial cells was very low in all regions of the plexuses (0 - 0.2%). Proliferative activity of the connective tissue cells, about which no reports have been found, is also very low (0 - 0.3%) in all of the plexuses of the adult hamster (Tables II, III).

It is of interest to note that although Shuangshoti and Netsky (1966), using "....deep multiple sectioning to rule out artifact due to plane of the cut....", suggested that in human plexuses a "....slow proliferation of choroidal epithelium occurs and is characterized by stratification and desquamation of superficial epithelial cells, followed by replacement from adjacent underlying cells...." (p. 290), no such phenomenon was noted in the plexus epithelium of the hamster.

Shuangshoti and Netsky (1966) reported that the epithelium of the choroid plexuses of the third and fourth ventricles in the human completed their differentiation before those of the lateral ventricle. This occurs at approximately 31 weeks of gestation. However, Klosovski (1963), in another investigation of the choroid plexuses of man, noted that the epithelium lining all of the plexuses acquire features characteristic of the adult plexuses during the third month post partum. Moreover, Purin (1963), in a study of the

developing plexuses of the cat, observed that the diencephalic plexus epithelium completes its differentiation first (end of first month post partum), followed by that of both the telencephalic and myelencephalic plexuses at 2 - 2.5 months post partum. In the hamster yet another sequence occurs. The first plexus to acquire features (adult shape of cell, adult mitotic index, and loss of glycogen) characteristic of the adult plexus is the diencephalic plexus (first week post partum), followed by the telencephalic plexus (second week post partum), and last by the myelencephalic plexus (end of fourth week).

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APPENDICES

Appendix I

Definitions of the terms used to designate the relative amounts of glycogen in the epithelial cells of the choroid plexuses.

1. Abundant - glycogen fills one-half to three-fourths of the cytoplasm of the cell.
2. Moderate - glycogen fills one-fourth to one-half of the cytoplasm of the cell.
3. Low - glycogen fills less than one-fourth of the cytoplasm of the cell.
4. Minimal - only a faint stippling of stainable glycogen granules is present in the cytoplasm of the cell.

Appendix XI

Mean mitotic indices of the epithelial and connective tissue cells of the telencephalic and diencephalic choroid plexuses of embryonic and neo-natal hamsters.

	Telencephalic		Diencephalic	
	Stalk Region	Folded Area	Proximal half of fold	Distal half of fold
Epithelial Cells	13.99	4.11	9.60	3.45
Connective tissue cells	13.88	11.05	9.73	10.56