QUALITATIVE AND QUANTITATIVE STUDY ON THE EFFECT OF NEONATAL UNDERNUTRITION ON THE POSTNATAL DEVELOPMENT OF THE VERMIAN CEREBELLUM OF MOUSE

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INTRODUCTION

Undernutrition is a known factor influencing the pattern of brain development. This factor was observed early as to cause a reduction of brain weight (Sugita, 1918 and Jackson and Steward, 1920). Several parameters have been used by which the effect of undernutrition on the development of the brain could be defined, among these are DNA content (Chase *et al.* 1969; Winick *et al,* 1968 and Winick, (1969), cell number (Winick *et al,* 1968 and Zomenhof *et al,* 1968), neuron size and ultrastrutural changes (Lowry *et al* 1962, and Olivito, 1977), myelination (Dobbing, 1964; Davidson and Dobbing, 1966; Dobbing and Widdowson, 1965; Cully and Mertz, 1965 and Benton *et al,* 1966) and on gyrification (Abdel Kader *et al,* 1976).

In this study an attempt is made to demonstrate the effect of neonatal undernutrition on the development of the cerebellum of mice at different stages of growth. The scope of the quantitative study was the growth of the whole cerebellum through measuring the surface area of the cerebellar cortex and the white matter, the cranio-caudal extent of the cortical sheeth and the thickness of the external granular layer and the whole cerebellar cortex. These parameters have not been dealt with before, at least, in the available litrature.

MATERIAL AND METHODS

A total number of 60 newly - born mice was used of which 48 were experimentally undernourished, while the remaining 12 were adequately fed and used as controls.

Undernutrition was achieved by breeding at least 12 offspring with one lactating mother until weaning. However, adequate feeding of the

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controls was ensured by housing only 3 newly-born litters with one lactating mother till weaning.

Both control and underfed animals were classified into 4 age-groups and were sacrified at 5th, 7th, 10th, 15th and 20th day postnatally. Ether anaesthesia was used, and the brains were extracted and fixed in Bouin's fluid. After proper dehydration, clearing and paraffin embedding, serial sagittal sections were cut at 10*u*. The mid - sagittal section of each series was chosen to be stained by the gallocyanine stain.

The stained mid-sagittal sections were projected by a projecting microscope in a dark room at a linear magnificantion of 46 times. The outline of the section and the line of demarcation between the cortex and the white matter were traced on a sheet of paper. From these projectons, the cranio-caudal extent of the cerebellar cortex of the mid-sagittal section was measured. Also the surface areas of the whole mid sagittal section, the cerebellar cortex and the white matter were measured by a planimeter.

From the stained mid-sagittal sections, the thickness of the external granular layer and that of the whole cortex were measured by an eye piece micrometer at the sides of the postcentral fissure, the horizontal fissure and fissura secunda. The mitotic figures in the external granular layer of the mid-sagittal section were counted.

RESULTS

1. Cerebellar Histogenesis :

A) The external granular layer (E.G.L.) : (Figs. 1..5)

In underfed animals, this layer appears much thinner than that of the controls of the corresponding age. Like in the controls, regional variation in the thickness of the E. G. L. is noticed till the age of 10 days. It is found to be thicker at the anterior region of the vermis (the lingula, central lobule and culmen). The thickness of E.G.L. in the caudal region (the uvula and nodule) is noticed to be less than that of cranial part. In the mid-vermian region (the declive, folium, tuber and pyramid) the external granular layer reaches its minimal thickness.

However, at the ages of 15 and 20 days, the thickness of the external granular layer only at the mid-region of the vermis in underfed animals exceeds that of the controls.

The E.G.L. continues to persist at the age of 20 days (mainly in the mid-vermian region) (Fig. 5), although it disappears at the age of 20 days in the controls. It reaches its maximal thickness at the age of 7 days (the same occurs with the controls).

The dividing cells in the outer zone of this layer (the germinal zone) in the underfed cerebellum appear to be less than those in the controls of the corresponding age. Mitotic figures (within the germinal

zone) continue to persist at the age of 20 days at which age they normally disappear.

B) The molecular layer: (Figs. 1 - 5)

This layer in all underfed animals is noticed to be less in thickness than that of the control mice of the corresponding age, inspite of its continuous increase in thickness till the age of 20 days. Regional variation in thickness of this layer is noticed. It is thinner in the region of the declive, folium and tuber (mid-region of the vermis) than in the cranial and the caudal regions.

The density of the migrating cells (from the E.G.L. to the internal granular layer) is some what less in the underfed animals, except in the mid-vermian region at the age of 15 - 20 days when it is noticed to exceed the controls.

C) The internal granular layer

(Figs. 1 - 5)

The thickness of this layer increases with the progress of age till the age of 20 days. The internal granular layer in the underfed animals is less in thickness than in the controls of the corresponding age in all the stages studied.

D) The Purkinje cell layer :

(Figs. 1 - 5)

The Purkinje cells appear as large pale nuclei surrounded by a moderately basophilic cytoplasm. Normally, at the age of 7 days and after the Purkinje cells are arranged into a separate layer formed of one row of cells.

In underfed animals Purkinje cells appeared to be smaller in size with smaller nuclei and decreased basophilia, compared with the controls of the same age. The Purkinje cells are arranged in more than one row and the cells are intermingled within the cells of the internal granular layer. The arrangement of Purkinje cells in one row is not reached till the age of 15 days (mainly in the mid-vermian region and the nodule).

E) The white matter :

(Figs. 4 & 5)

Both in control and undernourished animals, the white matter appears as a pale zone with nuclei arranged parallel to the surface. The density of the nuclei increases till the age of 7 days and it decreases at older ages. In underfed animals the thickness of the white matter is noticed to be less than in the corresponding controls.

11. Quantitative results :

1) Mitotic figures of the E.L.G. (Table 1) (Fig. 6)

The number of mitotic figures per mid-sagittal section of control cerebella increased up to the age of 7 days. The count then falls till the age of 20 days, when no mitosis could be detected. In underfed animals the counts of mitotic figures were much below those of the con-

trols of corresponding age. This holds true except at the age of 20 days. The counts of the mitotic figures per mid-sagittal section equaled 67, 71, 88, 67 and 200% of those of the controls in the ages of 5,7,10, 15 and 20 days respectively.

The apex of the curve of mitotic figures lies against the age of 7 days, both in control and underfed animals.

2) Thickness of the external granular layer : (Table 2) (Fig. 7)

The thickness of the E.G.L. at the sides of the postcentral fissure, in the mid-sagittal section, is taken as a representative example.

Both in control and underfed cerebella, the thickness of this layer increases till the age of 7 days. This is followed by a continuous drop till the age of 20 days.

In undernourished mice the thickness of the E.G.L. is less than that of controls except at the age of 20 days. The thickness of this layer equals 71, 64, 84, 83 and 600% that of the controls at the age of 5, 7, 10, and 20 days respectively.

The curve of growth of this layer in the underfed animals lies at a lower level than that of the controls. It declines sharply from the age of 7 days to the age of 15 days. Lastly it exceeds that of the controls. A gradual increase is noticed between the age of 5 and 7 days. The peaks of both curves lie opposite the 7th day.

3) Thickness of the cerebellar cortex : (Table 3) (Fig. 8)

Since the mode of growth of the cerebellar cortex is relatively the same at the sides of the 3 fissures (postcentral, horizontal and secondary fissures), the data given are those at the sides of the postcentral fissure; which is taken as a representative example.

Both in control and underfed animals the thickness shows a continuous increase till the age of 20 days. Those of the undernourished animals equal 81,86,83,81and 83% of that of the controls at the ages of 5,7,10,10 and 20 days respectively. In underfed animals the curve of growth in thickness of the cerebellar cortex against the age lies at a lower level than that of the controls.

4) The cranio-caudal extent of the cerebellar cortex. (Table 4) (Fig. 9)

In both control and underfed animals, the extent of the cerebellar cortex, per mid-sagittal section, increases with age. Those of the underfed cerebella are less than those of the controls at different ages. The cranio-caudal extent of the cortex in underfed animals equals, 70, 71, 86, 79 and 85% of that of the controls at the ages of 5, 7, 10, 15 and 20 days respectively.

The curve of growth of the cranio-caudal extent of the cortex, in both

control and underfed animals, shows a continuous increase till the age of 20 days. That of the undernourished mice lies at a lower level than in the controls.

5) The surface area of the mid-sagittal section :

(Table 5) (Fig. 10)

The surface area of the mid-sagittal section increases with age both in control and underfed animals. The surface area of the underfed cerebella equals 58, 59 77, 80 and 86% of that of the controls at the ages of 6,7,10, 15 and 20 days respectively.

The curve of growth of the surface area of the mid-sagittal section of the undernourished mice lies at a lower level than that of the controls.

6) Surface area of the cerebrellar cortex : (Table 6) Fig. 11).

The surface area of the cerebellar cortex per mid-sagittal section of the undernourished animals is less than that of the controls at all ages. It equals 59, 52, 77, 79 and 86% of that of the controls at the ages of 5,7,10,15 and 20 days respectively.

The growth curves, of controls and underfed, show a continuous increase till the age of 20 days. The curve of the undernourished animals, however, lies at a lower level than that of the controls.

7) Surface area of the white matter :

(Table 7) (Fig. 12)

In controls, the surface area of the white matter, per mid-sagittal sections, shows an increase till the age of 7 days followed by a sharp inclination between the age 7 and 10 days. Finally a progressive increase till the age of 20 days is noticed. A relative stationary phase of growth is noticed between the age of 15 and 20 days.

In underfed animals, the curve shows the same pattern as in the controls, but it lies at a lower level than that of the controls and also it shows a progressive increase between the age of 10 and 20 days.

The surface area of the white matter of the mid-sagittal section in underfed animals equals 60, 75, 75, 72, and 80% of that of the controls at the ages of 5, 7, 10, 15 and 20 days respectively.

DISCUSSION

The present work shows that undernutrition causes retarded growth of the cortical elements as proved by the histological findings.

The external granular layer :

The external granular layer (E.G.-L.) had the same pattern of arrangement in underfed animals, as that of the controls, although it shows a continuous reduction of layers

(rows of cells) compared with controls. The explanation of this phenomenon is that undernutrition causes a decrease in protein and DNA content. DNA synthesis in rat brain, stopped early at 17 days mainly in the cerebellum, the early restriction of diet interferes with cell division and the animal is left with a decreased number of cells (Winick, 1969). On the contrary, late underfeeding causes reduction in cell size with no effect on cell number (Sugita, 1918; Winick. *et al* 1968 and Winick, 1969).

Malnutrition during pregnancy will result in offspring whose brains contain a reduced number of cells (Zomenhof *et al,* 1968). Our present work confirmed these data, as we find that the count of the mitotic figures in the E.G.L. per midsagittal section is less than in the controls. The peak of the mitotic count lies at the age of 7 days (as in the controls). Reduced mitotic count was observed in hypothyroid cerebella (Haddara *et al,* 1975 and Matta *et al,* 1976) but the peak of the mitotic count occured at the age of 10 days. We could suggest that in undernutrition reduction in mitosis is due to reduced cellular proliferation due to decreased DNA content. However, in hypothyroidism elongation of mitotic cycle and blockage of differentiation, thus leading to accumulation of cells undergoing mitosis, are the causes of the shift of the peak to the age of 10 days (Matta *et al,* 1976).

It could be suggested that there is a certain period of the postnatal life during which undernutrition would inflict the severest effect on the nervous tissue. Dobbing and Widdowson (1965) and Brasel and Winick (1970) stated that underfeeding would be greatest if it occurs within the time of the rapid growth of the brain, which could be considered as the critical period of growth. However, the changes produced by neonatal undernutrition can be reversed with adequate feeding (Winick *et al,* 1968). Undernutrition only causes metabolic changes in the cells (Sharma and Sohan, 1976) causing hastening of ageing process. It causes minimal E.M. changes in cerebellar neurons (Olivito, 1977).

Eayr's and Horn (1955), Horn (1955), and Mulinos and Pomerantz (1940) stated that both h ypothyroidism and underfeeding cause retardation in brain development, and that undernutrition could lead to a decrease in thyroid function. However, Legarnd (1967 & 1971) disbelieved this view and stated that, unlikely underfed rats suffered from any degree of hypothyroidism. This view was supported recently by Krupp *et al* (1977) who stated that the thyroid gland of undernourished animals did not appear to be hypoactive as previously reported.

Bijlani *et al* (1976) claimed that cell proliferation and cell migration of E.G.L. and cell differentiation of cerebellar cortrx are laging behind

in low weight category of normal mice.

Surface area, thickness and extent of the cerebellar cortex.

The present finding of reduction of surface area of the cerebellar cortex of underfed animals could be attributed to two factors :

1) Firstly, the thickness of the whole cerebellar cortex is less than that of the corresponding controls. This is caused by decreased thickness of the E.G.L., decreased thickness of the molecular layer and internal granular layer. The documented suppression of proliferation, and meanwhile, migration of the cells of the E.G.L. could account for reduction of number of cells in internal granular layer and reduced number of axons of granule cells within the molecular layer.

2) Secondly, the cranio-caudal extent of the cortical sheet is less than that of the controls. Haddara *et al* (1975) stated that the dendrites of Purkinje cells are responsible for the expansion of the cortex in a cranio-caudal direction. Accordingly, we could suggest that underdeveloped Purkinje cell layer (more than one row of cells) is responsible for the decrease in the cortical extent.

The curves of growth of area of the whole mid-sagittal section and area of cerebellar cortex, show that the cerebellar cortex is the main factor for the growth of the cerebellum, and that any factor that could affect it will retard the development of the cerebellum.

The white matter :

Both in controls and underfed animals the white matter shows an increase in its surface area till the age of 7 days followed a sharp inclination till the age of 10 days. Lastly a continuous increase is noticed. The first increase could be due to the density of the white matter with the nuclei of the cells but due to the migration of the cells of the white matter to the internal granular layer a reduction in the surface area occurs. The following increase (after the age of 10 days) could be due to the beginning of myelination. Myelination is dependant, at least in part, on cellular growth of brain and is influenced by factors affecting cellular growth (Davidson and Dobbing, 1966 and Bensted *et al*, 1957). Malnutrition before weaning in rats produced retarded development of myelination (Dobbing, and Widdowoson, 1965; Dobbing, 1964; Cully and Mertz, 1965; Benton *et al*, 1966 and Noback and Rosso, 1976). So we could conclude that the decreased surface area of the white matter after the age of 10 days in underfed animals, could be due to retarded myelination.

SUMMARY

The effect of undernutrition on the postnatal development of the cerebellum of the mouse was studied.

There is retardation in the development of external granular layer in underfed cerebellum. It persists in most after 20 days, however, it disappears in the controls.

The molecular and the internal granular layers are thin in the underfed animals, and Purkinje cell layer is formed of more than one row of cells. The thickness of the E.G.L. is less than the control and its maximal thickness is reached at the age of 7 days, the same occurs with the count of mitotic figures.

The thickness and the surface area of the cerebellar cartex increase with age and are less than in the controls. The surface area of the white matter is less than in the controls.

The results are discussed in the light of the available data in the literature. A comparison is drawn between effects of undernutrition and hypothyroidism on the cerebellar development.

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Tabte (1)

Average mitotic figures counted in the external granular layer per mid-sagittal section in both control and underfed cerebellum (at different ages).

Age in days	Control	Underfed
5	230	154
7	324	231
10	210	184
15	32	21
20	Zero	2

Table (2)

Average thickness of the external granular layer of control and underfed cerebellum at different ages measured in microns at the sides of the postcentral fissure (C) the horizontal fissure (H) and fissura secunda (S) at the mid-sagittal section.

Age in days	Control			Underfed		
	C	H	S	C	H	S
5	56	—	52	40	—	36
7	66	63	60	42	—	38
10	38	40	32	32	42	32
15	12	26	10	10	36	10
20	Zero	6	Zero	Zero	14	6

Table (3)

Average thickness of the cerebellar cortex of control and underfed cerebellum at different ages, measured in microns at the sides of the poscentral fissure (C) the horizontal fissure (H) and fiissura seconda (S) at the mid-sagittal section.

Age in days	Control.			Underfed		
	C	H	S	C	H	S
5	138	—	136	112	—	110
7	161	164	171	138	—	130
19	232	168	240	192	136	190
15	296	252	280	240	192	232
20	320	300	312	264	218	272

Table (4)

Average cranio-caudal extent of the cerebellar cortex of control and underfed cerebella at different ages (measured at mid-sagittal section in mm)

Age in days	Control.	Underfed
5	12.82	9.01
7	16.00	11.43
10	22.64	19.48
15	27.87	22.00
20	29.10	24.73

Table (5)

Average surface areas of the mid sagittal section, of control and underfed cerebella at different ages (measured in mm^2).

Age in days	Control.	Underfed
5	1.93	1.13
7	2.79	1.65
10	4.80	3.69
15	6.27	5.01
20	7.04	6.02

Table (6)

Average surface areas of the cerebellar cortex of control and underfed cerebella at different ages (measured at mid-sagittal section, in mm^2).

Age in days	Control.	Underfed
5	1.32	0.77
7	2.10	1.09
10	4.28	3.30
15	5.73	4.53
20	6.38	5.47

Table (7)

Average surface areas of the white matter of control and underfed cerebella at different ages (measured at mid-sagittal section, in mm').

Age in days	Control.	Underfed
5	0.68	0.41
7	0.75	0.56
10	0.52	0.39
15	0.65	0.47
20	0.66	0.53

LEGENDS

Fig. (1) : Photomicrograph of the cerebellar cortex of 5-days old mouse (a), control (b) underfed (Gallocyanin-chrome, 40 X 12.5).

Fig. (2) : Photomicrograph of the cerebellar cortex of 7 - days old mouse (a) control (b) underfed (Gallocyanin-chrome, 40 X 8).

Fig. (3) : Photomicrograph of the cerebellar cortex of 10-days old mouse (a) control (b) underfed (Gallocyanin-chrome, 40 X 8).

Fig. (4) : Photomicrograph of the mid-sagittal section of 15 - days old mouse at the mid-region of vermis, (a) control (b) underfed (Gallocyanin-chrome, 20X8).

Fig. (5) : Photomicrograph of the mid-sagittal section of 20 - days old mouse at the mid-region of vermis (a) control (b) underfed, (Gallocyanine-chrome, 20X8).

Fig. (6) : A curve showing the change in the number of mitotic figures in mid-sagittal section of control and underfed cerebellum with age.

Fig. (7) : A curve showing the change in the thickness of the E.G.L. at the sides of the postcentral fissure of the mid-sagittal section of control and underfed cerebellum with age.

Fig. (8) : A curve showing the change in the thickness of the cerebellar cortex at the sides of the postcentral fissure of the mid-sagittal section of control and underfed cerebellum with age.

Fig. (9) : A curve showing the change in the cranio-caudal extent of the mid-sagittal section of control and underfed cerebellum with age.

Fig. (10) : A curve showing the change in the surface area of the mid-sagittal section of control and underfed cerebellum with age.

Fig. (11) : A curve showing the change in the surface area of the cerebellar cortex of the mid-sagittal section of control and underfed cerebellum with age.

Fig. (12) : A curve showing the change in the surface area of the white matter of the mid-sagittal section of control and underfed cerebellum with age.

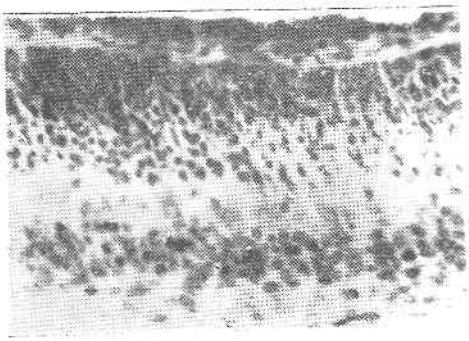
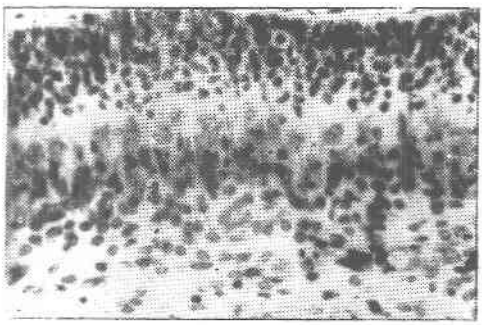


Fig. (1. a)



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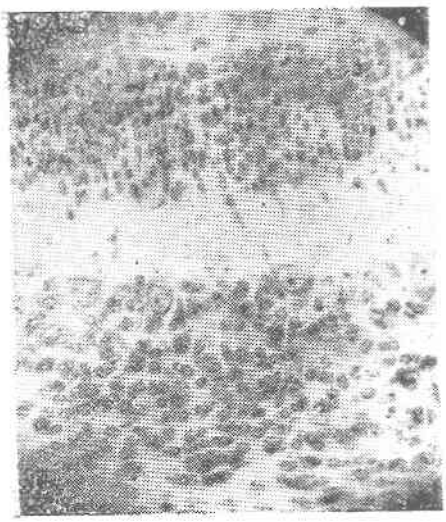
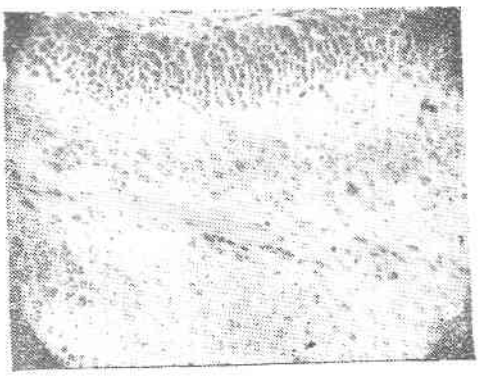


Fig. (2. a)



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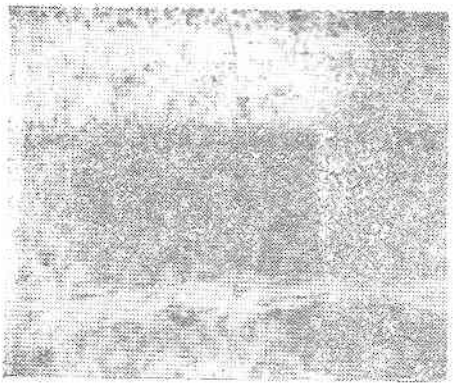

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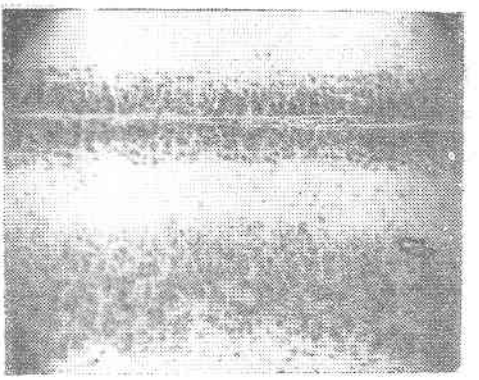

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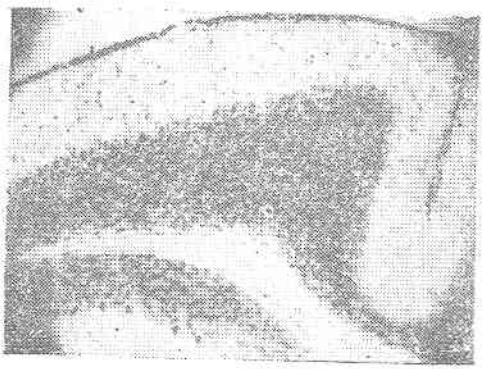

Fig. (4. a)

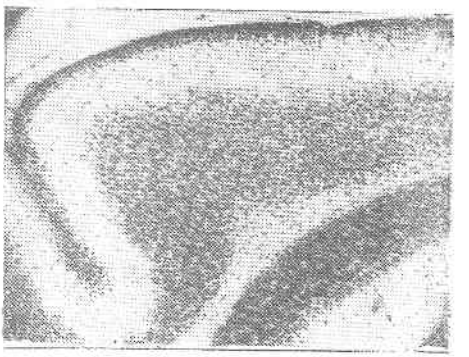

Fig. (4. b)

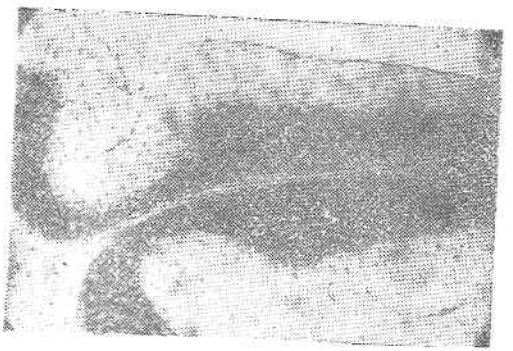

Fig. (5. a)

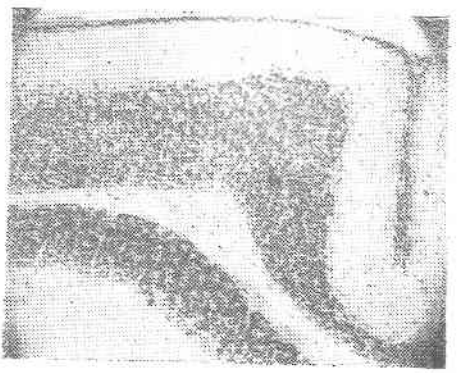

Fig. (5. b)

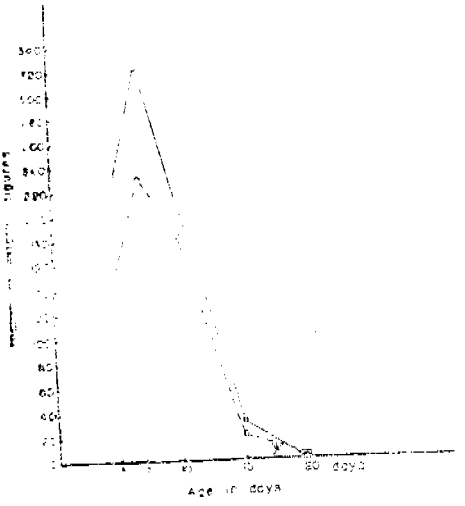

Fig. (6)

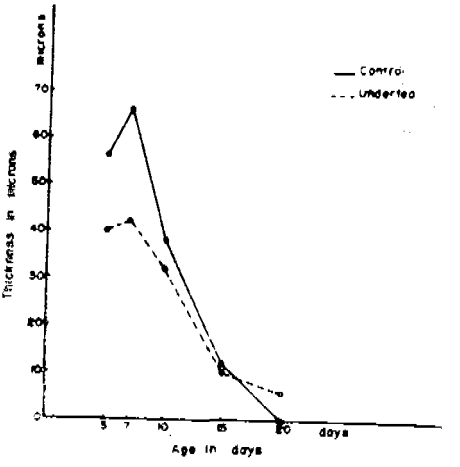

Fig. (7)

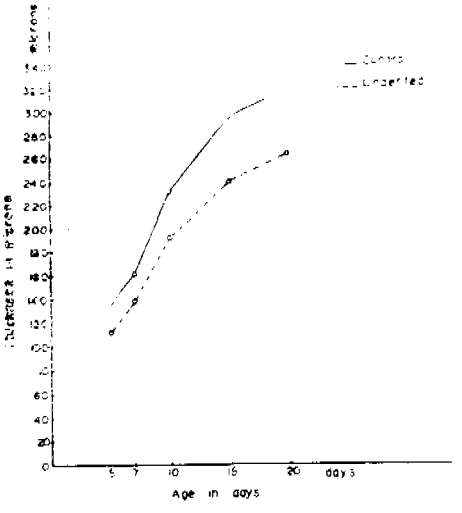

Fig. (8)

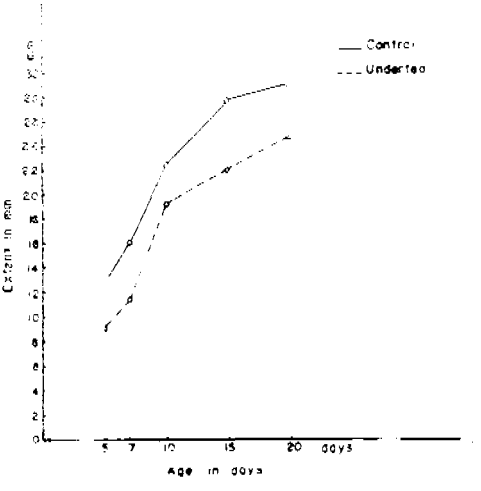

Fig. (9)

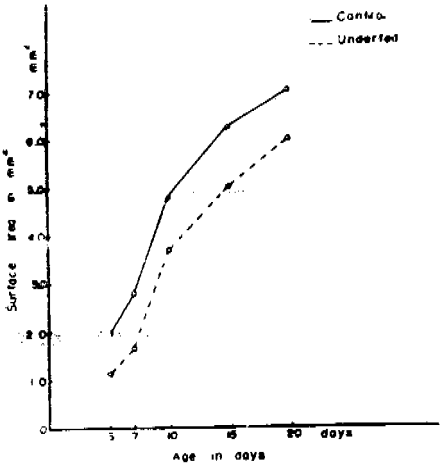

Fig. 10

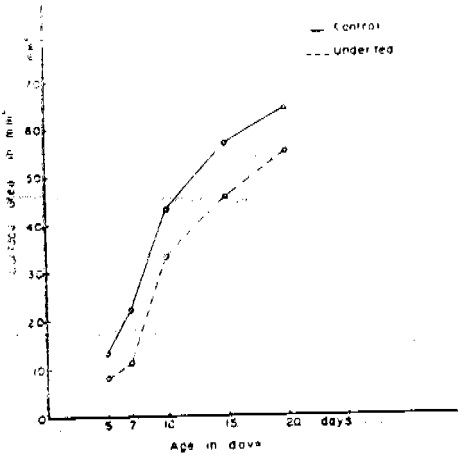

Fig. (11)

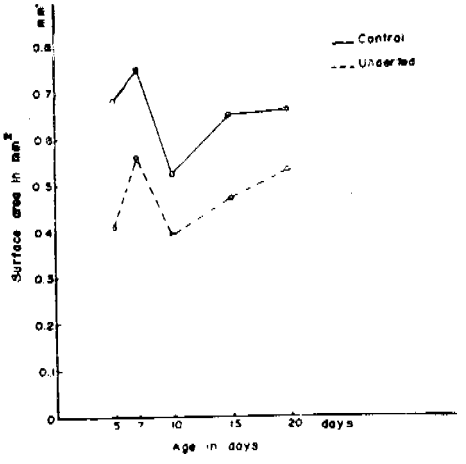

Fig. (12)