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EFFECTS OF ANOXIA ON THE DEVELOPING CEREBRAL CORTEX IN THE RAT

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Much experimentation and speculation have been directed at explaining what lack of oxygen may do to the developing brain and other organs of man and other mammals, and contrasting views have been expressed about the vulnerability of the embryo, fetus, and newborn to injury from anoxia. Some observations support the view that in early stages of development mammals are very resistant to harm from anoxia compared with adults. Other studies suggest that anoxia poses a serious threat to successful development. Part of the conflicting testimony stems from the broad use of the terms asphyxia and anoxia to embrace a variety of circumstances in which lack of oxygen is involved. These have included observations of the effects of clamping the umbilical cord of a fetal sheep or man *in situ*,[1,2] exposing a pregnant rodent (and her fetuses) to low atmospheric pressure,[3] or allowing an artificially delivered monkey to suffocate in its amniotic sac.[4] In these procedures more is involved than "simple" anoxia, and Windle [4] has recently emphasized that when monkeys are asphyxiated in their amniotic sacs after delivery, severe damage to the brain occurs, but when the baby monkey is immersed in nitrogen for a period and revived, no damage may be apparent. Correspondingly in our earlier experiments,[5,6] it was found that asphyxiation of pregnant rats by exposure to low atmospheric pressure occasionally led to acute destructive damage in the nervous systems or other tissues of the fetuses, but immersion of pregnant or newborn animals in nitrogen or in atmospheres of 5 per cent oxygen in nitrogen at normal pressure

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seldom damaged the developing nervous systems, so far as could be determined at the time.

It has long been known that newborn baby rats and mice can withstand periods up to nearly an hour without any oxygen, and they have seemed to recover apparently without harm.[7] Puppies, kittens, and some other mammals, including man, seem to have similar capacities for survival, but guinea pigs, which have a very mature brain metabolism at birth, resemble adults in their susceptibility to anoxic injury. Himwich[7] and his collaborators postulated that baby rats had alternative pathways to break down glucose, one aerobic and the other capable of running anaerobically for some time. When they gave iodoacetate or fluoride, which inhibit glyceraldehyde phosphate dehydrogenase and enolase respectively, the animals died immediately when immersed in nitrogen, yet they could survive in air. Racker,[8] however, has pointed out that this does not prove the presence of two pathways for glycolysis. Rather, glyceraldehyde phosphate dehydrogenase is a key enzyme in anaerobic production of adenosine triphosphate (ATP). A partial inhibition of its action, he says, can prevent maintenance of an ATP level sufficient for glucose phosphorylation under anaerobic conditions; yet in the presence of oxygen, oxidative phosphorylation can compensate for this inhibition of ATP production and mask the effect of iodoacetate as long as the dehydrogenase is not absolutely limiting in the pathway from fructose diphosphate to lactate.

Notwithstanding our ignorance of precisely what chemical mechanisms are involved, young animals do have this remarkable capacity to survive anoxia, and our laboratory long shared the prevalent view that they are unharmed by it. It occurred to us, however, that no experiments, including our own, had explored quantitatively the possibility that the brain or other tissues were damaged in some manner by this seemingly harmless procedure. Also, Racker's view that a distinct alternate "anaerobic pathway" may not exist at all suggested that the asphyxiated animals, with no real safety mechanism, might always be skirting disaster and often sustaining some injury that had gone unsuspected. We have, therefore, systematically immersed newborn rats and mice in nitrogen, with no oxygen, and then examined their brains in serial sections with matching controls at a series of stages from a few hours after anoxia until adult life. It turns out that the infants' ability to survive a period of anoxia carries a price tag, and they virtually never escape injury. We have concentrated only on the cerebral cortex thus far, and the injury there takes the form of inhibition of the proper development of the dendrites, yet no neurons are destroyed. The constellation of changes that does occur at this particular stage of development includes (a) in-

hibition of maturation of the nucleus in the less mature neurons of the outer cortex and delayed formation of their primary apical dendritic processes; (b) inhibition of the production of RNA along the apical dendrite of more mature neurons including sites where the dendrite will sprout branches; and (c) stunting of dendritic growth which leads to permanent changes in the architectural arrangement of the outer cortical layers.

It is the purpose of this paper to describe these changes and show how they come about.

Material and Methods

Infant rats and mice of several strains were immersed in pure nitrogen for 30 minutes, then removed and kept under normal laboratory circumstances for periods ranging from a few hours to 9 weeks. Seventy-three asphyxiated and 40 normal control animals were killed and necropsied. The brains were embedded in paraffin and prepared in serial sections and studied chiefly by conventional histologic methods. In certain instances ultraviolet microscopy, histochemical methods, and Cajal stains for dendrites in frozen sections were used as described later.

The rats were chiefly from an inbred albino line that was in its 36th to 40th successive brother-sister mating during these experiments. A few albino rats from the Harvard Biological Laboratory strain were also used. Though differing in certain gross characteristics, they both derived from the so-called Wistar stock many years ago. The mice were C57 black 6, DBA/2 and DBA/1, obtained from the Jackson Laboratory at Bar Harbor, Maine, during the experiments. They were kept in plastic cages with sawdust, given Purina Laboratory Chow and tap water *ad libitum;* partly shaded indirect daylight and concomitant artificial light were used in a room temperature range of about 72° to 80° F.

Asphyxiation was carried out in this same temperature environment about 12 to 20 hours after birth, the young always having been allowed to nurse in the meantime. For example, young born during the night were asphyxiated the following afternoon, but if they were born during the day, they were asphyxiated the following morning. They were placed on sawdust in a covered bell jar, through which nitrogen flowed at about 2 liters a minute. As described previously,[5] they became cyanotic, often urinated, and gasped for some minutes, but usually became motionless, rarely moving during the remainder of the asphyxia period. After 30 minutes they were returned to the normal atmosphere, jostled, pinched and agitated, which usually revived them within seconds or a minute or two. (Asphyxia for longer periods resulted in increasing mortality, nearly 100 per cent after 60 minutes.)

The rats were killed for study at 24 hours, 1 week, 5 to 6 weeks, or 9 weeks after asphyxia. Matching normal controls were prepared from different litters or more usually from litter mates. In two additional litters, asphyxiated animals and normal litter mates were killed as matching pairs 8, 24, 48, 72, and 96 hours after asphyxia in order that we might follow closely the early development of the anoxic changes. The mice were examined at similar intervals with more representation of 48, 72 and 96 hour stages and also 2 and 3 weeks after asphyxia.

Infant animals were decapitated; older animals were killed quickly in a jar of ether vapor. Usually, fixation was in Bouin's fluid made with 10 per cent formalin, embedding in paraffin, and brains were serially sectioned at 8 μ, and stained with cresyl violet, Klüver's cresyl violet and Luxol blue, or cresyl violet, fast green and acid fuchsin. Bodian protargol, certain other silver stains and hematoxylin and eosin were also used, but the silver stains of paraffin sections seldom lent themselves to

photography. Other organs were not usually studied histologically or histochemically in the present experiments because the acute changes following asphyxia (depletion of glycogen in heart and liver during the asphyxial period) were reported in earlier experiments.[5] The heart, liver, kidney and adrenal appeared normal in hematoxylin and eosin-stained sections in 4 representative young adult rats asphyxiated at birth in the present series.

To remedy the problem of silver stains in nerve cells and their dendrites, 6 rats from 4 different litters were subjected to asphyxia at birth and killed at 9 weeks with a corresponding normal litter mate control. The paired brains were fixed in Bouin's solution made with 10 per cent formalin or formalin buffered to pH 4.2 or 7 with sodium formate and formic acid (6 cc. of 88 per cent formic acid in 1 liter of 10 per cent formalin brought to neutrality with approximately 5.7 gm. of NaOH). Similarly, the brains were prepared from two infant rats killed 4 days after asphyxia with 2 normal litter mate controls, and from 2 rats killed 10 days after asphyxia with normal litter mate controls. Frontal slices extending from the forebrain between the anterior commissure and the posterior border of the habenula were sectioned in the freezing microtome at 20, 30 and 40 μ, and matching sections from the asphyxiated and normal animals stained together by Cajal's method (2 per cent $AgNO_3$-pyridine-alcohol mixture followed by reduction in hydroquinone-formalin-acetone). Bouin's fluid-fixed material provided over-all staining of virtually all apical dendrites and major cell processes, while formate-formalin gave somewhat more detail on fewer cells.

For ultraviolet microscopy, brains of rats removed 24 and 96 hours after asphyxia were fixed in Carnoy's acetic acid-alcohol-chloroform, dehydrated in ethanol, cleared in benzene, and embedded in paraffin. These were sectioned with the microtome set for 2 μ for ultraviolet investigation, and additional 5 and 8 μ sections were cut from the same blocks for staining. Sections treated with ribonuclease were incubated 1 hour at 37° C. in a 1:100,000 solution buffered with sodium phosphates to pH 6. California Biochemical Research "Ribonuclease 5 × crystal (pancrease), specific activity 50 units per mg. (Kunitz)," was used.

An additional litter of rats was subjected to asphyxia when 3 days old and killed for study 24 hours later. These showed a moderate degree of suppression of nuclear growth and cytoplasmic RNA formation corresponding to that of day-old animals. Two litters of rats were asphyxiated in the usual manner but for periods of 55 and 60 minutes; survivors failed to nurse well, lagged in growth, and became moribund after 1 to 6 days. Some were killed in these stages; growth of their cortical cells was inhibited in the manner to be described for animals with asphyxia for 30 minutes but was more severe. Another litter was asphyxiated for 45 minutes, and the effects on the cortex were not greater than those seen in some animals in the 30 minute group. In addition to the asphyxiated and control animals, our normal reference series of serial sections of the brains of developing rats (2 animals usually represented each day from birth to 10 days) and 2, 3, 4 and 5-week-old animals were used for further comparisons and for the study of normal cortical growth.

In all these investigations direct microscopic comparisons were made of matching sections of asphyxiated and normal brains, cortex area for cortex area, by projecting them simultaneously on a screen, comparing photomicrographs, or laying one microscopic section, on the slide, over another and focusing alternately on one and then the other. Simply measuring the thickness of the cortices or counting cells without attention to cytologic and architectural detail and other factors can be misleading. Small deviations in the planes of sections of the brains, variations in fixation, differences in terminal swelling of the brain when the animal was killed, and even the size and strain of the animal could conspire to make apparent differences in cortical thickness and density of cell population. As the experiments progressed, these pitfalls were avoided by using pairs of animals, usually litter mates, matched in age and processing of the brains. At the same time as we learned the morphogenesis of the

changes caused by asphyxia, the ultimate outcome, especially the consequences of the marked stunting of dendrites, became very easy to recognize.

Results

An account of some features in the cytologic maturation process of the rat and mouse cortex, not generally available, provides background for viewing the changes caused by anoxia. We have previously described some of the features of the migrations of cells that build the cortex[9,10] during fetal life by using radiation and tritiated thymidine in radioautographs to trace the movements.

Pertinent to the present study are the following:

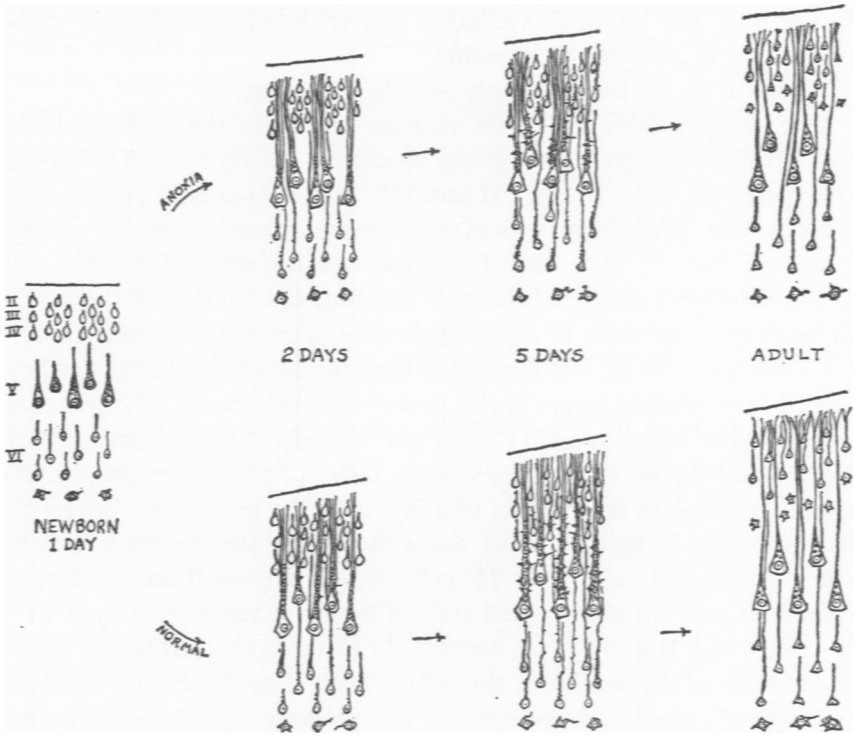
The last primitive cells that migrate out from the periventricular proliferative zones pass through the neocortex already formed to become principally the outer laminas II and III; hence these cells are youngest at birth. The cells migrate in waves, the last two waves arriving on days 21 and 23 (term). After birth few if any neurons are added to the neocortex although young glial elements continue to migrate and the medial hippocampus continues to receive primitive neurons for several days.

Unlike man, the rat has few migrating neurons adding to the numbers of its deeper cortical layers in this period of development. The postnatal sequences can be followed in the rat with the help of the epitome in Text-figure 1 and the normal sections in the series of photomicrographs. We use the convenient designation of 6 layers in the neocortex. Among the distinguishing features in most areas in the rat are the layer V cells, which are largest, and layer IV cells, which are small and stellate or pyramidal and not always well stained by silver methods. Layer VI is the widest and II and III are merged. At birth, in the vertex and dorsolateral slope of the neocortex, the cells in layer V are beginning to extend their apical cytoplasmic processes—the primary apical dendrite—upward into the less differentiated zone of cells which represents the future layers II, III, and IV. Proximally, this cytoplasmic process is diffusely basophilic and by 24 hours after birth its distal portion has formed a number of delicate fibrils, about 0.1 μ or less in diameter, that will reach progressively upward among the outer cortical cells. Normally these fibrils are spread rather evenly among the cells of the outer layers, but there is a tendency for the processes of 2 or 3 cells to run close together and commit the outer cells to a columnated architecture. This columnation is most evident in frontal sections caudal to the plane in which the hippocampal fimbria lies.

A principal general feature of the postnatal development of the cortex is that by the growth of fibers the cells spread themselves apart in a precise pattern, and disturbances in the developmental sequences lead to

disorder in the pattern. By 48 hours the longitudinally disposed fibrils have spread quite extensively into the outer layers, and the basophilic proximal stump of the dendrite has also lengthened and thickened. Layer VI cells have also begun to send their long fibrils up through V, and the outer cells themselves have begun to grow.

From about the second to third day, 24 to 48 hours after birth, another change has begun to occur in the more mature, deeply situated neurons.



TEXT-FIGURE 1. Diagrammatic summary of the principal changes in the cerebral cortex that follow asphyxia at birth in the rat. At 2 days there is a delay in growth of the young neurons of layers II to IV and delayed formation of aggregates of RNA material in the apical dendrites of the large pyramidal cells in layer V. These delays are still evident at 5 days and outer neurons have not spread apart as much as normal. Apical dendrites of layers II to V are stunted, and dendrites of layer V cells tend to run close together. The outcome in the adult is permanent stunting of dendrites especially in layers II to IV, with diminished thickness of these layers.

In the proximal part of their dendritic processes, particularly in layer V but also along the layer VI cell process, there appear little spots or collections of densely basophilic material. Some appear as little streaks within the proximal part of the dendrite, others form the bases of little pointed spurs or thorns on its surface. The cytoplasm of the dendrite at this stage has grown considerably and has become much less diffusely basophilic compared with its appearance when it was a mere embryonic

sprout. The appearance of the new basophilic granules is, then, seen against a rather faintly stained background, whether it be in a cresyl violet stain or cresyl violet with dilute fast green. The basophilic material is easily and completely removed by ribonuclease, and it absorbs ultraviolet light strongly at the wave length of 2,630 Å. It will, therefore, be referred to as ribonucleic acid (RNA) or as basophilic material, etc., although we do not know how it may be coupled with protein or incorporated into the structure of the endoplasmic reticulum, however much that structure may be developed in these young cells. Usually a dense accumulation of the material appears at the base of the spurs of newly forming branches of the dendrite, as might reasonably be expected where a good deal of new cytoplasm (and protein) is being manufactured. Minute particles and streaks of RNA also appear to be disposed along the neurofibrils in their most proximal situation in the base of the primary apical dendrite, but we cannot place them more accurately with light microscopy. Occasional neurons fairly bristle with minute RNA spurs as early as 2 or 3 days, but the tiny processes themselves are at just about the limits of the resolution of light microscopy and hard to follow at length.

The formation of the basophilic thorns or spurs increases rapidly through the first postnatal week. It is most advanced in the layer V and VI cells from 3 to 6 days, the outer cells being slower to differentiate in this and other respects than those in the deeper layers. This differentiation process begins a little earlier in the dorsolateral neocortex than in the vertex. Since the spurs parallel the formation of lateral dendrite processes to a considerable degree through the cortex, formation of the interlacing feltwork of dendrite neuropil starts in the deep layers and develops last in the outer layers. On day 5, the close packed neurons of layer IV, still rather undifferentiated, begin to show little spurs, and day 6 shows a great increase in the characteristic feltwork of this layer. Part of this may be due to increased arrival and possibly branching of thalamocortical afferent fibrils, but we do not know the details of this yet. (Thalamocortical afferents start arriving in the cortex on the 16th fetal day and continue for at least some days, maybe for a long time, after birth.) The last cells to develop spurs and lateral dendrites are the cells in layers II and III.

Tissue fixed in Carnoy's fluid, cut at 5 μ and stained with cresyl violet and dilute fast green, is favorable for observing what may be called the axons of the young cortical neurons. Most of the cells in layer V as well as in adjacent layers in 5-day-old animals, show a delicate process arising from the bottom of the cell body, that is, pointing away from the brain surface. These almost always taper immediately from the cell body and

are about the diameter of the fine apical dendrite fibrils. They always show some RNA at the origins of the processes and occasionally contain particles along the first few μ of their extent, seldom further. Such axons cannot usually be traced more than 50, rarely 100 μ, toward the subcortical white matter in these thin sections. It is not unusual to find in the normal young cortex a few neurons in V and VI which are upside down as compared with the usual cells, that is, their apical dendrite processes point downward. Also, some cells, right side up, have 2 or 3 basilar processes all resembling what have just been described as axons.

The nuclei of the nerve cells undergo certain changes in parallel to, and presumably in relation to, the development of the cytoplasm, and these normal nuclear changes were altered by anoxia. At birth, in the rat and mouse, the outer half of the cortex, composed of elements that will ultimately be layers II to IV, is least differentiated. Although some really quite mature looking neurons develop in the normally sparsely populated layer I long before birth, cells in layers II, III, and many in IV are quite undifferentiated at birth. On the average these youngest neurons are oval with a beginning tapered apical cytoplasmic process, and the nucleus is oval with a diffuse granular chromatin. In cross section they do not yet look like nerve cell nuclei with the characteristic concentration of chromatin in the rim and less chromatin showing within. They contain one, two, or more round bodies which we call nucleoli, and similar nucleoli are seen in the more mature young neurons. They take a dark red stain with cresyl violet, but this is only partly removed by ribonuclease. A fairly considerable amount of granular basophilic material removed by ribonuclease is scattered in both the immature and more mature nuclei, but also concentrated close to the nuclear membrane. Nuclei of the deeper cells in layers V and VI are already more mature. In the outermost cells, in conventional paraffin sections, the nuclei are elongate, granular, and dusty, and about 5 to 7 μ in long diameter on the day of birth, enlarging slowly until the fifth day. On the sixth day they are all quite round, about 7 μ in diameter and getting to look like nerve cell nuclei. By 10 days they are about 10 μ in diameter. In layer V the nuclei begin to resemble those of mature neurons at the end of fetal life and those in VI at the time of birth. Those in layer VI are about 6 to 7 μ in diameter through the first week, enlarging to 10 μ in 10 days. In V, the diameter increases from 8 or 10 μ at birth to 13 μ by the end of a week and a little larger at 10 days.

Generally, then, nuclear maturation, paralleling cytoplasmic maturation begins in the deeper layers, and overtakes the outermost layer II cells last. Although the beginnings of apical dendrite processes are formed by cortical cells when they are in a very primitive stage in late fetal life, the deposition of the dots, particles, and spurs of RNA follows

only after the nucleus has matured. In layer V cells, for example, this begins a day or two after the nuclei have rounded up into the more mature form. However, aggregates of basophilic material that might fairly be called Nissl substance gradually materialize in the perinuclear and basal cytoplasm of layer V cells over the period from about the fifth to seventh day, progressing in the following weeks. This relative shift of concentration of basophilic substance from the dendrite to the cell body region is spread over a couple of weeks at least, and may, therefore, be slower in the rat than in the guinea pig, where it has been described, with the use of ultraviolet microscopy, by Hughes and Flexner.[11] Some focal concentrations of RNA at the base of major dendrite branches persist well into young adult life, but this becomes relatively inconspicuous by the second and third postnatal week. Although the cortex appears fairly mature by 5 weeks, there is still considerable extension of dendrites and spreading apart of cell bodies in the ensuing 4 weeks; we have used 9-week animals to represent the adult for the most part.

The effects of anoxia may now be presented against this normal background by tracing the changes from the initial stage just after asphyxia to the adult. Characteristic changes were seen in all animals, although in 2 animals at 2 weeks of age we could only see the lag in nuclear growth about to be described. About 24 hours after the animals were deprived of oxygen for 30 minutes, it could be seen that (Fig. 1) the cells in the outer layers had failed to show elongation of their primary apical processes. The nuclei in cells in what were presumed to be layers III and IV, which would normally begin to show a change, remained compact and dark staining. By 48 to 72 hours after asphyxia these nuclei were measurably smaller than the normal at the same stage. Although this was a matter of beween 1 and 2 μ in long diameter, it represented a considerable lag in size when translated into nuclear volume. By the end of a week the nuclei had caught up in size to a considerable degree in many but not all animals examined at this stage. (viz., the two animals noted earlier).

Normally the ascending fibrils from layer V cells and to some extent those in layer VI would have been threading toward the outer layers by the second day after birth, but they failed to do this in normal fashion after asphyxia (Fig. 2). Rather, where the apical dendrite of a layer V cell normally sent up a half dozen or more long delicate tendrils, it now often sent up fewer and sometimes much coarser ones which tended to stay close together. At the same time, neighboring layer V cells often sent their fibrils up in close proximity with the result that the ascending dendrites formed fascicles coursing through the outer layers, and in turn the outer cells were formed into relatively compact cell clusters.

Together with these alterations in growth was a diminution of the RNA material in the proximal dendrite in layer V and VI cells. This diminution persisted throughout the period when RNA would normally be prominent in the dendrite. The RNA deficiency became more and more difficult to recognize after the first week when normally RNA in the dendrite would have begun to diminish anyhow.

Ninety-six hours after anoxia the characteristic changes seen earlier continued to be prominent. The nuclei of the outer (II to IV) cells were still more immature looking, and smaller, than the corresponding normal (Fig. 3). The apical dendrites of layers V and VI continued the tendency to run together in bundles, exaggerating somewhat the normal vertical columnation of the outer cortical neurons. The fibrils at this stage were often coarser than normal. A contrasting appearance between the proximal dendritic processes of normal and injured cells could be seen. Normally the concentration of the RNA particles tapered off gradually from the most proximal part of the dendritic trunk and showed prominent concentrations at the sites of spurs for a considerable distance along the process. The asphyxiated neurons showed less material along the proximal part of the dendrite, and the more distal part of the main dendrite often looked somewhat like a hollow tube with few RNA spurs, virtually no particles, and fewer fine branches. Figure 3 was chosen to illustrate this change in well developed form.

After 6 days, the neuropil feltwork normally formed an increasingly dense matrix that characterized the mature brain, and both the fibrillar and RNA changes became difficult to see. Asphyxiated animals, however, showed the aftermath of these earliest anoxic changes as they became adults. The most obvious finding in adults was a reduced thickness of the outer half of the cortex, laminas II, III and IV (Figs. 4 to 6). Cytologically this was the result of the marked stunting in the growth of the apical dendrites extending from the pyramidal cells of these layers, and failure of the neurons to spread apart (Figs. 7 and 8). That the reduction in the thickness of the cortex was not due to a loss in numbers of cells was confirmed by a few comparative counts of the number of neurons in the columns of cortex with constant width in normals and abnormals. Although the cortex of asphyxiated animals was sometimes only about 4/5, or less, as thick as the normal, the same numbers of cells were present. For that matter, the packing is evident simply by inspection. In Figure 6 there are more neuron bodies per square unit area in the outer cortex of the asphyxiated animal.

Other characteristics of the adult cortex asphyxiated at birth were an exaggeration of the normal vertical columnated appearance of the cells in the outer layers and some tendency for the outer cells to be

clustered, residua of what went on in the first few days after anoxia. Bundling of long apical dendrites, especially from layer V, was occasionally prominent. The outermost cell bodies of the cortex normally show a somewhat irregular border at their junction with the relatively acellular lamina I, but in asphyxiated brains this line was often too straight. In asphyxiated brains the medial wall of the cerebral cortex above the corpus callosum sometimes sloped away from the midline more than in the normal, and the total bulk of brain in that region where cingular cortex, vertex neocortex and cingulum were in contiguous relation to each other was reduced. This reduction was similar to that in the adjacent neocortex—it was the result of cells not spreading out, or conversely, deficiencies of fiber development. In the cingular cortex the notable finding was the shorter dendrites of the pyramidal cells making up most of the thickness of this cortex.

The observations in sections examined with the Land Color Translating ultraviolet microscope confirmed a number of alterations seen with the conventional microscope and added one useful finding. The finer resolution of the ultraviolet microscope showed numerous dots of material scattered along the distal fibrils of young apical dendrites from normal cells, principally those in the deeper layers (V, VI) which absorbed strongly at 2,630 Å. This material was less prominent or much reduced in fibrils 24 hours after anoxia, and it was still reduced, compared with a matching normal, after 96 hours. It will be recalled that the fibrils were much thinner than 2 μ, the measurement set on the microtome for these sections. The whole thickness of fibrils was, therefore, always seen so that variations in section thickness should not invalidate comparisons as they might for larger structures. Although occasional spots of basophilic substance, removable by ribonuclease, were seen with the conventional microscope in these finest fibrils, they were close to the limit of resolution and difficult to examine. Other wave lengths were explored, including 2,800 Å, the absorption pattern of which qualitatively resembled that at 2,630 Å. Absorption at 2,800 Å might be interpreted as reflecting concentrations of certain proteins which in turn would reasonably be expected to be associated with RNA in regions of rather rapid growth.

Discussion

The inhibiting effects of anoxia on maturation and growth of nuclei in young nerve cells, the suppression of RNA propagation in dendritic cytoplasm, and the stunting of dendrite growth were unexpected findings. It is hard to relate these changes to available biochemical data, but it is known that protein synthesis measured *in vitro* by the incorporation

of amino acids into the process, is coupled to ATP production, and the over-all synthetic process is inhibited by oxygen lack.[12] RNA, in turn, is closely bound to protein synthesis.[13,14] In the view of some workers, small RNA molecules act as transfer agents in the cell, conveying the amino acids to the more complex RNA templates in the ribonucleoprotein of the ribosomes, where the protein is made. Whether the nucleus only initiates the formation of cytoplasmic RNA or whether it continuously builds it and supplies it to the cytoplasm wholly or in part does not have complete agreement. In the case of the rapidly differentiating infant nerve cell, where the nucleus is also changing, few precedents serve as the basis for speculation. What the present experiments do not distinguish, among other things, is whether the trouble begins in the nucleus and the consequences to RNA and protein synthesis follow, or whether several steps in protein synthesis may be affected because they are especially dependent, for example, on a critical level of ATP.[8] As a first step toward a possible better understanding of these matters, as well as the related problem mentioned in the final paragraph, we have begun experiments in which tritiated uridin is given to infant rats and the time and place of its uptake in the various parts of the brain cells in normal and asphyxiated animals are studied autoradiographically. In preliminary investigations, the uridin was rapidly taken up during the first hour by nerve cell nuclei, both immature and more mature, but little appeared in the cytoplasm. In subsequent hours and days it largely disappeared from the nucleus but appeared in the cytoplasm of the cell body and along the cell processes. The effects of anoxia on this incorporation have not yet been observed.

Considering the experiments in another light, it is reasonable to wonder whether the sensitivity of dendrite development to anoxia may be applicable to other mammals and to man, where resuscitation of asphyxiated infants is accepted practice. Although the rodents studied experimentally were closely related to each other, the possibility of a generalization about the sensitivity of growth of nerve cell processes is one that is worth exploring further because of some far-reaching implications. The first is the obvious one, that seemingly harmless asphyxia at birth in man may impair the fullest development of the individual's cerebral cortical structure. Knowledge of this will hinge on two things: one is the question whether the human cortex, which develops differently in some respects from the rodent[9] and is relatively a little more advanced at birth, is really as susceptible to anoxic injury as the rodent's. The other is the difficulty in obtaining closely matched sections of cortex from asphyxiated and normal babies. The success of the experiments in animals, especially where the changes are not severe, depends much on pre-

cise matching, which in turn is made possible by serial sections. Nonetheless, the lead is an important one and deserving of pursuit.

The second implication related to the possible general susceptibility of nerve cell processes to anoxic injury is more speculative. There is a growing concept among some neurobiologists, though it is not a universal one, that the neuron is a rather self-contained unit in which the cell body is the center for metabolic activity and that cytoplasmic constituents made there are propelled from the body out along the axonal and presumably also the dendritic fibers.[15,16] Embodied in this concept also is the idea that the nerve cell retains part of its embryonic status throughout life, and that in health and disease the protoplasm of the fibers is constantly being replenished, and, indeed, constantly being altered by the cell body center. Although there are experimental pathologic circumstances which suggest that the fibers may sustain local metabolic injury apart from any primary involvement of the cell body, the idea that "embryonic" growth is a perpetual characteristic of neurons leads to the question whether subtle alterations in the dendrites and axons should not be much more carefully sought for in various adult conditions in which anoxia is a factor. Continued study of the early stages in the embryo and neonatal animal, when the nerve cell fibers are still relatively easy to see, may show how best to go about this.

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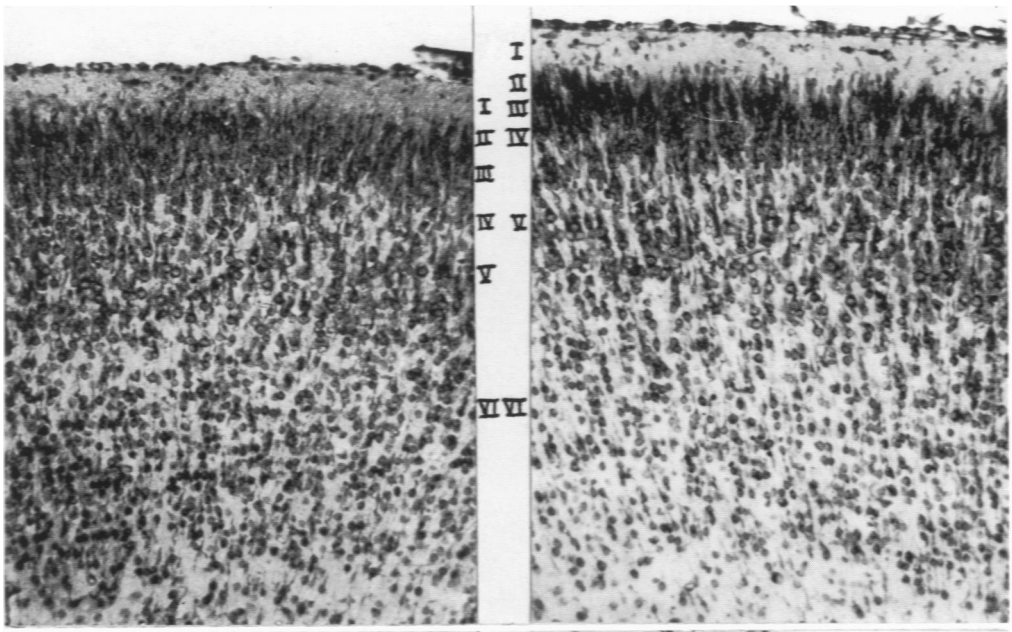
LEGENDS FOR FIGURES

FIG. 1. Vertex neocortex of a baby rat (left) one day after 30 minutes of anoxia induced when it was 24 hours old, compared with that of a normal litter mate. Cresyl violet stain. × 150.

FIG. 2. Vertex neocortex, slightly lateral, of a baby rat (left) 4 days after 30 minutes of anoxia induced when it was 24 hours old, compared with that of a normal litter mate. Cresyl violet and fast green stain, Carnoy's fixation, 5 μ. × 150.



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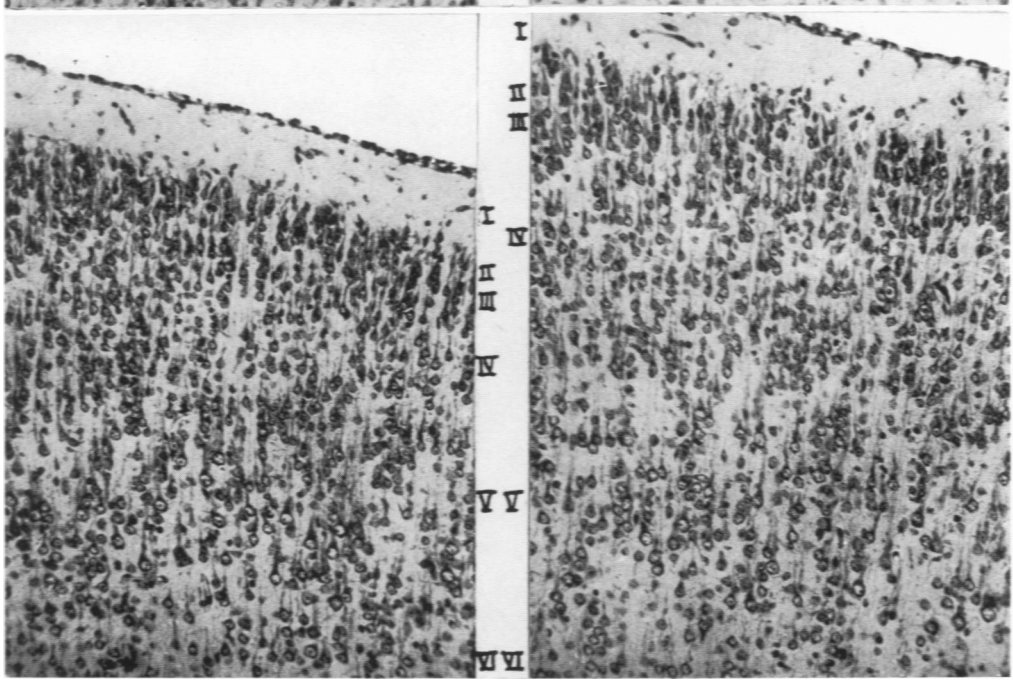

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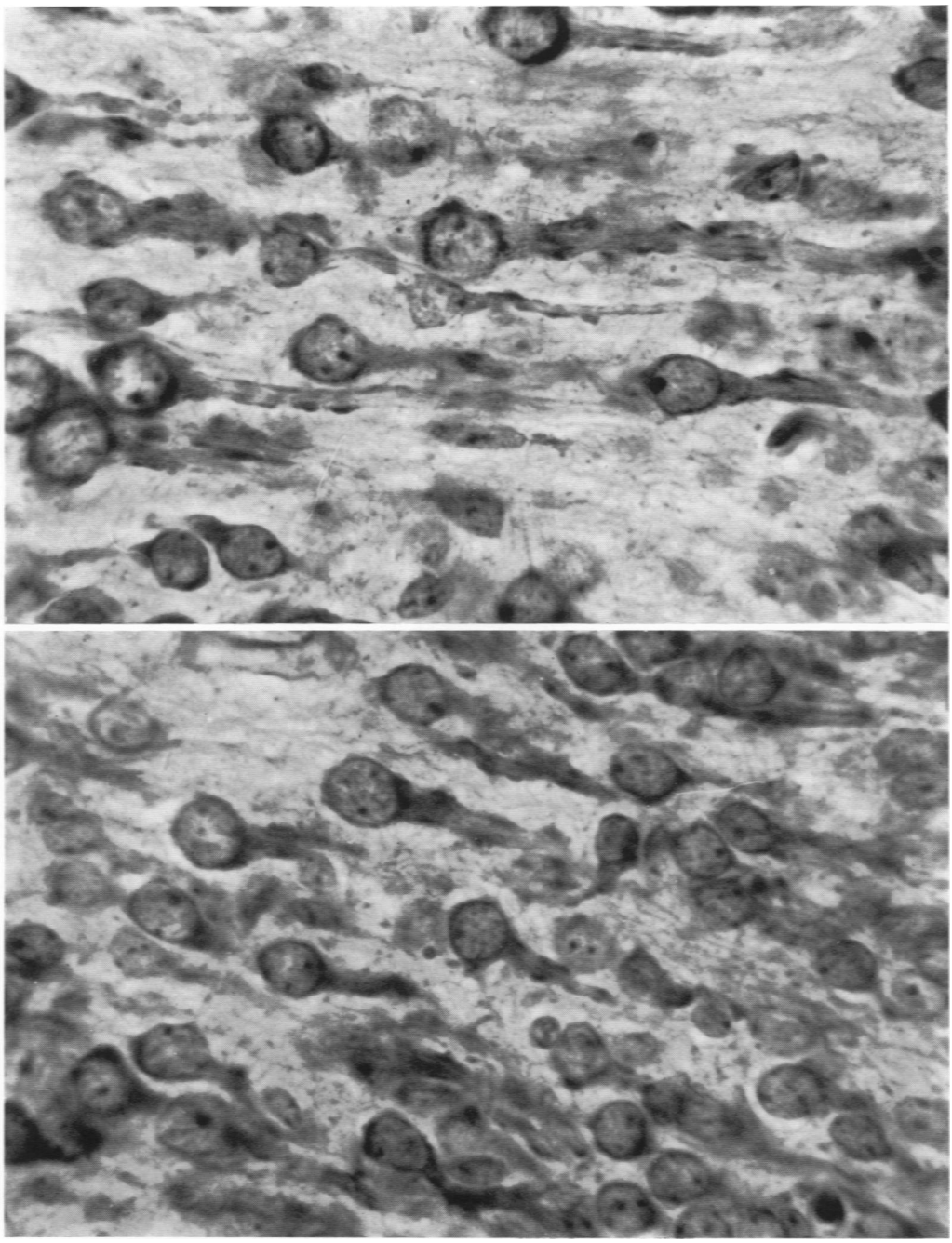

FIG. 3. Neurons in lamina V in a baby mouse (below) 3 days after 45 minutes of anoxia induced when it was 24 hours old, compared with that of a normal. The distal parts of the asphyxiated dendrites are almost transparent and shorter than normal. Cresyl violet and fast green stain. × 1,200.

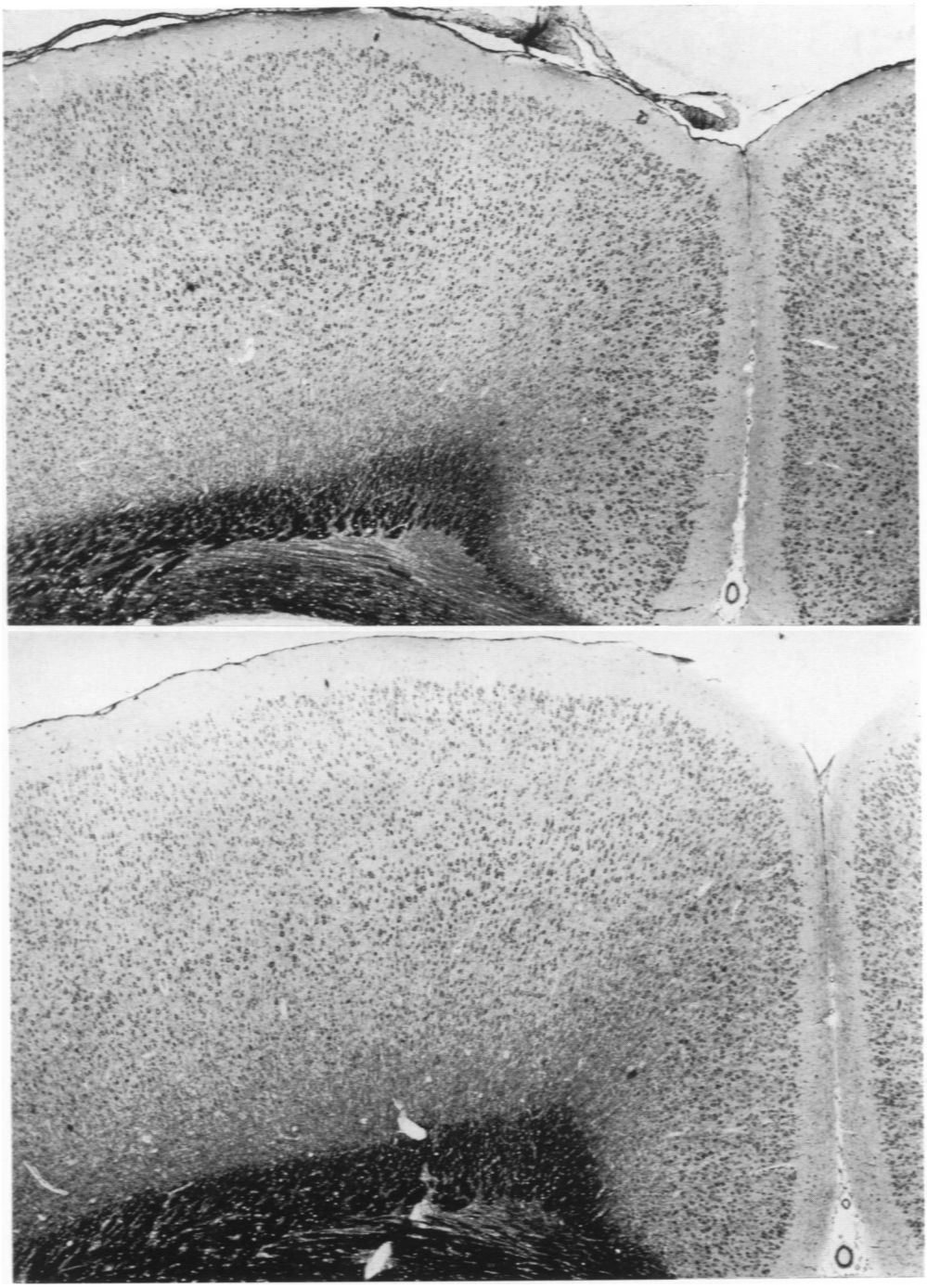

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FIG. 4. The cortex of a young adult (9 weeks) rat (above) subjected to 30 minutes of anoxia when it was 24 hours old, compared with a normal litter mate. The outer cortex from lamina V (large central neurons) outward is diminished in thickness in the asphyxiated brain. Cresyl violet and Luxol blue stains. × 40.

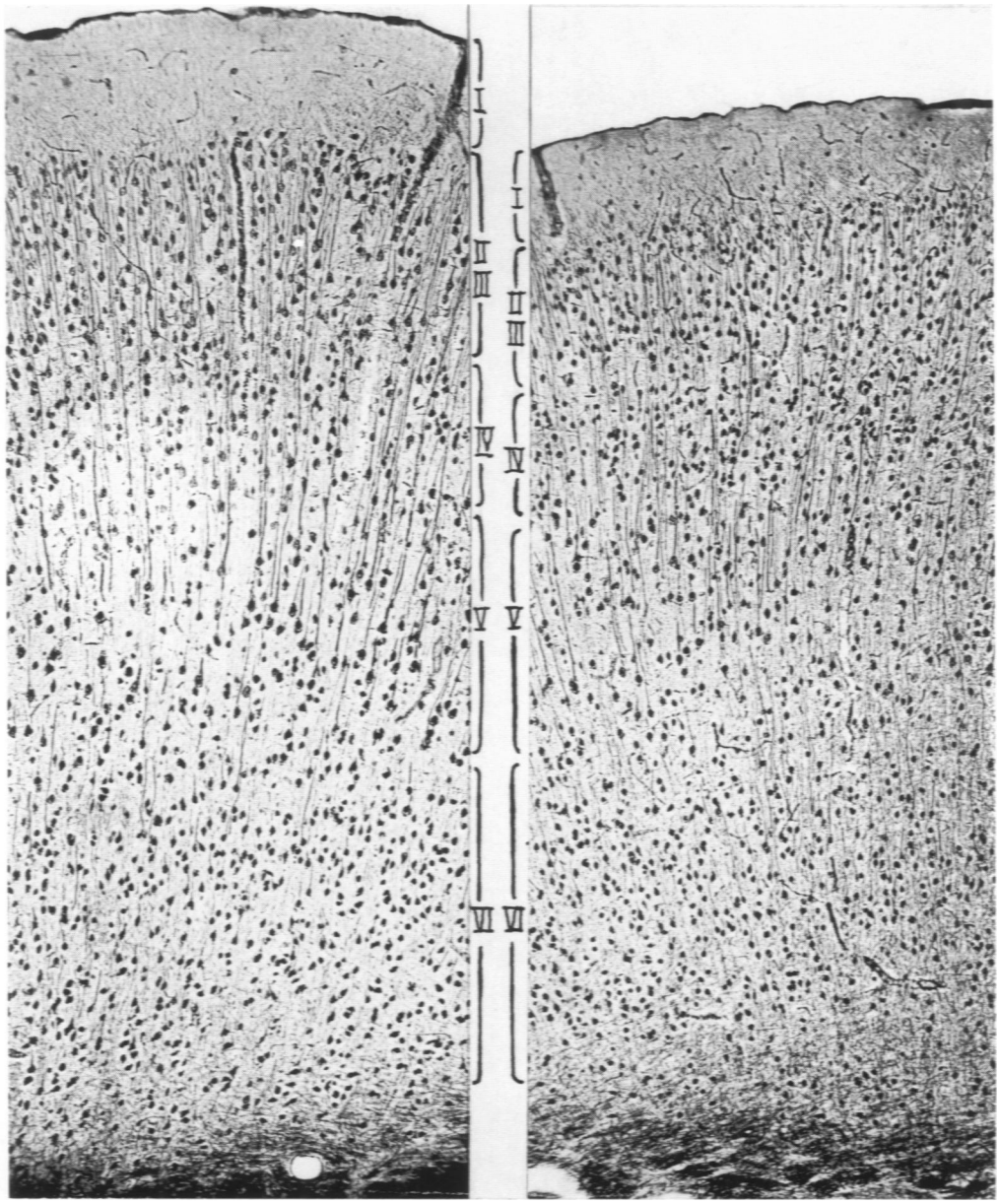


FIG. 5. The lateral neocortex, rotated 90 degrees, in a normal adult (9 weeks) rat compared with that of a matched animal (right) asphyxiated for 30 minutes at birth, to show apical dendrites. Cajal stain, Bouin's fixation, frozen sections, 20 μ. $\times$ 125.

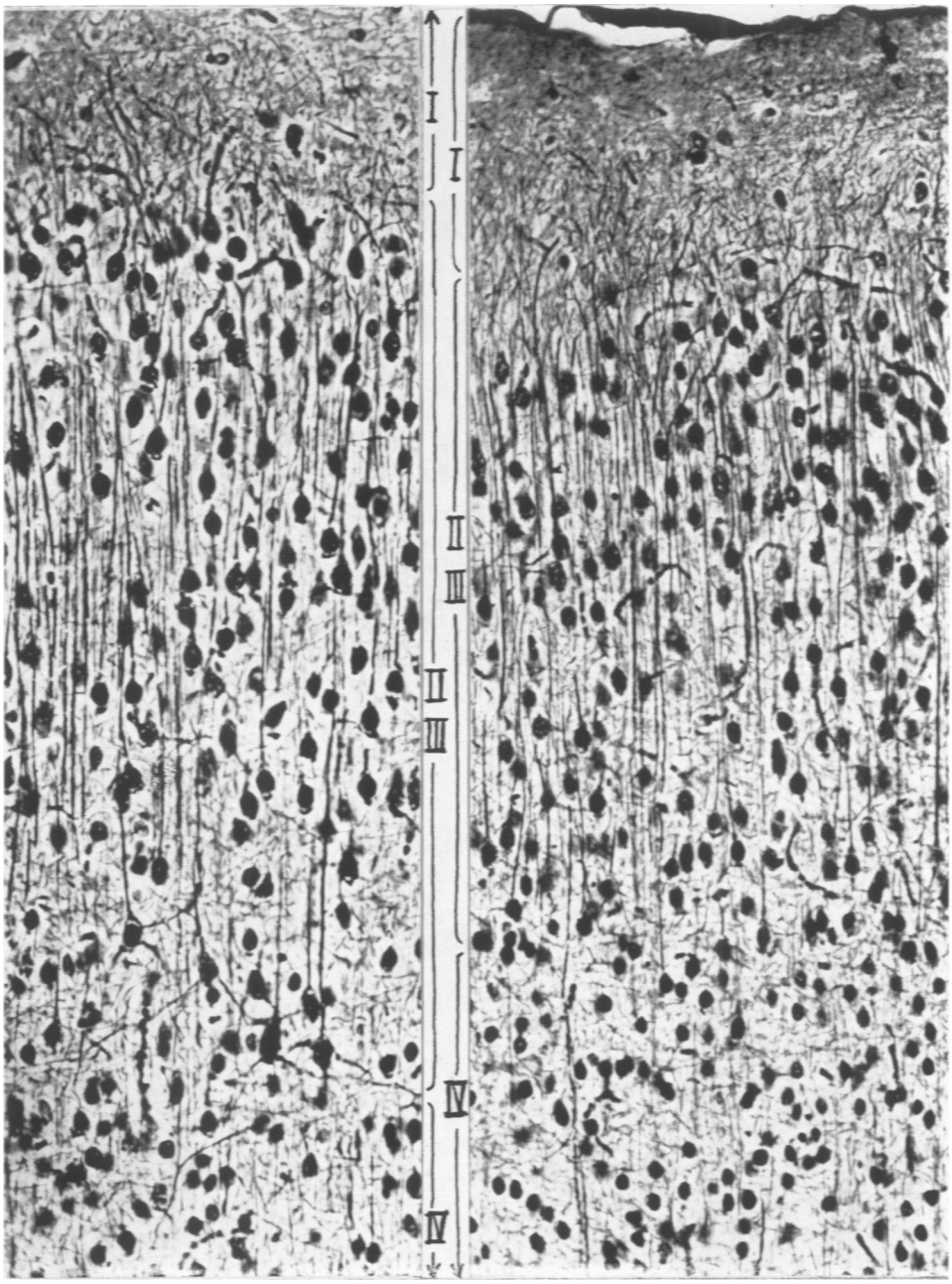
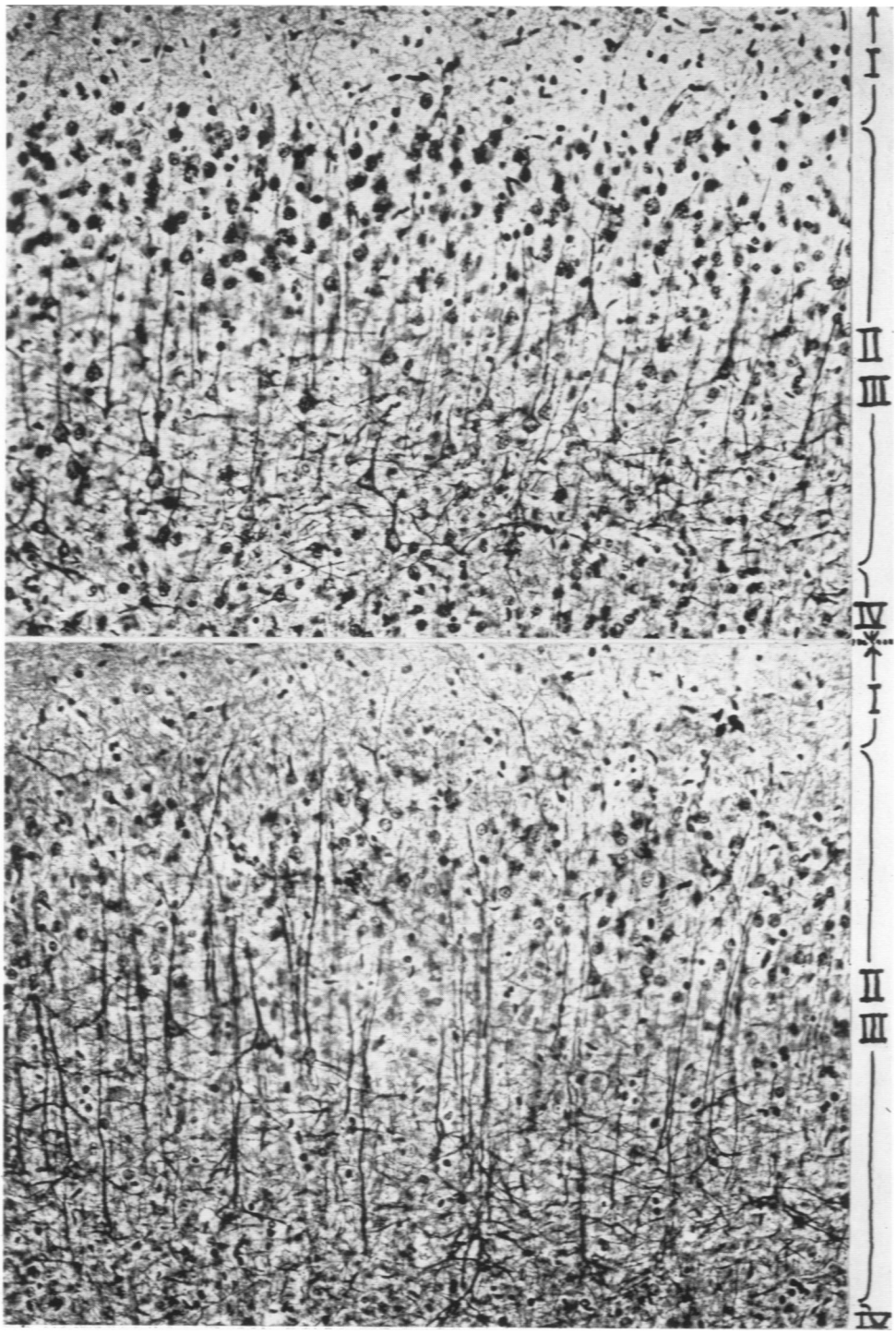


FIG. 6. Vertex cortex in a normal adult (9 weeks) rat compared with that of a matched animal (right) asphyxiated for 30 minutes at birth. Evident are closer packing of cells, shorter apical dendrites, and relative thickness of cortical layers. Cajal stain, Bouin's fixation, frozen sections, 20 μ. × 400.



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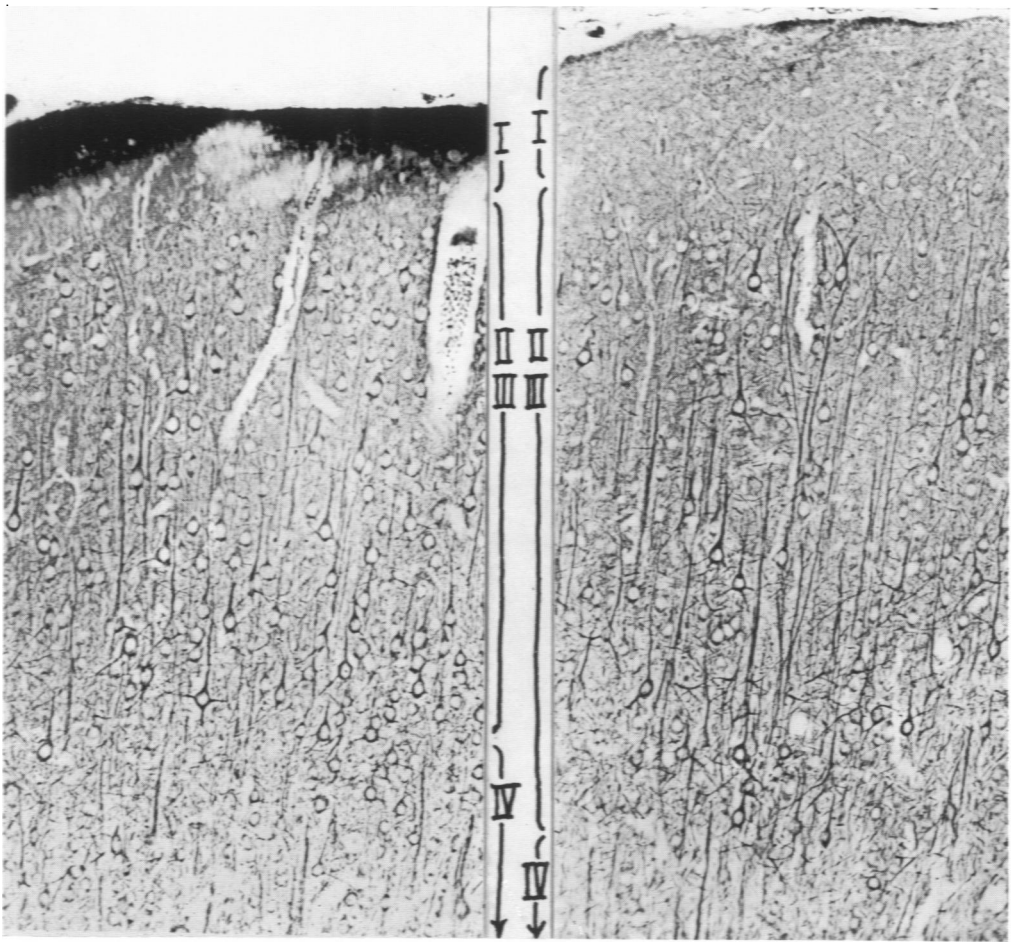

FIG. 7. Dorsolateral cortex in an adult normal (9 weeks) rat (below) compared with that of a litter mate asphyxiated for 30 minutes at birth. Thick sections with focus on some of the deeper pyramidal cells of layers II and III show the stubby dendrites of the asphyxiated cells. Layer IV is just visible at the bottom of each photograph. Cajal stain, formate formalin fixation, frozen sections, 30 μ. × 250.

FIG. 8. Vertex cortex, slightly lateral, in a normal adult (9 weeks) rat compared with that of a litter mate (left) asphyxiated for 30 minutes at birth. Cajal stain, paraffin sections, 15 μ. × 250.