ONTOGENY OF THE INFERIOR OLIVARY COMPLEX IN THE FETAL RHESUS MONKEY *(Macaca mulatta)*

by

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INTRODUCTION

An extraordinary number of investigations dealing with the various processes of development of either the whole brain or brain parts have been studied anatomically, behaviourally, biochemically, electrophysiologically, and pharmacologically in lower vertebrates, birds, and mammals, including man (Himwich and Himwich, 1964). Although most of these studies have emphasized the whole biological approach to normal development, some have shown an interest in the pathological development of the brain. Despite this proliferation of work, almost no data have been reported on the morphological changes in the developing brain of the rhesus monkey. This is surprising since this animal has proved to be a useful model for various other kinds of brain research.

Ontogenetic investigations have long been used to acquire an understanding of the organization of the central nervous system. Probably their most important single advantage is the visibility of neuronal groupings, interneuronal connections, the characteristics of individual neurons, and the migration of neurons and fibers before the infiltration of the dense networks of fibers which characterize the adult brain. In addition, the ontogenetic approach clarifies the mechanical conditions that bring about the major structural changes of the brain.

Recently, Portman, Alexander, and Illingworth (1972) investigated the changes in weight and chemical composition associated with development in 135 rhesus monkeys, ranging from 58 days gestation to over 10 years. They reported that (1) the relationship between brain weight and age forms a sigmoid curve, the greatest rate of increase occurring at about 125 days of gestation; (2) at birth the brain, which is about 70% of the adult size, reaches maximal weight within one year and remains nearly constant until

senescence. Kerr, Kenna, Waisman, and Allen (1969) reported similar findings for brain weight at birth, and Zuckerman and Fisher (1937) noted no significant change in brain weight after the permanent incisors and second molars are in place (about two years). In addition to whole brain weight, Portman et al. (1972) reported growth curves for parts of the brain (Fig. 1). Starting about 90 days of gestation, the cerebral hemispheres showed the first rapid increase in weight and continued to grow until about 170 days (five days after parturition) when there was a gradual increase until about one year of age. At a later age, the cerebellum of the rhesus monkey, like that of man and other species (Larsell, 1934, 1947; Jansen, 1954), grows more rapidly than the cerebrum and continues to increase in size after parturition. The brain stem grows very gradually until about 130 days gestation, when there is a rapid increase in weight until it reaches its maximal weight at parturition.

The present investigation is concerned with some of the major morphological changes within the brain stem and cerebellum during the period of maximal growth. In general, the sequential maturation of individual neurons, the formation of neuronal groups, and the distribution of nerve fibers are described. Specifically, the inferior olivary nuclear complex is examined in detail as a prototype of neuronal development in the lower brain stem since the spatial orientation of its cells, dendritic arbors, and axons as well as its structural formation is one of the most complicated, yet constant, aspects of the nervous system (Scheibel and Scheibel, 1955). Moreover, the inferior olivary complex was selected because it reaches its greatest size and internal complexity in man (Ariens Kappers, Huber, and Crosby, 1936) and thus suggests phylogenetic differences within the complex and behavioural changes in the function it subserves. In addition, the

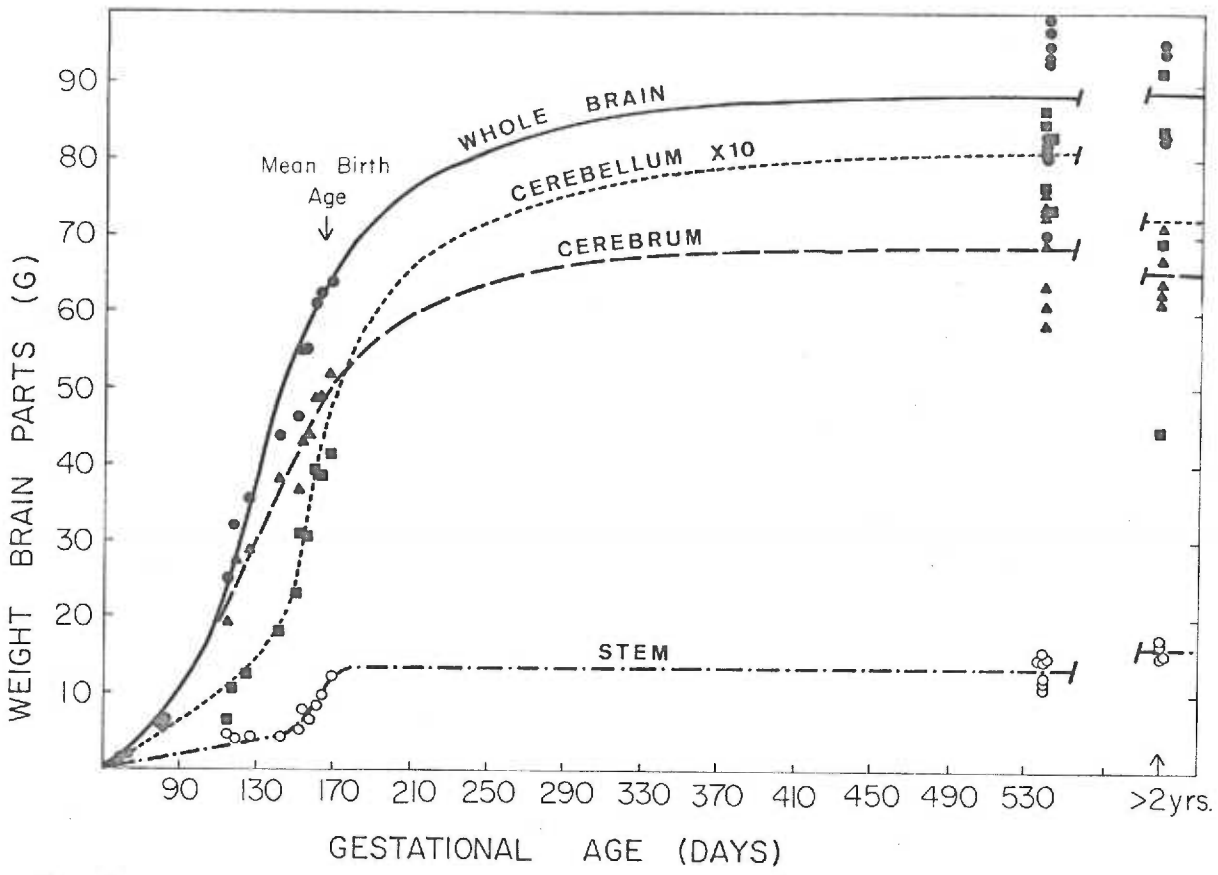


Fig. 1 The relationship of the weight of the brain and its parts to gestational age of rhesus monkeys. Weights from animals of the present study are included in the data for this figure. (Portman et al., 1972, Fig. 2, p. 200).

ontogenetic approach may provide additional insight into the distribution of its afferents whose areas of termination have been extensively studied. Finally, the inferior olivary complex is closely related through its efferents with the slower maturing cerebellum, which also has phylogenetically different regions.

The inferior olivary nucleus is a conspicuous cell group in the ventral portion of the lower brain stem. The important aspects of its phylogeny and comparative morphology have been summarized by Papez (1929), Verhaart (1970), Tateishi (1959), and Ariens Kappers et al. (1936). Kooy (1917) distinguished three divisions of this nucleus: a medial accessory nucleus, found in birds; and a principal nucleus which first appeared in birds (Vogt-Nilsen, 1954) and became progressively more prominent in the mammalian series. The principal nucleus can be further subdivided into a dorsal lamella and a smaller ventral lamella. The principal portion is largest in the primate brain where, as its name implies, it constitutes the major portion of the inferior olive (Ariens Kappers et al., 1936). The cells of the nucleus arise from the caudal part of the rhombic lip of the alar plate and migrate in a ventromedial direction to form, in higher vertebrates, its three divisions (Essick, 1912; Harkmark, 1954a, 1954b).

The cellular morphology of the inferior olivary nucleus was initially described in 1909 by Ramon y Cajal who described cells with small bodies and spherical dendritic patterns and afferents which he believed were collaterals from large ascending and descending fibers coursing over and around the olivary nucleus (Scheibel and Scheibel, 1955). Basing their report on an extensive study of a large group of animals, including a young monkey and a human infant, and using the Golgi method of impregnation, Scheibel and Scheibel (1955) gave a more complete description of the

olivary complex. They reported regional differences between various cell types within the inferior olive. For example, cells with large, simple, and relatively unramified dendritic arbors are generally found in the caudal regions of the dorsal and medial accessory nuclei. Another type has a smaller, highly ramified, spherical dendritic pattern, similar to that described by Cajal. These cells are located mostly in the rostral portions of the accessory nuclei and of the principal nucleus, which are the phylogenetically newer regions of the olive. Additional cell types were reported for the olivary nucleus of the monkey and man. One cell, occasionally observed in the dorsal accessory nucleus, has an extremely small cell body and an unramified dendritic pattern. Another cell, occasionally found in the middle of the gray matter of the principal nucleus, has a very extensive dendritic arbor spread over perhaps 100 or more olivary cells (Scheibel and Scheibel, 1955).

Although not as distinct as the dendritic patterns, regional variation in the distribution of different types of afferent terminals has been demonstrated by the Golgi method (Scheibel and Scheibel, 1955; Scheibel, Scheibel, Walberg, and Brodal, 1956). Three types of axonal endings have been observed in most areas of the inferior olive: the bushy terminals originally described by Cajal; a heavy rosette-bearing type; and a thinner, bouton-bearing type. The first appear to be everywhere; the heavy rosette fibers are particularly abundant in the ventral lamella of the principal nucleus; and the sparsely distributed bouton-bearing type are observed in the dorsomedial part of the dorsal accessory nucleus, in the ventrolateral regions of the medial accessory nucleus, and in both the dorsal and ventral lamella. For the most part, boutons impregnated with the Glees method do not show clearcut regional differences. Only the ventral lamella, especially the caudal half, shows

a very high density of boutons; the other regions show scarcely any (Blackstad, Brodal, and Walberg, 1951).

Many different parts of the nervous system have been suggested as possible sources for the inferior olivary afferents (Crosby, Humphrey, and Lauer, 1962). The use of various techniques and many different species of animals may partially account for the widely discrepant data on the subject. Three fairly large groups of fibers, which have been reported by a number of investigators, project onto distinct regions of the olivary complex: (1) the first ascends from the spinal cord and projects onto the caudal regions of the dorsal and medial accessory nuclei; (2) a large descending connection from the red nucleus and surrounding area is localized primarily in the rostrodorsal portions of the principal nucleus and, to a limited degree, in the rostral sections of the accessory nuclei; (3) another descending small group of fibers from the cerebral cortex, especially the frontal and parietal lobes, is mainly distributed to the ventral lamella and the medial accessory nucleus.

One of the first experimental demonstrations of the spinal cord fibers terminating within the olivary complex was by Wilson and Magoun (1945). Using the Marchi method, they reported that cats showed degeneration in the dorsal accessory nucleus after hemisection of the cord at C1 and C2. Similar findings were observed in a more extensive study in which the brain stems were examined with the Glees method after lesions had been made at various regions within the cord at several different levels (Brodal, Walberg, and Blackstad, 1950). Except for the most rostral region of the dorsal accessory nucleus, they found terminal and preterminal degeneration throughout the nucleus, particularly in the caudal, ventrolateral portions. Although not reported in the Wilson and Magoun (1945) paper, possibly because of the

use of the Marchi procedure, extensive degeneration was also observed in the ventrolateral parts of the caudal half of the medial accessory nucleus (Brodal et al., 1950). In addition, they reported that whereas the spino-olivary fibers originate in the posterior horn in all levels of the spinal cord, most of the afferents are from the lumbosacral region, only a sparse number being from the cervical and upper thoracic segments. Most of the spino-olivary fibers ascend in the ventral and, to a limited extent, in the lateral funiculi of the spinal cord. Using other techniques, several authors have reported similar patterns of distribution of spino-olivary fibers in the cat (Anderson and Berry, 1959; Johnson, 1954; Kusama, 1961), in the rabbit (Mizuno, 1966), and in the monkey (Mehler, Feferman, and Nauta, 1960).

Despite much contradictory evidence, the caudal regions of the principal nucleus have also been reported to receive spinal afferents (Anderson and Berry, 1959; Bowsher, 1962; Nauta and Kuypers, 1958). Minimal degeneration was observed bilaterally in the caudal parts of the principal nucleus in four of seven human cases of spinal cordotomy or medullary tractotomy performed to relieve intractable pain (Bowsher, 1962). No degeneration, however, was seen in either the medial or dorsal accessory nuclei. In another human case, Mehler (1962) did not observe any degeneration in the principal nucleus after a bilateral middle cervical cordotomy. The absence of degeneration in the principal nucleus after lesions of the spinal cord was also noted by Mizuno (1966) in the cat and by Mehler, Feferman, and Nauta (1960) in the monkey.

A large descending fiber bundle from the red nucleus to the rostral inferior olivary complex has been reported for normal and pathological material and for experimental work on both monkeys and cats (Bebin, 1956; Herrman and Brown, 1967; Hinman and Carpenter, 1959; Lapresle and Hamida,

1970; Papez and Stotler, 1940; Poirier and Bouvier, 1966; Stotler, 1954; Verhaart, 1949; Walberg, 1954, 1956, 1958; Woodburne, Crosby, and McCotter, 1946). In general, these investigators have shown that interruption of rubro-olivary fibers results in terminal degeneration in the rostrodorsal portions of the principal olivary nucleus, especially the dorsal lamella (Stotler, 1954; Walberg, 1956). Most investigators agree that the tract originates in the rostral, small-cell part of the ipsilateral red nucleus (parvicellularis), which, incidentally, is better developed than the caudal region in higher mammals (Arien Kappers et al., 1936). The morphological study of Poirier and Bouvier (1966), which included retrograde cellular degeneration of more than nine months duration and short-term fiber degeneration, showed that monkeys with lesions that interrupted the rubro-olivary fibers, with or without a partial or complete involvement of the ipsilateral superior cerebellar peduncle, experienced a complete cell loss in the parvicellular part of the ipsilateral red nucleus. If the rubro-spinal fibers were spared, such lesions did not produce any cell loss in the contralateral red nucleus nor in the ipsilateral magnocellular (caudal) part of the red nucleus. The rubro-olivary fibers project to the inferior olivary complex via the central tegmental fasciculus (CTF), which includes fibers from other origins. This pathway apparently is the same as the tegmento-olivary tract of Woodburne, Crosby, and McCotter (1946), and the anulo-olivary tract of Mettler (1944), who contended that no rubro-olivary fibers exist. Although the rubro-olivary tract is now well documented, the anulo-olivary tract, which consists of afferents to the olive that originate from the periaqueductal gray, also has its supporters. Lesions of the periaqueductal gray have been associated with degeneration of terminals not only in the dorsal lamella but also in the ventral lamella and medial accessory nucleus of the

cat (Hinman and Carpenter, 1959; Mabuchi and Kusama, 1970; Walberg, 1956) and the monkey (Poirier and Bouvier, 1966).

Initially, the cortico-olivary fibers were briefly described as part of an extensive series of investigations of cortical projections (Mettler, 1935, 1947). After large lesions made in various parts of the frontal and parietal cortex in the monkey, Marchi-prepared sections of the lower brain stem showed degeneration in the "medial segment of the inferior olive" (Mettler, 1935, p. 536). The cortico-olivary fibers were described as accompanying the corticospinal efferents. Using electrophysiological techniques, Snider and Barnard (1949) recorded evoked potentials in the inferior olive of the monkey and cat after unilateral application of strychnine to what they vaguely described as "various areas of the cerebral cortex" (p 253). A more precise distribution of cortical projections within the olivary complex of the cat was reported by Walberg (1956) who used the silver impregnation technique of Glees. The majority of cortico-olivary afferents originated from the sensorimotor part of the cortex, with a few fibers arising from the temporal lobe and almost none from the medial surface of the hemispheres. Again the cortico-olivary fibers were seen to travel in conjunction with the corticospinal tract and to project bilaterally upon the olivary nucleus. Most of the afferents terminated on cells in the ventral lamella, a smaller percentage within the medial and dorsal accessory nuclei. Mettler's use of the Marchi method probably accounts for the limited terminal distribution observed by him (Mettler, 1935, 1947).

A clear somatotopical organization within the cortico-olivary projections to the three main divisions of the olivary complex was demonstrated in a series of experiments in the cat (Sousa-Pinto, 1969; Sousa-Pinto and Brodal,

1936; Walberg, 1956).

The anatomical studies of the efferent fibers of the inferior olivary complex in the cat (Brodal, 1954) greatly expanded and confirmed the work of Holmes and Stewart (1908) in man. Both studies demonstrated that efferents of inferior olivary cells form a distinct bundle within the inferior cerebellar peduncle and then project solely to the cerebellar folia and most of the deep cerebellar nuclei. A most striking point-to-point relationship exists between specific parts of the inferior olive and specific lobes of the cerebellum, which sets it apart from all other afferent cerebellar pathways. In general, the accessory nuclei project to the vermian part of the cerebellum. The anterior lobe receives olivary fibers from the dorsolateral parts of the dorsal accessory nucleus and from the intermediate and caudal, ventrolateral sections of the medial accessory nucleus; the principal nucleus sends efferents to the cerebellar hemisphere, where there is similar medial-lateral distribution of fibers in both structures (Brodal, 1954). Since the experimental anatomical investigations in 1959 of Szentagothai and Rajkovits (as described in Hamori and Szentagothai, 1966) and of Fox, Hillman, Seigesmund, and Dutta (1967), and the electrophysiological investigations of Eccles and his collaborators (Eccles, Llinas, and Sasaki, 1964, 1966), there is now general agreement that the olivocerebellar fibers terminate as climbing fibers around the Purkinje cells. This recalls Dow's (1942) earlier opinion, which was not subscribed to by all investigators, that the logical source of climbing fibers is the inferior olivary nucleus. However, in a recent study of the discharges evoked in Purkinje cells by climbing fiber activity produced by afferent stimulation in a group of intact animals and in a group of animals with total bilateral chronic lesions of the olivocerebellar pathway, Batini and Pumain (1968) demonstrated

that only a part of the climbing fibers in the posterior lobe of the vermis had their origin in the inferior olivary nucleus. In a more specific electrophysiological study in the frog, Llinas, Precht, and Kitai (1967) reported that vestibular root fibers end as climbing fibers.

In the present study the main focus has been upon the morphological changes in the inferior olivary nucleus as a single entity; in more general terms, those areas of the lower brain stem that are either directly or indirectly neuronally related to the inferior olive were also examined. Therefore, the present observations have attempted to answer the following questions:

1. What are the general structural and internal morphological changes in the inferior olivary nucleus at different stages of fetal life? Do different parts of the inferior olive develop at different rates? Are there identifiable mechanical forces that influence the shape of either the whole olivary complex or of any of its divisions?

2. At the various stages of inferior olivary development, what other parts of the lower brain stem are showing similar neuronal organization?

3. At what age do the various afferents project upon the inferior olive? Do afferents from different structures have distinct terminal areas, or do some of these contingents share projection areas within the olivary complex? How do the various patterns of terminal distribution change with age?

4. When do the olivary efferents become identifiable and is the time the same for all parts of the inferior olivary complex? How does

appearance of the olivary efferents correlate with the morphological development of the cerebellum?

METHOD

Brain stems from 26 rhesus monkeys (*Macaca mulatta*) ranging from 60 days gestational age to 10 months after parturition were examined (Table I). The brains were the byproducts of several different experiments by a large group of investigators over a four-year period. Complete histories were available for all specimens, and exact ages of all animals except one were known. Fetus #18, which died in utero a short time before being aborted, may have been as much as two days younger than the recorded age. In all cases, the mothers had been maintained during pregnancy under standard conditions of light, temperature, and humidity and had been fed a commercial monkey chow with supplements of vitamins and seasonal fruit. Medications had been prescribed only for specific illnesses.

Twenty of the brain stems were from fetuses ranging in age from 60 days gestation to parturition, which in this species is 164 ± 4 days (Hartman, 1932). Except for the stillbirth, the fetuses were obtained either by Caesarean section or experimentally induced abortion. It should be noted that experimental procedures on four of the fetuses may have altered the normal neuronal development; however, as yet, no obvious changes have been detected. The 92-day fetus was aborted two days after ligation of an interplacental vessel. The 146-, 147- (#15), and 153-day fetuses were part of an experiment in reproductive biology, in which the 146- and 153-day fetuses had bilateral orchiectomies at 109 and 105 days of gestation respectively, and the 147-day fetus underwent a sham bilateral oophorectomy in utero at 105 days gestation.

The remaining six brain stems were from monkeys of natural birth. On the basis of morphological and behavioural development, these monkeys can be divided into neonates (from parturition to one month, #21 and #22)

	Subject #	Days of Gestation	Sex	Body Wt. (gm)	Brain Wt. (gm)	Plane of Section: Sagittal	Coronal	Horizontal
FETUS	1	60	M	11	-	x	x	
	2	82	F	52	-	x	x	
	3	92	M	82	12	x		
	4	100	F	129	16.3	x		
	5	100	F	122	16.5		x	x
	6	102	F	114	16.0	x		
	7	107	M	154	19.9	x	x	
	8	109	M	200	25.7	x		
	9	121	M	238	34.5	x	x	
	10	127	M	289	39.5	x		
	11	129	M	271	40.7		x	
	12	134	M	302	47.0	x		x
	13	137	M	244	41.9		x	
	14	146	M	376	50.3	x		
	15	147	F	370	47.0	x		
	16	147	F	351	44.1		x	
	17	153	M	379	58.8		x	x
	18	154	M	402	56.4		x	
	19	155	M	486	56.5	x		
	20	157	F	393	56.0	x		
Parturition								
INFANTS	21	195	M	487	62.4	x		
	22	195	F	371	51.1		x	
	23	245	F	525	61.5	x		x
	24	255	M	960	77.6	x		
	25	257	F	600	67.2		x	
	26	475	F	1150	75.6	x		

Table 1 Distribution of monkeys from which the brain stems were analysed. In some cases animals of the same or approximately the same age were considered as a single developmental stage, as indicated by shading and lack of shading, e.g., #4, 5, and 6 are one developmental stage, whereas #7 and 8 are another stage.

and infants (from one month to ten months, #23, #24, #25, and #26). All the monkeys had been under close observation and were in good health before the terminal experiment or illness.

Complete autopsies and histologic examinations were performed on all animals as soon as possible after death. No monkeys that showed any developmental malformation or obvious disease were included. Total body weights were recorded, and organs were carefully dissected free and weighed. "Brain weight" represents the combined weights of the cerebral hemisphere, cerebellum, and brain stem. To reduce the risk of misinterpretations because of postmortem changes, the brains were rapidly dissected free of the skull and processed early in the autopsy. The brain stem was disarticulated from the rest of the brain by a straight cut through the peduncle and midbrain and then in most cases was sectioned sagittally in the midline. One-half of the brain and stem was generally used in various neurochemical analyses, whereas the remaining parts were placed into a 10% neutral formalin solution and saved for morphological examination. Usually 15 to 20 minutes elapsed between death and the beginning of formalin fixation. Fixation continued for at least two weeks, in most cases over a month, and in a few instances for as long as three years. After adequate fixation, the cut brain stems were dehydrated in various concentrations of ethyl alcohols and infiltrated with paraffin (Appendix I).

Since one of the primary objectives of the present investigation was to identify the various afferent pathways of the inferior olivary complex, at each age level one-half the brain stem was sectioned sagittally, proceeding from the midline laterally. Whenever possible, either the contralateral symmetrical half of the brain stem or a brain stem from animals of the same or approximately the same age were cut in either the

transverse (coronal) or horizontal plane. In all cases, the embedded brain stems were sectioned serially at either 10 or 15μ with a Spencer rotary microtome. Every tenth section was stained by the silver reduction method developed by Stotler (1958); in some cases, the adjacent series of sections were stained for cells with cresyl fast violet (Appendix II).

Interpretations of various fiber connections were made only after verification of adjacent slides and similar areas in subsequent ages. In addition, an attempt was made to identify, with the aid of oil immersion, the terminal processes of the neurons.

Parts of the data were analysed with the aid of an IMLAC PDS-1 Graphics Computer (IMLAC Corporation, Needham, Mass.) which was interfaced with a Computex GT50 Graphical Tablet (Computex, Inc., Cambridge, Mass.) and a teletype for data output. The PDS-1 computer was programmed to calculate areas of images drawn on the computer graphical tablet. As the given image was drawn on the tablet, the coordinate points (1000 lines on the X-axis, and 1000 lines on the Y-axis) were stored by the computer. The areas of the images were then calculated by the computer at a signal from the operator and the answers printed out on the teletype.

Microphotographs were made on a Zeiss Ultraphot II. The negatives were made on 4" by 5" Kodak Pantomic X sheet film, developed in Microdal X, and printed for medium and high contrast on Ektamatic-SC paper. All cell measurements were made with a stage micrometer and calibrated ocular. Drawings were made from microphotographs of the brain stems for each age group. Three-dimensional models were constructed from serial drawings of the inferior olivary complex.

OBSERVATIONS

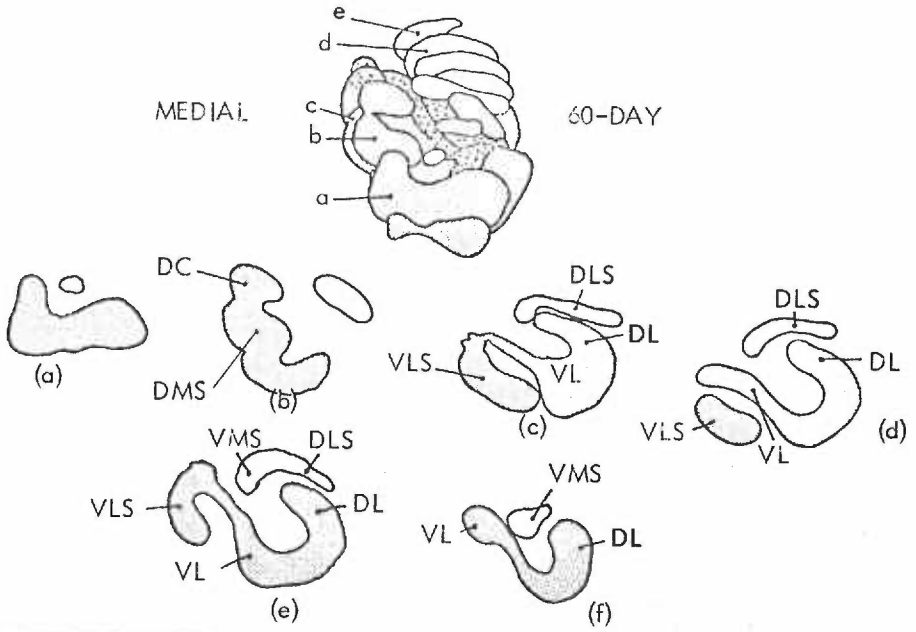
A complete understanding of the inferior olivary complex necessitates a detailed examination of its structure and various subdivisions and a comprehension of its relationship to the rest of the lower brain stem and cerebellum. Consequently, these observations will deal with (1) the general architecture, including maturational changes of major divisions and smaller subdivisions at different fetal ages; (2) developmental changes of the whole lower brain stem, including descriptions of the main morphological landmarks of the lower brain stem, with special emphasis on those structures that send afferents to different parts of the olivary complex or that share common innervation, and on the cerebellum, which is the recipient of the olivary efferents.

A. Structural Organization of the Inferior Olivary Complex

By 60 days of gestation, the inferior olivary nucleus can be clearly delineated from the surrounding undifferentiated lower brain stem. Although the internal complexity of the nucleus increases with age, its overall pattern persists throughout the various developmental stages. Three-dimensional diagrams of the different states show the relationship of the three main divisions: (1) the principal nucleus, (2) the medial accessory, and (3) the dorsal accessory nuclei, so-called because of their relationship to the principal nucleus (Figs. 2 and 3). Immediately apparent is the consistent relationship between divisions: the principal nucleus has the most complex structural arrangement and the greatest width; the medial nucleus extends the farthest caudally and, since the divisions have the same approximate rostral poles, has the greatest length; and the dorsal accessory nucleus is the least complex and the smallest.

Fig. 2 Structural changes with age are illustrated in three-dimensional diagrams of the inferior olivary complex of the 60- and 129-day fetuses. Below them are representative coronal sections from the model showing the main subdivisions of the complex. Because of the orientation of the model, sections "f" of the 60-day fetus and "d" of the 129-day fetus cannot be seen.

DLS	dorsal-lateral segment
VMS	ventral-medial segment
DL	dorsal lamella
VL	ventral lamella
DC	dorsal cap
VLO	ventral-lateral-outgrowth
DMS	dorsal-medial segment
VLS	ventral-lateral segment


MEDIAL
60-DAY
e
d
c
b
a
DC
DMS
(a)
(b)
DLS
DL
VLS
VL
(c)
(d)
VMS
(e)
(f)

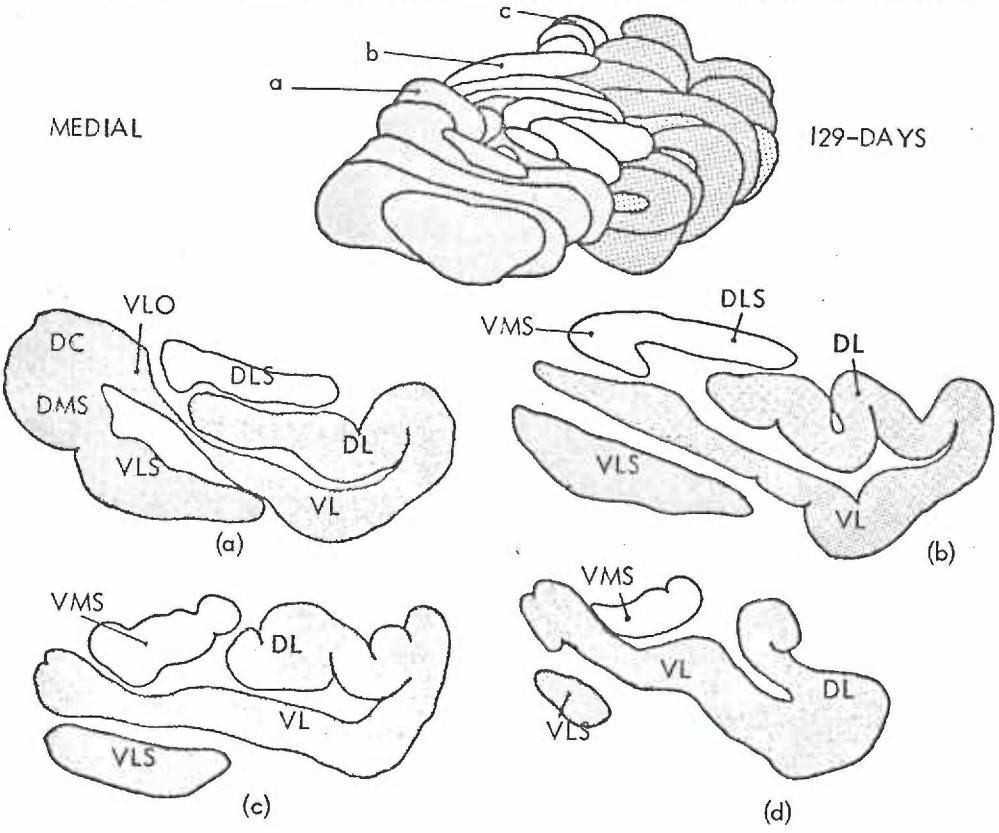
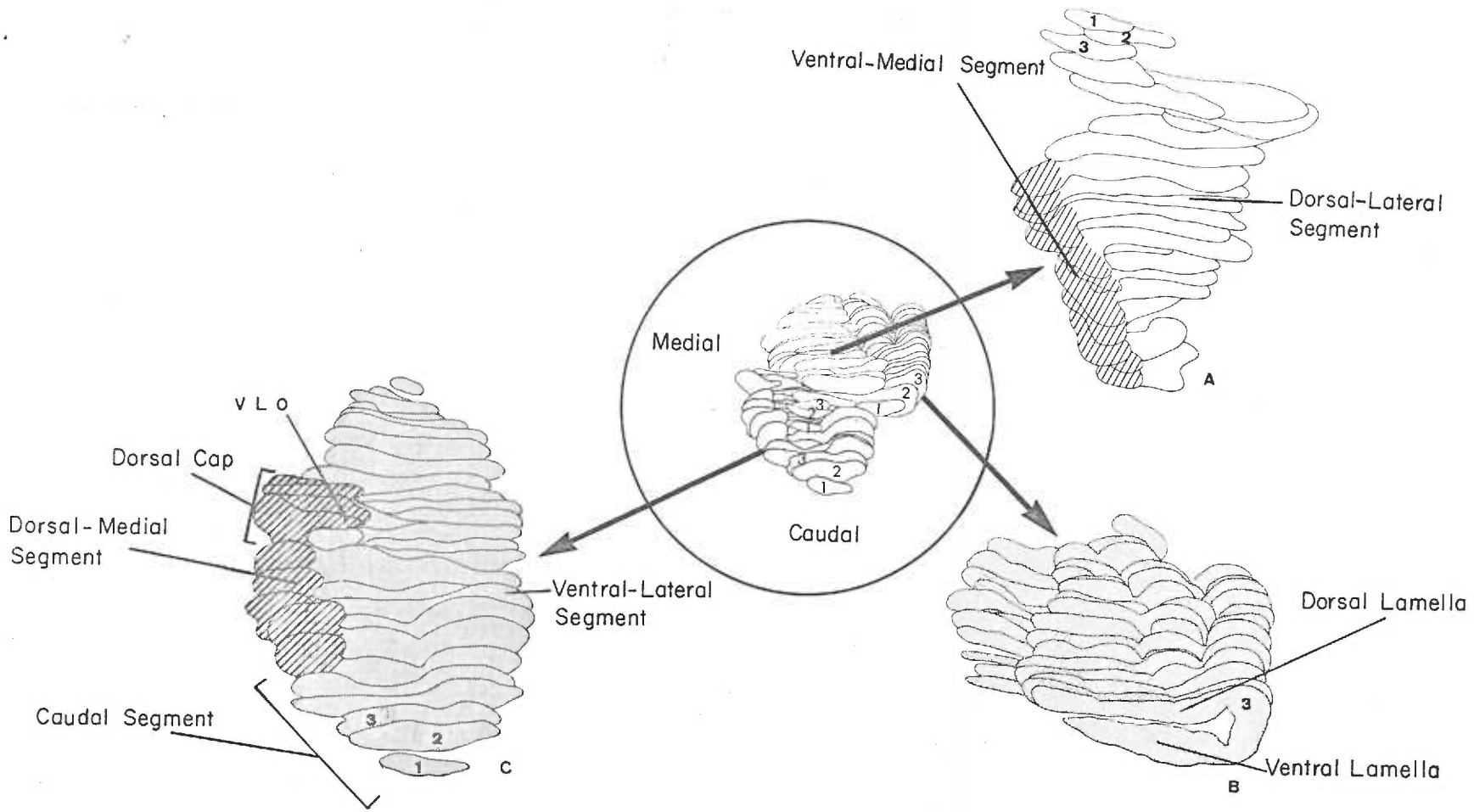

MEDIAL
129-DAYS
c
b
a
VLO
DC
DMS
DLS
DL
VLS
VL
VMS
(a)
(b)
(c)
(d)
Dorsal accessory
Medial accessory
Principal

Fig. 3 Three-dimensional diagram of the inferior olivary nucleus and its three main divisions of the 147-day fetus. The first few caudal sections of each division are numbered to aid orientation. The dorsal accessory nucleus (A), depicted from its ventral surface, can be divided into a dorsal-lateral segment, which extends over the dorsal lamella, and a ventral-medial segment that is rostrally attached to the medial extension of the ventral lamella. The principal nucleus (B) has had its first and second caudal segment removed to demonstrate its dorsal lamella, lateral band, and ventral lamella. The medial accessory nucleus (C) can be subdivided into a small caudal segment, a dorsal-medial segment that is topped by the dorsal cap and the ventral-lateral-outgrowth (VLO), and a large ventral-lateral segment, which extends rostrally under the ventral lamella.


Ventral-Medial Segment
Dorsal-Lateral Segment
A
Medial
Caudal
V L O
Dorsal Cap
Dorsal-Medial Segment
Ventral-Lateral Segment
Caudal Segment
C
Dorsal Lamella
Ventral Lamella
B

Changes in the length and area of the three nuclei can be quantified. An approximation of their length can be made from the number of sections (150μ between stained sections) on which each nucleus appears (Fig. 4). A prominent feature is the constant relationship between each nucleus. At all stages of development, the medial accessory nucleus is always the longest, the dorsal accessory nucleus is shorter, and the principal nucleus is the shortest. The length of the total olivary complex closely corresponds with that of the medial accessory nucleus because the greatest rostrocaudal variation between the nuclei is the change in length of the caudal portion of the medial accessory nucleus. The greatest period of growth for all nuclei occurs between 60 and 121 days, when the dorsal accessory and principal nuclei more than triple their length and the medial accessory nucleus almost triples its length. After 121 days, the dorsal accessory nucleus generally maintains a constant length until one month after parturition when a gradual increase (450μ) occurs until three months. The principal and medial accessory nuclei, on the other hand, show some variability between 121 days and parturition, although the principal nucleus shows almost no increase in length after 137 days. However, by three months after parturition, the medial accessory nucleus has increased its length more than 1000μ from that of the 121-day stage.

The total area of each nucleus increases with age; however, the proportional area between the three divisions remains almost constant (Table 2). At every stage of development, the principal nucleus constitutes proportionally the greatest area of the olivary complex, the dorsal accessory nucleus the least. The principal nucleus shows a slight increase in its proportional area between 60 and 257 days, whereas the medial and dorsal accessory nuclei tend to decrease slightly during the same period.

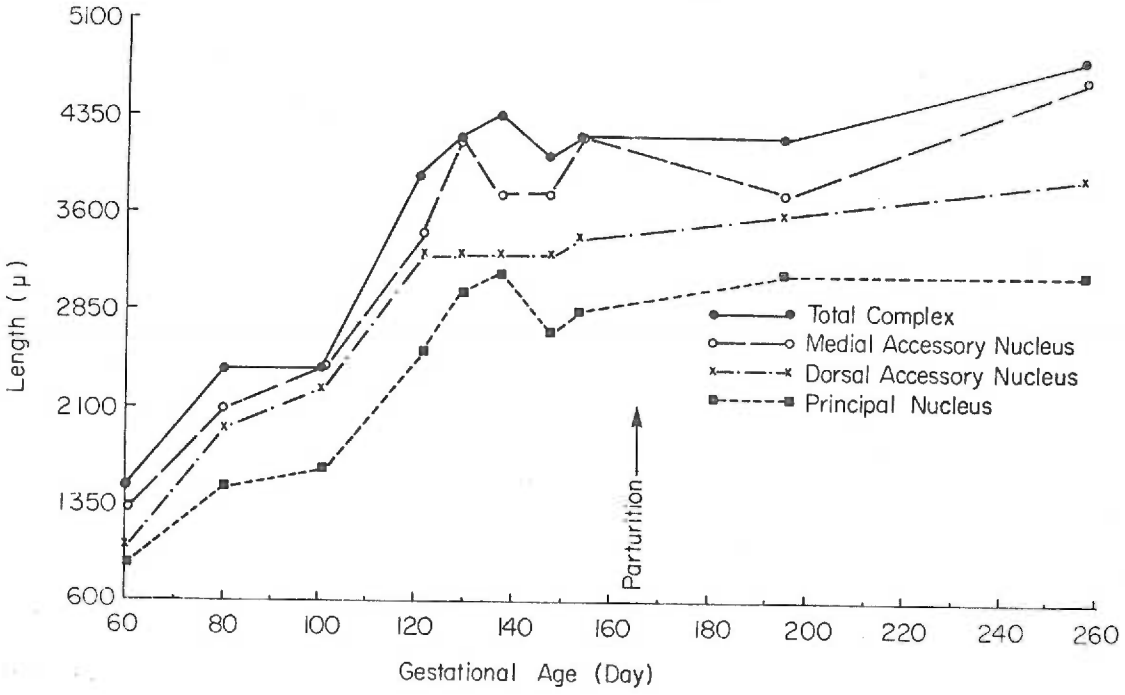


Fig. 4 Relationship of the rostrocaudal length of the total olivary complex and its three main divisions at different ages.

Table 2. Percent of the total olivary complex for the three main nuclei at various days of gestation

Age	Nucleus		
Days Gestation	Principal	Medial	Dorsal
60	46.2	38.8	14.9
80	47.1	36.7	16.2
100	49.0	36.2	14.8
121	47.7	35.4	16.9
129	55.0	32.1	12.9
147	50.7	35.8	14.6
257	53.1	34.0	12.5

The medial accessory nucleus, the larger of the accessory nuclei, can be subdivided into a ventral and a dorsal area (Fig. 3). The ventral divisions can be further separated into a massive, oval-shaped caudal region and a smaller, gradually narrowing, ventral-lateral segment, which extends rostrally under the principal nucleus. The proportions between the caudal region and the ventral-lateral segment change with age. Under 100 days, the caudal region accounts for about half of the total length of the medial accessory nucleus whereas afterwards it constitutes only about a third of the nucleus. The caudal region maintains about the same shape throughout development, except that it moves more laterally within the brain stem as the corticospinal tract enlarges into a more medial, triangular-shaped bundle. The caudal region develops a slight dorsal, concave appearance at the level of the caudal pole of the dorsal accessory nucleus. Slightly more rostral, a dorsal sulcus develops, which then becomes the line of demarcation between the caudal region and the dorsal area, the dorsal-medial segment. This demarcation is only partially represented in the younger two fetuses, but becomes progressively more obvious, especially with increased fiber development, in older fetuses. The angle between the two components is obtuse, since the dorsal-medial portion is situated on a slanting plane at

about a 45° angle with the midline. In the 60- and 80-day fetuses, the dorsal-medial segment is rather small, extending for only a couple of sections, but after 100 days its length increases until it can be clearly identified in almost a third of the medial accessory sections. However, with continued development its borders become highly diffuse because of the maturational increase in the olivary efferents that pass through the nucleus.

The dorsal area of the medial accessory nucleus can be still further divided to include a small patch of cells, usually referred to as the "dorsal cap of Kooy" (1917), which extends for a few sections on the top of the dorsal-medial segment (Figs. 2,3; Plate 4b) and projecting from it is a small, narrow, ventral-lateral-outgrowth which, in turn, connects briefly with the ventral lamella of the principal nucleus. At 60 and 80 days the ventral-lateral-outgrowth and the ventral lamella is proportionally a large connection, but by 129 days it extends for no more than 300μ and is only a few cells thick (Plate 16d, e, f). Likewise, by 129 days the dorsal cap also persists for only a few sections. Most of the cells of these structures are in the center part of the dorsal cap; thus, the borders are somewhat diffuse, and as in the dorsal-medial segment, many fibers primarily efferent from the principal nucleus, bifurcate this area.

Rostral to the dorsal cap, only the ventral-lateral segment is seen. Maintaining a ventral position under the medial half of the ventral lamella of the principal nucleus, it gradually decreases rostrally in width. In only the 60- and 80-day fetuses, the rostral pole establishes a distinct connection with the rostromedial extension of the ventral lamella (Fig. 2; Plate 3a). In older fetuses the rostral pole of the ventral-lateral segment comes close to the same area of the ventral lamella but never makes any

contact.

Throughout all stages of development, the principal nucleus is basically U-shaped, with the dorsal and ventral lamella connected by a lateral band, although both rostrally and caudally, the lamella join together to form a common pole (Fig. 3). The whole structure is at a 30° inclination to the midline. Medially the lips of the lamella are encased by the accessory nuclei, whereas the lateral parts of the lamella, which constitute its greatest area, form a lateral bulge on the side of the brain stem. With age the principal nucleus tends to extend more and more in a lateral rather than a caudal-rostral direction. The dorsomedial border is the site of the hilus, which is a long, narrow, slit-like opening between the lamellar leaflets, which occupies almost the entire extent of the dorsomedial border and is also covered by the accessory nuclei.

The main developmental changes are represented by an increased thickness of the lamella and the acquisition of deep folds (gyri) which increase the overall area of the nucleus. At 60 days the lamella are smooth and concentrated around the lateral band (Fig. 2), although the caudal part of the ventral lamella extends enough medially to make contact with the ventral-lateral-outgrowth. Throughout development, the ventral lamella also makes a rostral connection with dorsal accessory nucleus, which will be described in more detail under the dorsal nucleus. By 80 days the dorsal lamella is considerably thicker, includes a small sulcus on its rostrolateral border, and projects more medially. In addition, in both the 60- and 80-day fetuses a connection exists between the caudal, medial extension of the dorsal lamella and the dorsal accessory nucleus (Fig. 2, Plate 3c). Like that between the ventral lamella and the rostral part of the ventral-lateral segment described above, the dorsal lamella-

dorsal accessory connection cannot be identified in older fetuses. At 100 days a number of dramatic changes have occurred. Most apparent are two large longitudinal sulci that course through the rostral three-fourths of the dorsal lamella (Fig. 3).. The more lateral sulcus is much larger than the medial and in later stages extends the full length of the dorsal lamella. The walls of the dorsal lamella are thin enough, however, to allow the interior of the nucleus an elevation that complements the external depression. On the other hand, the caudal part of the ventral lamella extends medially, as in the younger fetuses, and establishes contact with the ventral-lateral-outgrowth, after which it recedes; then about 300 to 400μ more rostrally, it again projects medially where at its rostral third it makes contact with the dorsal accessory nucleus. This small gap, where the medial extension of the ventral lamella does not reach, can be found in fetuses of all ages but does not appear to increase in size with age as do most other parts of the olivary complex. At 107 days, the ventral lamella displays a small sulcus in its lateral edge. A second, smaller, more medial sulcus is also apparent in the 121-day fetus. Both sulci and corresponding gyri increase in size with development, but never attain the size of the dorsal sulci.

The dorsal accessory nucleus shows the least amount of change throughout the various developmental stages (Figs. 2 and 3). Its caudal pole is slightly more rostral than the medial accessory nucleus, after which it is quickly separated by the caudal pole of the principal nucleus from the dorsal surface of the medial accessory nucleus. It then extends rostrally as a broad, thin lamella over the medial aspect of the principal nucleus. About midway, or at the approximate level of the ventral-lateral-outgrowth, the dorsal nucleus curves ventrally over the medial surface of

the dorsal lamella of the principal nucleus (Fig. 2). Rostrally, this ventral-medial segment becomes larger, whereas the dorsal-lateral segment decreases in size. In the rostral fourth of the nucleus, the dorsal-lateral segment totally disappears, but the ventral-medial segment connects with the dorsomedial border of the ventral lamella. In 60- and 80-day fetuses, this provides a common junction with the principal and medial accessory nuclei. Finally, since the cellular organization in this area is extremely diffuse in older fetuses, its exact boundaries are hard to identify and appear to fuse in many places into the surrounding cells and fibers.

B. Developmental organization of the lower brain stem and cerebellum

Like the structural maturation of the inferior olivary complex, the lower brain stem, except the cerebellum, shows its greatest neuronal differentiation between day 60 and day 129 of gestation, after which it morphologically resembles the adult organ. During this period of rapid growth, the boundaries of nuclei groups become apparent, cellular differentiation occurs, and various long and short fibers establish their connections. After 129 days, development is much slower and much less discernible and consists primarily of an enlarging of established fiber systems and, to a limited degree, of some cellular elements. The cerebellum, on the other hand, develops much more gradually. That is, a number of major structural changes occur between 60 and 107 days gestation, whereas changes in various cellular and fiber developments can be observed from 60 days gestation to almost a year after parturition. Consequently, a more detailed report is presented of the main features of the lower brain stem for fetuses between 60 and 129 days of age; after this the observations focus primarily upon the inferior olivary complex and the distribution of its afferents and

efferents. The cerebellum, however, is described in detail at each age level.

60-day fetus. At this stage of development, the basic form of the adult brain stem has been established. Regional proliferation of neuroblasts has formed recognizable major nuclear masses, the more distinct of which are all the nuclei of the cranial nerves, the pons, the superior olivary nucleus, and the inferior olivary complex. Identifiable also are some of the longitudinal fiber systems, such as the corticospinal tract and the medial lemniscus as well as some of the shorter transverse fibers like the cranial nerve rootlets. Consequently, the lower brain stem, at this stage, can be divided into distinct regions: mesencephalon, metencephalon, and myelencephalon (Fig. 5).

The mesencephalon, proportionally the largest area of the lower brain stem, consists of three major regions: a tectum dorsally, a very large tegmental area medially, and a very small peduncular region ventrally (Plate 1a). The tectal zone shows a graduation of undifferentiated cells into loosely defined superior and inferior colliculi. Medially there is a very large mesocoele, the rudimentary cerebral aqueduct, whose walls are only slightly reduced from those of the lumen of the metencephalon and myelencephalon. The mesocoele is lined with a thick layer of darkly staining neuroblasts. Although most of the tegmentum consists of undifferentiated neuroblasts, there are recognizable cellular masses. Very apparent are the oculomotor and trochlear nuclei, including their distinct nerve rootlets. Another conspicuous gray mass is the rudimentary red nucleus, which consists of a small oval mass of early developing motor neurons which in places are separated by descending oculomotor nerve rootlets (Plate 1b). The more anterior small cell portion, which is characteristic of the adult

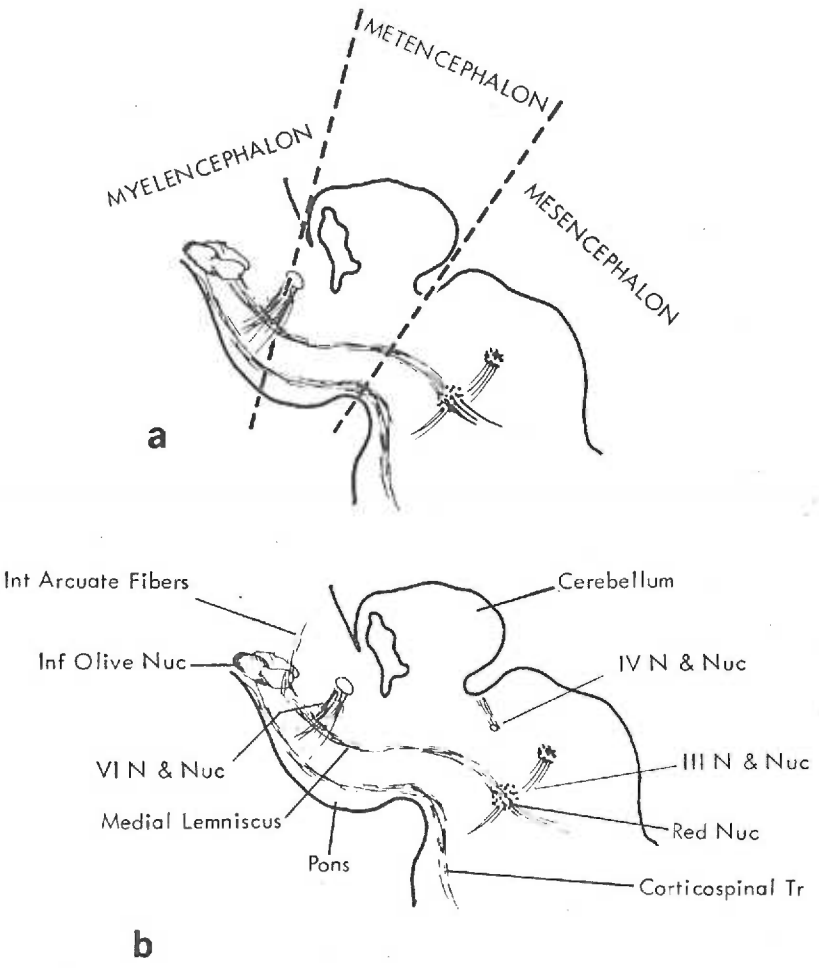


Fig. 5 Representation of a sagittal section of a 60-day fetal brain stem that illustrates: (a) the three major subdivisions, and (b) some of the main identifiable structures.

nucleus, is not discernible. On the ventral-lateral edge of the tegmentum there is an increase in cell density; subsequent development shows it to be the substantia nigra, which caps the very small peduncular region consisting of a small number of longitudinally-running corticospinal tracts.

At this stage, the major divisions of the metencephalon are proportionally the reverse of the mature organ; a rudimentary cerebellum, a large tegmental region, and a very small pons. The chief external features of the cerebellum are a primary fissure that subdivides it transversely and a posteriolateral fissure that separates the relatively large flocculonodular lobes from the rest of the cerebellum (Plate 2a). Attached to the laterocaudal border of the floccular lobe is the choroid plexus, which fills most of the lateral expanse of the fourth ventricle. The most distinctive internal trait is the embryonic or external granular cell layer, which consists of dark-staining cells that form the outer border of the whole cerebellum. It is thinnest on the rostrodorsal surface and becomes progressively thicker laterally and caudally. The remaining cellular elements consist of undifferentiated cells of various densities, including a suggestion of an internal granular layer, the early formation of the lateral cerebellar nucleus (dentate), and just a hint of the other deep nuclei (Plates 2a, 2b, 22). Two small fiber systems enter the cerebellum. The larger consists of vestibulo-cerebellar fibers, some of which project to the floccular lobe and others, the larger number, approach the midline where a few fibers continue on the contralateral side via the lateral commissure (Plate 1d). The other recognizable fiber system can be followed from the spinal cord, along the dorsal surface of the myelencephalon, as part of the inferior cerebellar peduncle (restiform body),

and then into the cerebellum where it intermingles with the vestibular fibers from the region of the juxtarestiform body.

The metencephalic tegmentum includes many undifferentiated neuroblasts, which have coalesced to form recognizable cranial nerve groupings, a large superior olive formation, and a few longitudinally disposed fibers, including a small, but identifiable, medial lemniscus and a few cranial nerve rootlets. Ventral to the tegmentum is the pons, which consists of a dense collection of undifferentiated neuroblasts through which run the small corticospinal tract (Plate 1c).

The myelencephalon is characterized by a few undifferentiated cellular masses: the cranial nuclei, a conspicuous inferior olivary nuclear complex, and, most ventrally, a small corticospinal tract, the rudimentary pyramids (Plates 3a and 4a). Although the gracillis and cuneate nuclei are not clearly delineated, their efferents, the internal arcuate fibers, are discernible throughout the lower tegmental region. These fibers course ventromedially, passing through the undifferentiated reticular formation and inferior olivary complex and crossing in the midline to form the small medial lemniscus. Crossing the internal arcuate fibers in a ventrolateral direction are the coarser hypoglossal nerve rootlets, which also pass through the inferior olivary nuclear complex.

At this stage of development, the inferior olivary nucleus consists primarily of undifferentiated neuroblasts and a few fibers (Plates 3 and 4). An extremely thin fibrous matrix, which is basically part of the adjacent fiber systems, surrounds the nucleus. For example, ventromedially is the corticospinal tract, dorsally and medially are portions of the medial lemniscus, and laterally the spinal cord afferents. On sagittal sections, however, the caudal region of the olive has a dense network of fibers that appear to

be contiguous with fibers of the spinal cord. In this region, a few fibers also appear to enter the olive (Plate 4c). In addition, a few spinal fibers course along slightly anterior to the lateral border of the medial accessory olive and enter its dorsal border and the caudal region of the principal olive. These fibers project, almost at right angles to the border of the olive, to the approximate center of the nuclei. None, however, can be seen terminating on any of the immature olivary cells.

80-day fetus. The lower brain stem of the 80-day fetus is larger and much more complex than that of the 60-day fetus (Fig. 6). Although some cellular differentiation has occurred, primarily among the cranial nuclei, the most striking changes are the definite organization of some of the larger nuclear groups, such as the superior olivary nucleus or the deep cerebellar nuclei, which were previously just barely detectable, and the tremendous increase in the number of both large and small fibers. Many longitudinal fibers course throughout the tegmental region. Some form distinct bundles, such as the medial longitudinal fasciculus (MLF) and the medial lemniscus, which can be followed from the rostral half of the myelencephalon to the rostral mesencephalon. The corticospinal tract has also greatly enlarged to act as a major mechanical or structural influence on the organization of the lower brain stem. For example, the subthalamic nucleus is forced more caudally toward the edge of the rostral mesencephalon, the ventral aspect of the substantia nigra is delimited, the pons is greatly enlarged, the inferior olivary complex is raised off the floor of the medulla, and the caudal extent of the nuclear groups of myelencephalon is influenced by its decussation.

In the mesencephalon, fibers course in all directions. The tectospinal tract arises from the ventral border of the superior colliculus, where it

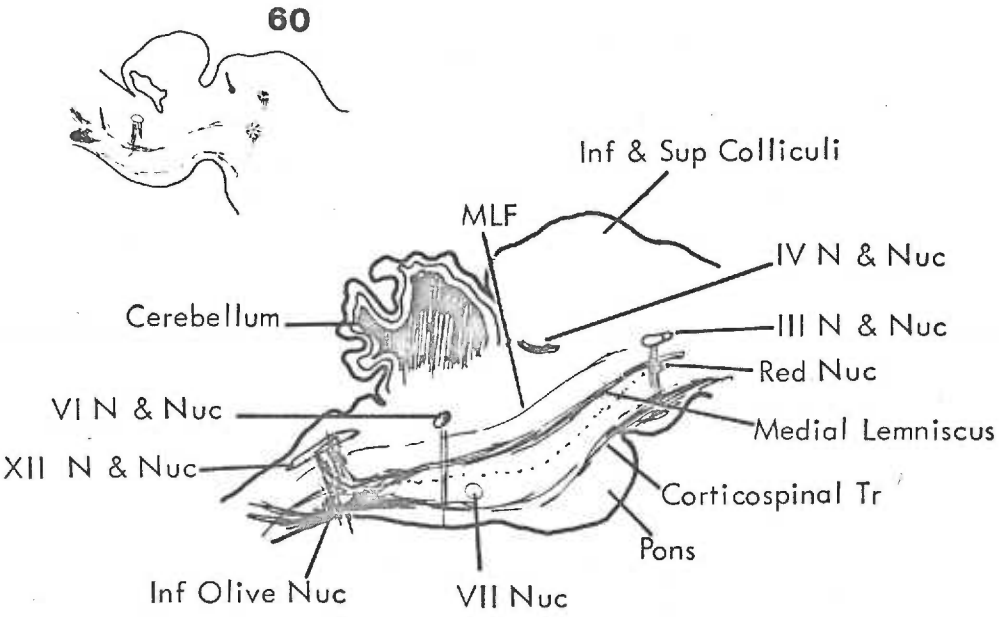


Fig. 6 A representation of a sagittal section of the lower brain stem of an 80-day fetus, showing its main structural features.

forms a distinct bundle that curves in a ventral convex direction and thereby clearly separates the periventicular gray from the superior colliculus. Within the tegmentum, the bundle becomes more diffuse and projects toward the midline where it undergoes complete decussation; on the contralateral side, it becomes interwoven with the MLF and cannot be further separated. Some of the tectospinal fibers, it should be noted, pass through the caudal portion of the red nucleus and the ventral region of the oculomotor nuclear complex. At this stage of development, it is impossible to determine whether any of the tectospinal fibers terminates or gives off collaterals to these nuclei.

The area of the red nucleus is also the crossing point, and perhaps the area of termination, for a number of other fibers. A large bundle, seen most distinctly on sagittal sections, originates in the caudal half of the subthalamic nucleus and projects caudally between the ventral surface of the red nucleus and the dorsal border of the substantia nigra; many of the fibers then intermix with those of the medial lemniscus whereas other fibers can be followed through the medial lemniscus to the undifferentiated cellular area just lateral to the red nucleus. Another group of fibers that pass through the rostral part of the magnocellular region of the red nucleus is the habenulointerpeduncular tract. However, no fibers from this tract appear to project to the cells of the red nucleus.

The tegmental region, with its many longitudinal fibers, is still proportionally the largest area of the metencephalon, although both the cerebellum and the pons have increased in size. The greatest degree of development in the cerebellum is the midline vermis and the deep nuclei (Plate 5a). The vermis is characterized by a deep primary fissure and a number of small fissures, which separate the many small folia. The flocculo-

nodular lobe shows about the same development as in the 60-day fetus. There is a distinct external granular layer, from which cells migrate centrally to coalesce as the precursor of the internal granular layer (Plate 22). However, all four deep cerebellar nuclei can now be clearly delineated (Plate 5b). The smallest and most medial, the fastigal nucleus, is heavily infiltrated with fibers, whereas the largest and most lateral, the dentate, is relatively free of fibers within its oval boundary. Between these two are a lateral, wedge-shaped emboliform nucleus and medially the globose nucleus, both of which have many fine afferent fibers entering along the dorsal surface. Except for an occasional large round cell, seen predominately in the fastigial nucleus, little cellular maturation has occurred. Most of the cerebellar afferent fibers enter via the restiform body. As in the 60-day fetus, there is a large vestibulo-cerebellar tract that projects to the floccular lobe and the fastigial nucleus. Present for the first time, however, are a large number of spino-cerebellar fibers that can be traced from the dorsolateral region of the myelencephalon to the cerebellum where they curve around the anterior border of the dentate nucleus and project primarily upon the anterior lobe. The other cerebellar afferent system, the middle cerebellar peduncle, consists of a small bundle of fibers that originates mainly from the rostral pons. The superior cerebellar peduncle, the brachium conjunctivum, is fairly small and appears to arise mainly from the emboliform and globose nuclei, descend into the mesencephalon and then to decussate, after which it can no longer be followed.

In addition to the longitudinal fibers of the metencephalic tegmentum, there are a number of transverse oriented fibers, such as those of the superior olivary nucleus, and numerous vertical running fibers, which are

primarily related to the cranial nerves (Plate 6b). The cellular organization of the tegmentum is similar to that of the 60-day fetus. However, in the pons there is an increase in the number of cells, which is related to the pontocerebellar afferents. Here again, the most noticeable change in the pons is not cell population, but the tremendous enlargement of the corticospinal tract and cerebellar afferents.

The general organization of the myelencephalon is still fairly ambiguous, consisting principally of undifferentiated neuroblasts that have coalesced in diffuse nuclear groups (Plate 6c). There is, however, a proliferation of fibers. For example, it is difficult to distinguish between the boundaries of the gracile and cuneate nuclei, yet there is a noticeable increase in the number of internal arcuate fibers, especially in the caudal part of the brain stem. The cellular boundaries of the vagal complex are enigmatic, but the nerve rootlets are very apparent. There are a few giant cells scattered in the lateral expanse of the tegmentum, but no recognizable reticular nuclear groupings. Therefore, the hypoglossal nucleus and its tract, the inferior olivary complex, and the pyramids are the chief traits of the myelencephalon at this stage. The hypoglossal nucleus consists of a longitudinal column of neurons. Bundles of fibers emanating from the nucleus transverse the tegmentum, pierce the dorsal accessory nucleus and the principal olivary nucleus, and exit from the brain stem between the principal olive and pyramid.

The inferior olivary complex, like the rest of the lower brain stem, shows a multitude of fibers of all types. The increase in the number of internal arcuate fibers that transverse the dorsal accessory olive and the dorsal-medial segment of the medial accessory nucleus is such that artificial separations occur. There is a general profusion of a fine fibrous matrix

within all divisions of the olive, especially in the medial region of the principal olive, the early formation of the hilus (Plate 7c). In addition, a thin fibrous capsule surrounds the rostrolateral expanse of the principal olive (Plate 7a).

The most noticeable change, however, is the massive influx of fibers from the lateral funiculus of the spinal cord although most of the spinal fibers project over the dorsal and lateral surfaces of the olivary complex to more rostral parts of the brain stem (Plate 7b). Most of the spino-olivary afferents project around the dorsal and lateral borders of the caudal segment of the medial accessory nucleus and terminate on the middle third of the ventral-lateral segment at about the level of the caudal extension of the principal olive. These form a rather dense fibrous matrix around the cells in the middle third of the ventral-lateral segment, whereas both the rostral and caudal thirds are relatively free of any fibers.

A small group of afferents enters the small sulcus at the rostral pole of the principal olive, where most of them become part of the fine matrix of the dorsal lamella (Plate 7a). Subsequent development shows these to be precursors of the rubro-olivary tract. Another bundle of fibers, identifiable for the first time, runs transversely between the ventral border of the caudal part of the medial accessory nucleus and the pyramids. At this stage of development, it is impossible to determine whether these fibers are afferents or efferents of the olive; they can be identified only between the olive and the midline, where they intermingle with the medial lemniscal fibers and the cells of the midline arcuate nucleus (Plate 6a).

Although a few efferents can be traced from the hilus at this stage of development most of them originate from the ventral-lateral segment. After leaving this segment, the fibers project to the contralateral side

of the brain stem, around the ventral and lateral borders of the medial accessory and principal nuclei, and then along the lateral border of the tegmentum. Efferents from the rostral third of the principal nucleus form a small hilus and then project through the dorsal-medial segment into the midline fibers, where they cannot be separated from the medial lemniscal and internal arcuate fibers.

92-day fetus. At this stage, marked cellular differentiation and fiber maturation have occurred throughout the lower brain stem. Morphologically, more and more cells and fibers, especially the cranial nuclei, resemble those of the adult. Both large and small tracts such as the MLF, medial lemniscus, brachium conjunctivum, lateral lemniscus, and mesencephalic root of the trigeminal, can easily be identified and their courses traced (Plates 8a,b).

Cellular proliferation is particularly evident in the mesencephalon where there is a distinct separation of the superior and inferior colliculi. The latter shows a dense concentration of cells, forming a recognizable nuclear unit. Coinciding with the enlargement of the colliculi is the decrease in size of the lumen of the mesencephalon, the cerebral aqueduct, which now forms a narrow canal that joins the lumina of the fourth and third ventricles. However, a thick ependymal layer still coats the aqueduct.

Within the tegmentum, internal differentiation of the red nucleus forms two morphological regions. The smaller caudal portion, seen in the 60-day fetus, is now represented by large multipolar neurons that are interspersed by a dense network of fibers of the oculomotor nucleus, the brachium conjunctivum, and, in dorsolateral areas, the medial lemniscus (Plates 8c,d). Some of the axons of the brachium conjunctivum form terminal nets around many of the large multipolar neurons. The conspicuous efferent bundle from

the caudal red nucleus is the rubrospinal tract, which emerges from the large multipolar cells, crosses to the contralateral side, and projects caudally along the dorsolateral border of the pons where it blends with a number of longitudinal fibers of the metencephalon. A small rostral region of the red nucleus, the pars parvocellularis, consists of a cluster of closely packed smaller neurons. It is also traversed by many fibers whose origin cannot be determined, except for a few of the larger tracts, such as the habenulointerpeduncular tract and the brachium conjunctivum. The rubro-olivary tract, an uncrossed descending efferent system from the parvocellularis, is a very small bundle of fibers that project caudally through the uncrossed brachium conjunctivum and descend down the brain stem via a diffuse central tegmental fasciculus (CTF). A few of the fibers of the CTF can be followed through the metencephalon to the rostrodorsal aspect of the principal nucleus of the inferior olivary nucleus.

In the metencephalon, the rapid growth of the vermian component of the cerebellum is particularly noteworthy (Plate 8a). It has developed numerous secondary and tertiary lobules, unlike the neocerebellum which despite an increase in overall size, is still relatively small and free of lobulation. The external granular layer is present through the whole cerebellum and waves of cells migrate from it centrally. At this age, the cellular organization of the cerebellar cortex consists of a thick external granular layer and a fairly diffuse array of undifferentiated cells that compose the internal granular layer (Plate 22). The external granular layer is a dense collection, about 6 to 8 cells deep, of early neuroblasts and that covers the outer surface of the cerebellum. Beneath it is a small region of a few cells, the precursors of the molecular layer. Finally, in the larger lobules, a number of fine fibers course throughout the central region of

the internal granular layers. The deep cerebellar nuclei are clearly represented; structurally, the dentate is changing and now has a central core of efferents. The other nuclei also show an increase in cellular organization and fiber development. All the peduncles are well developed, and afferents can be traced from the restiform body into the large folia of the vermis although, even under high magnification, only a few small fibers enter the secondary and tertiary lobules. As in the 80-day fetus, about the same number of afferents from the pons enter the cerebellum. The superior peduncle displays a continued increase of efferents, especially from the emboliform and globose nuclei. Only a few efferents are directly traceable from the dentate nucleus.

Structurally most of the nuclear groups of the myelencephalon can be clearly identified, e.g., all the components of the lemniscal system: the gracilus and cureatus nuclei, the internal arcuate fibers, and the medial lemniscus. The hypoglossal nucleus, along with some of its associated nuclei, has developed as a longitudinal column of multipolar neurons. The restiform body has increased in size, and fibers can now be traced from the lateral cuneate nucleus to it. The reticular formation is still composed mostly of undifferentiated neuroblasts and intervening longitudinally oriented fibers. However, occasional neurons, larger than the surrounding cells, are noticeable; there is a cluster of cells in the region where the adult lateral reticular nucleus is located. In addition, fibers appearing to originate from this general area project into the restiform body.

The inferior olivary nucleus of the 92-day fetus shows a dramatic increase in total size, in differentiation of its cells, and in the number of fibers both within and surrounding the olive (Plate 9). The olivary complex, particularly the dorsal lateral border of the principal olive,

is covered with a thin fibrous capsule. The outermost portions of the dorsolateral and ventral aspects of the capsule are made up of fibers running in a rostral to caudal direction; the inner layer consists mainly of fibers encasing the olive in a dorsal to ventral direction. Because of the extensive fiber development, the medial accessory olive can be clearly separated into a ventral-lateral segment and a dorsal-medial segment. Furthermore, the ventral-lateral segment can be subdivided into two discrete parts by their afferents. That is, the caudal portion has relatively few fibers, except those, already described in the 80-day fetus, that enter via the ventral bundle that courses between the ventral olive and the pyramids. However, slightly more rostrally, at about the caudal extent of the principal olive, the ventral-lateral segment becomes so heavily infiltrated by spino-olivary fibers that each cell is totally surrounded by a dense fibrous matrix (Plate 9a). More rostrally, the afferents become fewer and fewer, until at the most rostral fourth of the ventral-lateral segment they almost disappear. The course of the spino-olivary tract has also widened the distance between the dorsal aspect of the ventral-lateral segment and the ventral lamella of the principal olive.

A number of spino-olivary fibers can be identified for the first time entering the caudomedial part of the dorsal accessory nucleus, where they form a rather loose latticework of fine fibers in its caudal-most region, and, to a more limited degree, the caudal region of the principal nucleus (Plate 9b). Some fibers may also enter the ventral-lateral-outgrowth, but it is difficult to separate the spinal afferents to the inferior olive and those that project to more rostral regions of the brain but transverse through the olivary complex.

The other major afferents are rubro-olivary fibers carried in the CTF.

These fibers can be traced from the rostral red nucleus to the rostral pole of the principal olive, where most of them now enter the rostral fourth of the dorsal lamella, forming a loose intermesh of fine fibers. A few other rubro-olivary fibers can also be traced to the most rostral tip of the dorsal accessory olive.

The only other olivary afferents, seen for the first time, are from the corticospinal tract. Consisting of a few, small, distinct fibers, these afferents turn abruptly, at about a 45° angle from the corticospinal tract, and enter for a short distance into the medial accessory nucleus, its most caudal segment and the caudal part of the ventral-lateral segment, where they are immediately lost in the internal fibrous matrix (Fig. 7).

At this stage of development, most of the efferents emerge from the ventral-lateral segment of the medial accessory and course in a ventromedial direction to the midline where they cross to the contralateral side. They then traverse ventrally to the contralateral medial accessory nucleus. Although a few of the fibers penetrate the lateral regions of the principal nucleus, most efferents can be followed around the lateral border of the principal nucleus and then along the lateral border of the brain stem until they reach the restiform body. A few efferents can also be traced from the dorsal-medial segment of the medial accessory nucleus and from the dorsal accessory olive. These fibers likewise cross to the opposite side and penetrate the dorsal-medial segment and the dorsal lamella before crossing the diffuse reticular formation lateral to the internal arcuate fibers and entering the restiform body. In addition, more rostrally there are a few efferents from the principal nucleus, most of which originate from the rostral dorsal lamella. These fibers form a small hilus and project medially, but they cannot be traced beyond the midline because of the

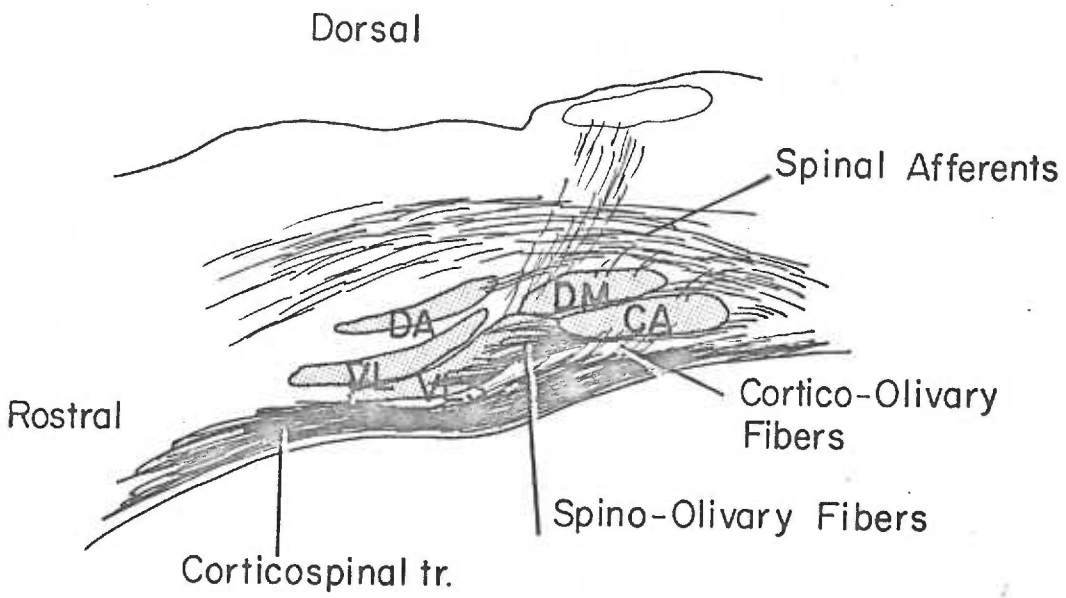


Fig. 7 A sagittal section of the medial myelencephalon of the 92-day fetus. Spino-olivary afferents can be identified entering the medial accessory nucleus, especially its ventral-lateral segment (VLS) and, to a more limited degree, its dorsal-medial segment (DM) and caudal segment (CA). A few spinal afferents also enter the caudal part of the dorsal accessory nucleus (DA). Although not illustrated here, some spinal fibers also project into the medial region of the ventral lamella (VL). A few cortico-olivary fibers can be traced from the corticospinal tract into mainly the caudal segment (CA).

density of fibers in this area.

100- and 102-day fetuses. Since the degree of development and the general appearance of the lower brain stem appear to be similar in 100- and 102-day fetuses, they will be discussed together. Although some signs of an immature brain stem are still present, such as many undifferentiated neuroblasts throughout the tegmentum, the lack of myelination of most tracts, and the small size of many nuclear groups, the general landmarks of the adult organ are present (Fig. 8). In the mesencephalon, cells of the oculomotor and trochlear nuclei, the caudal portion of the red nucleus, and the substantia nigra are noticeably enlarged compared with those of the 92-day fetus.

Coinciding with the cellular growth is the continued increase in the number of fibers, which is such that the large multipolar cells in the caudal part of the red nucleus are like individual islands among a maze of interlinking fibers (Fig. 9, Plate 10c). In addition, tectospinal fibers curve around the central gray and through and around the oculomotor and red nuclei. In fact, many of the large multipolar neurons of the magnocellular region appear to line up, so to speak, along the traversing tectospinal fibers (Plate 10d). The rubrospinal fibers also project in conjunction with the tectospinal fibers, so that in many places they cannot be separated from tectospinal fibers, nor can their exact neuronal origin be established. It is still very difficult to completely follow the rubro-olivary tract, but there are a few more fibers than in the 92-day fetus, which originate from the parvocellular region and project straight caudally through the magnocellular region and the brachium conjunctivum (Fig. 9), Plates 10a, c, e). Another small fiber system, finally, is the decussating subthalamicoreticular fibers, which traverse the ventral portion of the

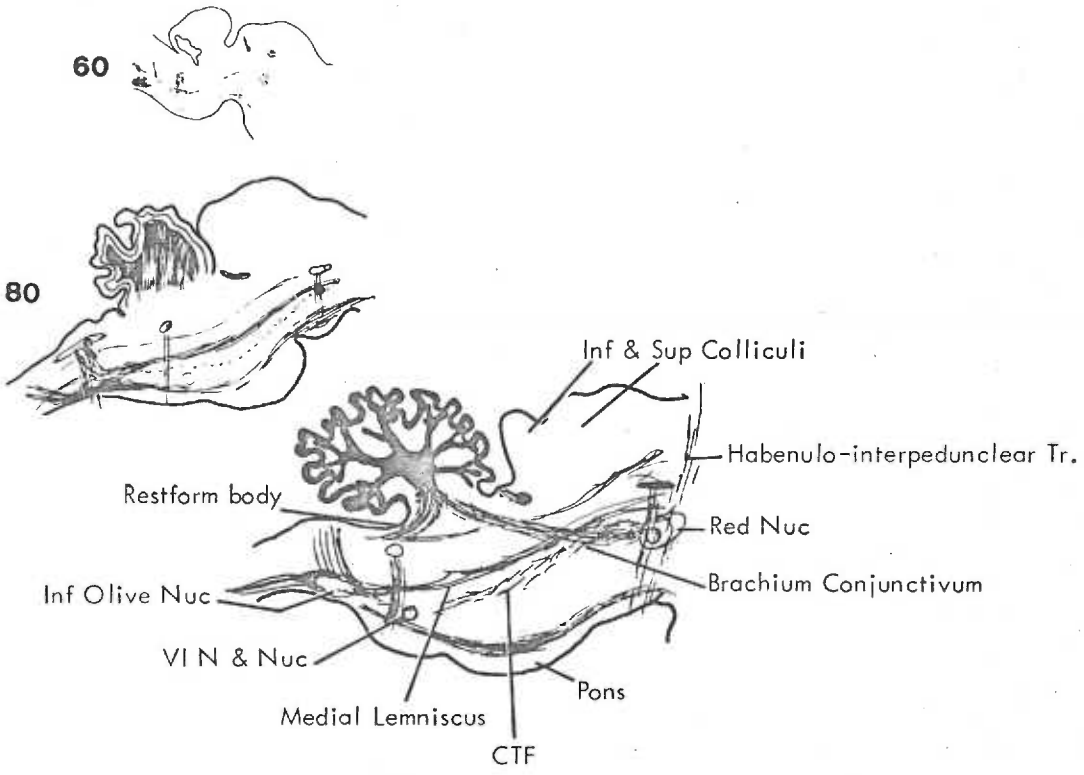


Fig. 8 A representation of a sagittal section of the lower brain stem of a 100-day fetus and a comparison with the 60- and 80-day fetal brain stems.

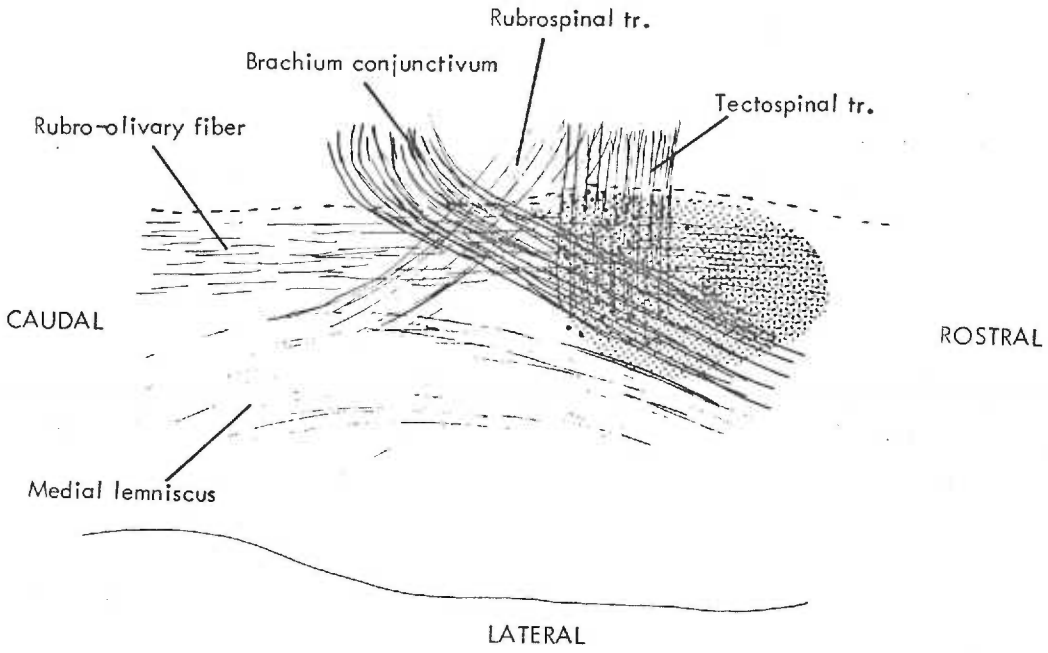


Fig. 9 Schematic horizontal section of the mesencephalon at the level of the red nucleus of a 100-day fetus. The two regions of the red nucleus are illustrated. The rubro-olivary tract originates from the rostral part; the rubrospinal tract develops from the caudal part and then crosses to the contralateral side. The brachium conjunctivum and the tectospinal tract also contribute to the maze of fibers within this region.

red nucleus. Many of these fibers appear to become part of the loose fibrous matrix of the caudal parvocellularis, just rostral to its junction with the magnocellular region.

In the metencephalon, the cerebellum shows the greatest development. As in younger fetuses, the vermian portion shows more morphological change than the neocerebellum. However, the surface of the whole cerebellum is covered with a thick external granular layer, which contains cells in different stages of mitotic division. These cells can easily be observed migrating centrally through a discernible molecular layer into a diffusely packed granular layer. On the peripheral edge of the internal granular layer is a line of single large cells whose subsequent development shows them to be immature Purkinje cells. The white matter still consists of a mesh of fibers, but a few longer fibers can be followed to the periphery of the larger lobules (Plate 22). All the deep cerebellar nuclei are easily delineated; as in younger fetuses, the dentate nucleus shows the greatest degree of development.

A conspicuous gradation of cellular differentiation in the myelencephalon is evident in the cranial nuclei, cuneate and gracile nuclei, and the reticular formation. Also conspicuous is the enlargement of the inferior olivary nuclear complex, including a slight further maturation of its cells (Plate 11).

Examination shows an increase in the number and complexity of the afferent fibers to the inferior olivary complex. Fibers from the spinal cord enter the caudal portion of the dorsal accessory olive and form a thin fibrous matrix on the caudal one-third. To a limited degree, the spino-olivary fibers also invade the most caudal portion of the medial accessory olive, but most of the spino-olivary fibers still project to the middle

part of the ventral-lateral segment. However, by this time, only the rostral fourth is free of the afferents. More and more spino-olivary fibers course between the ventral-lateral segment and the corticospinal tract to enter the nucleus ventrally (Plate 11).

As in the 92-day fetus, fibers from the corticospinal tract enter the posterior regions of the medial accessory; however, the more rostral section of the nucleus now receives fibers also. These fibers are relatively few in number and their path is more diffiult to trace as more and more spino-olivary fibers course through the region between the ventral-lateral segment and the corticospinal tract.

The rostral area of the principal olive is covered by a thin capsule, about as thick as in the 92-day fetus, from which a number of afferents enter the most rostral pole. Most of the afferents enter the rostral-dorsal lamella, but a few also enter the more rostral border of the ventral lamella. In addition, a few fibers can be traced over the dorsal surface, where they enter the dorsal lamella via the lateral longitudinal sulcus. However, the rubro-olivary afferents are difficult to distinguish just rostral to the olivary complex because of the enlargement of the superior olivary nucleus and the trapezoid body, as well as the continued increase of medial lemniscial fibers.

107- and 109-day fetuses. By this stage, a number of salient developmental changes have occurred in the mesencephalon of these fetuses. Most obvious are the increases in size of the oculomotor and trochlear cranial nerves, of the brachium conjunctivum, and of the peduncle, which now clearly contains laterally corticopontine fibers. Closer examination reveals many changes in the nuclear groups and smaller fiber systems; for example, subdivisions within the oculomotor complex, separation of the substantia

nigra into a compact and reticular zone, and organization of the nucleus of the lateral lemniscus within its fiber system. Analysis of the smaller fiber systems reveals their close association with the red nucleus. More small fibers arise from the parvocellularis, particularly the dorsal-lateral region, which as in the 100-day fetus, projects caudally through the middle portion of the brachium conjunctivum and then into the diffuse tegmental area of the metencephalon (Plates 12a,b).

Corresponding to the development of the corticopontine division of the mesencephalic peduncle is a dramatic increase in the pontine nuclei. This increase is such that the corticospinal tract as it passes through the pons is now totally surrounded by pontine nuclei. In the rostral pons are many terminating corticopontine fibers, and, likewise, a number of transverse pontocerebellar fibers, which can be traced along the lateral contour of the tegmentum and into the cerebellum where they mix with afferents from the restiform body. Most of the pontocerebellar fibers are restricted, however, to the more rostral and dorsal areas of the pons (Plate 12c).

The tegmental portion of the metencephalon has much the same arrangement as in the 100- and 102 day fetuses, except for a definite increase in the medial and lateral lemnisci. In addition, there is a further organization of the statoacoustic nuclear complex and a graduation of neurons from the ventral tegmental reticular nucleus. For the most part, however, the tegmentum remains primarily a longitudinal fiber system; separation of small fiber systems, such as CTF, is very difficult (Plate 12d).

At this stage, both external and internal changes have occurred in the cerebellum. Structurally, the most noticeable is the enlargement of the hemispheres, which now display large, rounded folia which, for the most part, appear to follow the pattern already established by the vermis. A

consideration of the internal organization reveals that the vermis is also more advanced morphologically than the hemispheres (Plate 22). First, the folia of the vermis have a white layer with many fibers projecting through the rudimentary granular cells which is lacking in the undifferentiated cells of the hemispheres where only a few fibers course. In the vermis, there is a visible layer of large multipolar cells (immature Purkinje cells) under the distinct molecular layer, whereas in the hemispheres there is only a suggestion of a molecular layer and only undifferentiated neuroblasts are present. Finally, although still present in the vermis, the external granular layer is thinner with fewer cells migrating centrally than in the younger fetuses, whereas the external granular in the hemispheres is much thicker and there are many migrating cells. In the deep nuclei, which provide an internal transition from the vermis to the hemispheres, the dentate nucleus continues to show the greatest development. Although its cells are still undifferentiated neuroblasts, the change in distribution is such that a rudimentary hilus with efferents is now recognizable. The medial nuclei, on the other hand, show considerable cellular differentiation and obvious fiber development (Plate 13c).

The myelencephalon shows a further neuronal differentiation, particularly of the reticular neurons. Some of the numerous giant multipolar cells throughout the tegmental area form distinct groups, such as those at the lateral reticular nucleus. Most, however, are spread randomly among the many fibers of the tegmentum. Further cellular differentiation also discloses a definite lateral cuneate nucleus and a recognizable graduation of cells within the vagal complex. In conjunction with the cellular changes, there is a profusion of fibers. The restiform body now contains not only spinocerebellar afferents but also efferents of the lateral reticular and

cuneate nuclei, plus a few olivocerebellar fibers, which originate mainly from the medial accessory nucleus. A multitude of fibers runs in all directions within the diffuse reticular formation. The reticular cells have likewise increased in size (Plate 13e). As might be expected, the medial lemniscus has also become a distinct ventral midline bundle, which displaces the inferior olivary complex laterally.

The external structure of the inferior olivary complex is similar to that in the 100- and 102-day fetuses; however, there is evidence of additional fibers. A distinct fibrous capsule forming around the rostral third of the principal olive is thickest at the ventrolateral surface and then diminishes as it progresses caudally. Rubro-olivary afferents compose most of the anterior part of the capsule, and can be traced from the olive into the tegmentum of metencephalon as the CTF (Plates 13c, d). At this stage of development, most of the rubro-olivary afferents appear to project to the most rostrolateral region of the dorsal lamella and to enter the large dorsal sulcus. From this point, the fibers tend to move to the more medial sections of the dorsal lamella and a few enter the dorsal-lateral area of the dorsal accessory olive. Another part of the bundle curves ventrally around the most lateral extension of the principal olive to the under side of the ventral lamella, giving off afferents all along its course. In addition, a few afferents project onto the lateral extent of the medial accessory olive, but only in its rostral third (Fig. 11).

The spino-olivary afferents follow the same pattern already established in the younger fetuses, but disproportionately more and more fibers are now distributed to the medial accessory olive. Even the caudal-most portion of the medial accessory, which previously was relatively free of any afferents, now has a fine matrix of spino-olivary afferents, but the

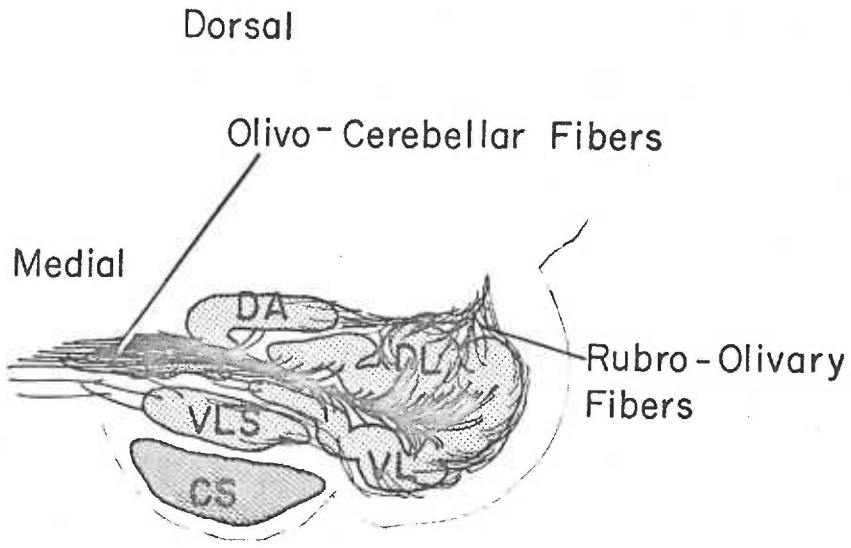


Fig. 10 Coronal section of the rostral part of the inferior olivary nucleus of a 107-day fetus. Rubro-olivary fibers are shown entering the principal nucleus, primarily the dorsal lamella (DL) and, to a more limited degree, the ventral lamella (VL). A few afferents also enter the lateral part of the dorsal accessory nucleus (DA) and the ventral-lateral segment (VLS) of the medial accessory nucleus. The distribution of the olivocerebellar fibers corresponds with that of the rubro-olivary afferents, that is, most originate from the dorsal lamella and then cross to the contralateral side. CS: corticospinal tract.

majority still enter the middle region of the ventral-lateral segment. In fact, the density of spino-olivary fibers along the ventral border of the medial accessory is so great that afferents from the corticospinal tract are no longer visible.

Not only is there an increase in the number of afferents into this region but all the other fiber systems have likewise increased. It is very difficult, for example, to identify exactly the efferents for those regions of the olivary complex that are near the midline, such as the medial part of the dorsal accessory of the dorsal-medial segment of the medial accessory, because passing through these structures, all in the same plane, are many internal arcuate fibers and efferents from the other divisions, both ipsilaterally and contralaterally, of the olive. However, efferents for the principal olive and the lateral parts of the medial accessory can be clearly identified (Plate 13f). The distribution of efferents from the principal olive is similar to the distribution of afferents, that is, most of the efferents arise from the lateral region of the dorsal lamella and only a few come from the ventral lamella. Similarly, most of the efferents for the medial accessory olive come from those areas that receive many afferents.

121-day fetus. In the mesencephalon, the major maturational changes of this stage are an increase in fiber density, particularly the smaller internuncials, and the continued organization of the reticular nuclei. The increase in the number of internuncials makes it exceedingly difficult to clearly delineate the various fiber systems, particularly within the tegmentum, and obscures some of the boundaries of the nuclear groups. The caudal region of the red nucleus is still conspicuous because of its large multipolar cells, but the increase in the brachium conjunctivum, which appears to give off

collaterals that end around the large multipolar cells, makes identification of the nucleus efferents difficult. Some rubrospinal fibers, however, can be seen to leave the large cells and then decussate in the midline where they then take a position ventral to the contralateral red nucleus. As seen in younger animals, these fibers are closely associated in many places with tectospinal tracts, which can now be seen to arise from cells in the superficial as well as in the middle gray stratum of the superior colliculus and to pass in an arch-like manner around the central gray. The rostral contour of the red nucleus, like its efferents, is even harder to demonstrate because of the large influx of fibers from many sources. However, a few fibers, about the same amount as seen in the 107- and 108-day fetuses, can be traced through the caudal portion of the nucleus and through the dorsal region of the descending brachium conjunctivum.

A reticular nucleus, the deep tegmental nucleus of the mesencephalon (also called the nucleus profundus mesencephali), is just lateral to the red nucleus and the descending rubro-olivary tract. It is further enclosed by the medial lemniscus on its ventral side and the spinothalamic tract on its lateral side. It receives afferents from a number of sources, including the subthalamic nucleus, that pass through the ventral region of the red nucleus. The nucleus also receives fibers from a lateral bifurcation of the tectospinal tract and from the lateral corner of the substantia nigra. In addition, its diffuse organization and its large number of internuncials suggest that it also connects with the red nucleus or contributes fibers to the CTF.

Sections through the metencephalon show little change from that of the previous developmental stage. The pons are a little larger, and additional pontocerebellar fibers arise within the pontine nuclei and cross

and condense laterally to form the brachium pontis, which enters the cerebellum. On the dorsal surface of the pontine nuclei are the distinct tracts of the medial lemniscus, on its lateral side the spinothalamic and the lateral lemniscus. Rubro-olivary fibers can be seen in sagittal sections as a diffuse array just dorsolateral to the medial lemniscus, but in cross-section it is difficult to identify this tract (Plate 14a).

The development of the cerebellum has changed little from that of the previous maturational stage. The external granular layer closely resembles that of the 107- and 109-day fetuses. The molecular layer is slightly larger and fewer cells migrate through from the external granular layer. The white matter is slightly more dense in the vermis but about the same in the hemispheres (Plate 22). The deep nuclei, on the other hand, show an increase of the internal fibrous matrix, particularly around the fastigial nucleus, and a slight enlargement of the multipolar neurons, most noticeably in the dentate.

The basic structure of the mylencephalon in general, and of the inferior olivary nuclear complex in particular, is established; therefore, the maturational changes that are now discernible consist of a further elabouration of the various fiber systems. For example, with the progressive increase in the rubro-olivary fibers, the rostral portion of the principal olivary nucleus is becoming more encapsulated. The capsule is thicker in the lateral and ventral area of the rostral nucleus and becomes progressively thinner toward the caudal pole of the nucleus. As would be expected, the internal matrix of afferent fibers corresponds with the distribution of the capsule, that is, most of the rubro-olivary afferents project upon the rostral half of the principal olive, the greatest increase being within the dorsal lamella, whereas the caudal half of both the dorsal and ventral lamella, particularly

the lateral regions, shows a diminished number of afferent fibers.

As in younger fetuses, the spino-olivary fibers project primarily upon the mid-region of the ventral-lateral section of the medial accessory nucleus. The density is so great that each cell is completely surrounded with fibers (Plates 14b, 15d), but it gradually decreases toward the rostral pole. The more medial part of the olivary complex, which includes the dorsal-medial segment and the dorsal cap is relatively free of afferents (Plates 15c, e). The lateral expanse of the caudal section, however, shows a conspicuous increase in spino-olivary afferents.

Spino-olivary afferents also project upon the caudal third of the dorsal accessory nucleus, but this relationship is becoming obscure because of the increased number of other fibers, such as the internal arcuate and olive-cerebellar efferents, which penetrate this nucleus. The rostral part of the nucleus, especially the ventromedial part, receives efferents from the reticular formation. These fine fibers parallel those of the hypoglossal (Plate 14c).

127- and 129-day fetuses. Although the lower brain stem still has many immature characteristics, the adult pattern is apparent (Fig. 11). The most obvious developmental feature is the further cellular differentiation that has occurred throughout every area of the lower brain stem. Particularly conspicuous is the maturational change of many reticular neurons within the tegmentum, whereas others have coalesced into definite nuclear groups. Corresponding with the maturation of the reticular neurons is a profusion of intermingled fine fibers throughout the tegmentum.

In the mesencephalon, the tectal region resembles that of the adult. The superior colliculus consists of laminations of longitudinal rows of cells alternated with rows of small fibers. The inferior colliculus

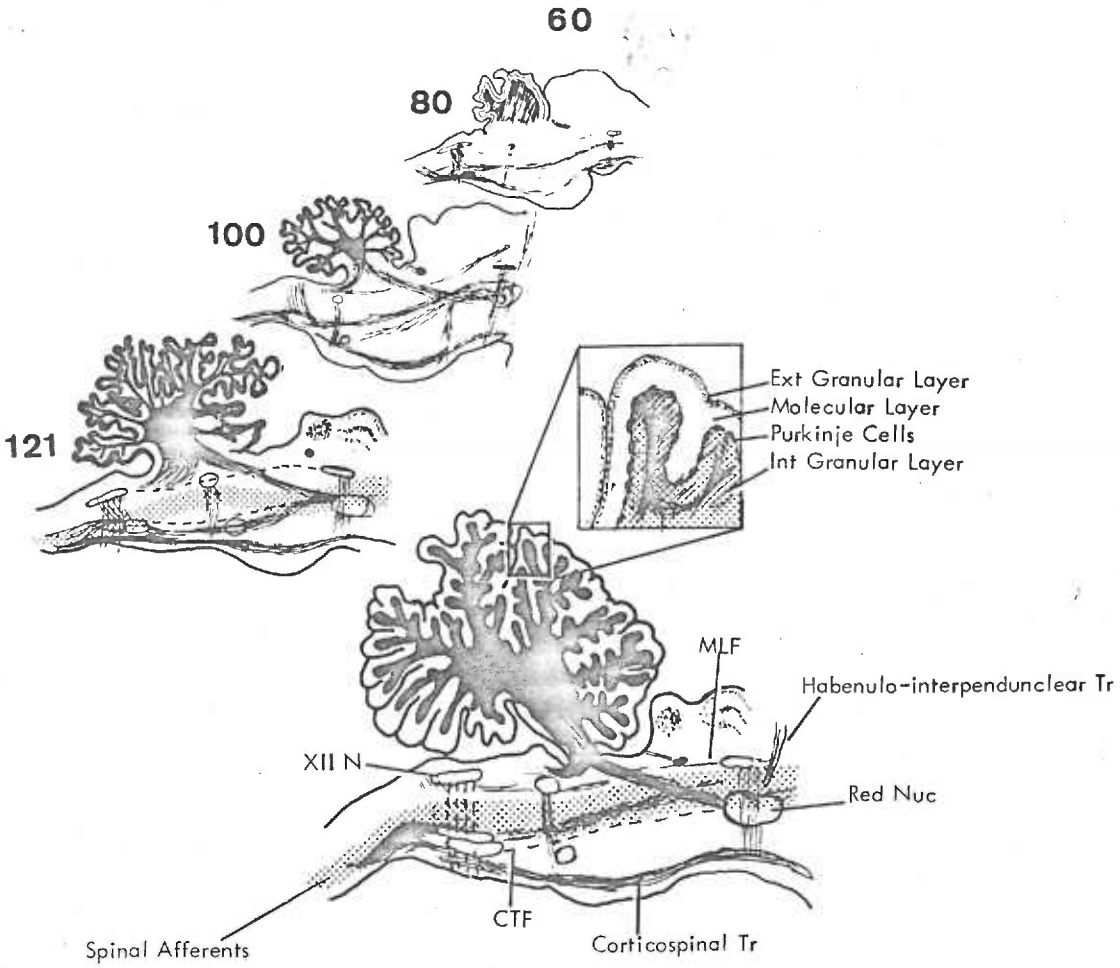


Fig. 11 Representation of the development of the lower brain stem of a 127-day fetus in comparison with younger stages. The enlargement of the cerebellar cortex illustrates its four cellular layers.

exhibits a densely packed nucleus surrounded by a fibrous capsule. The nucleus consists of small and medium-sized compact cells throughout which terminals of the lateral lemniscus can be identified. More medially, the central gray is clearly delineated laterally by fibers of the colliculi and the mesencephalic trigeminal fibers and ventrally by cells and fibers of the oculomotor and trochlear nuclei. The tegmental region consists medially of the larger red nucleus, which shows an increase in multipolar cells, laterally of a diffuse mesencephalic reticular nucleus which contains a few giant multipolar neurons, and ventrally of the substantia nigra, which has a distinct dorsal region of large pyramidal cells and a ventral zone of scattered cells of irregular shapes. The peduncular region has greatly enlarged because of the continuous addition of corticopontine fibers and the growth of the corticospinal tract.

Of particular interest to the present investigation, however, is the continued morphological maturation of the cerebellum. Although the hemispheres are still relatively small compared with the adult organ, the discrepancies between the internal morphology of the hemispheres and vermis are less. Its four cellular layers, which are apparent throughout the cortex of the whole cerebellum, are much more discernible than in the 121-day fetus. There is a decrease in thickness of the external granular layer and only a few cells are seen migrating through to deeper layers. The molecular layer is much larger and contains a few lightly staining fibers and a few darkly staining small cell bodies. The Purkinje cell layer can be clearly distinguished from the dorsal surface of the granular layer. They now have a more pearshaped cell body and a single large dendritic process, which projects for a short distance into the molecular layer. The granular cell layer shows a greater density than before as well as an increase of inter-

mingling small fibers. The granular cell bodies are almost completely filled with a nucleus, and only a thin strip of cytoplasm exists near the cell membrane. The center of the granular layer is the medullary layer. A profusion of both long and short fibers can be traced almost to the end of all folia.

The four deep cerebellar nuclei are clearly delineated and show considerable cellular differentiation. The fastigial nucleus consists of a few darkly staining, large, triangular-shaped neurons and an array of smaller round ones intermeshed with short and long fibers. As in the younger fetuses, many of the longer fibers can be traced to the vestibular nuclei and the stato-acoustic nerve. The next most medial nucleus is the globose, which is a large rounded mass of cells. On its lateral border is the emboliform nucleus, a small wedge-shaped group of cells. Both of these two nuclei have similar cellular morphology. They consist mostly of undifferentiated neuroblasts with a few small round neurons and a few large multipolar neurons randomly dispersed. The globose nucleus, however, shows a heavier infiltration of fibers. The most lateral and largest cerebellar nucleus is the dentate, which also extends farthest rostrally and caudally. It is almost totally surrounded by the white matter and from its center comes a large bundle of fibers. Examination of transverse sections shows it to be generally pearshaped with one large, rounded sulcus on its dorsal-lateral border; its center is composed of a fibrous matrix and many long efferent fibers. The nucleus consists of a large number of small, darkly stained cells and a moderate number of medium to large multipolar cells, many of the latter arranged along the border of the nucleus. In addition, the cells are interspersed by many long fibers arising from the granular Purkinje cell layers, and by many short fine fibers.

Like the rest of the brain stem, the myelencephalon has the gross features of the adult organ. On the dorsal surface, the cuneatus and the gracillis nuclei and the enlarged restiform body are conspicuous, whereas ventrally the medullary pyramids are prominent; in between, the medullary tegmentum, which includes the prominent inferior olivary complex, has expanded laterally. Internally, as in the higher brain stem, there is continued cellular differentiation in all cellular groups and an obvious increase in fibers of all sizes.

A number of significant developmental changes have taken place in the inferior olivary complex. Perhaps the cardinal feature is the cellular maturation throughout the whole olivary complex, which is particularly evident in the lateral portion of the dorsal and ventral lamella (Plate 16b). The cells are 15 to 20μ in diameter. The nuclei of the cells are relatively large and each contains one dark, slightly eccentric nucleolus and very fine reticulum. The cytoplasm is filled with coarsely granular, darkly staining Nissl bodies. Scattered among these cells are numerous smaller ones (5 to 10μ in diameter) with darkly staining nuclei and a very small amount of surrounding cytoplasm.

The lateral region of the principal olive is a rather loosely organized stratification of cells, many of which are arranged along the periphery of the olive; their nuclei on the outside and their large dendritic processes project toward the center. There are also regional changes in the density of cells, such as the concentration of cells in the most rostral part of the dorsal cap (Plate 16a). On the other hand, the density of the large neurons is relatively low in the medial segment of the ventral lamella in the region between the connection with the ventral-lateral-outgrowth and the more rostral medial extension of the ventral lamella (Plate 16d, e, f).

Regional differences in the distribution of afferents and efferents are also evident. As in the younger fetuses, the principal afferents to the olivary complex are those from the spinal cord and those from the red nucleus which travel via the CTF. Most of the afferents that can be traced from the ventrolateral funiculus of the spinal cord pass over the dorsal surface of the olivary complex to become part of the longitudinal fibers of the tegmentum, and only about one-sixth of them project directly into the olive. Most of these enter the medial accessory olive; a small number enter the caudal dorsal accessory and an even smaller amount enter the caudal ventral lamella. The increase of spino-olivary fibers is so great that they encapsulate and heavily infiltrate the internal structure of the ventral-lateral segment of the medial accessory olive. In each succeeding developmental stage, the spino-olivary fibers project more and more rostrally into the ventral-lateral segment where at this age only about 450μ of the rostral pole remain free of spinal afferents.

The rubro-olivary afferents follow the same general pattern as in the younger fetuses. They enter the olivary complex at the dorsal lateral border of the rostral dorsal lamella, where most of them enter the lateral sulcus and a few project both medially and ventrally. Thus, a few afferents enter that part of the dorsal accessory that entends over the dorsal lamella, whereas a few encircle the lateral border of the principal olive and enter the ventral lamella; as in the younger fetuses, a few also project into the most rostrolateral part of the ventral-lateral segment. The most noticeable change, however, is the tremendous increase of rubro-olivary fibers to the rostral dorsal lamella and of the fine matrix within the dorsal lamella. As one progresses more caudally into the principal olive, there are fewer and fewer afferents, until there are almost no fibers in the most caudal

region.

Another afferent system, which is more clearly discernible than at the 121-day age, is the extremely thin fibers from the diffuse reticular formation that project into the dorsal accessory olive. These fibers can be seen running in a dorsal to ventral direction in a number of parallel rows. Some of them appear to arise from large reticular cells throughout the medial portion of the tegmentum; others appear to originate from cells associated with the hypoglossal nucleus (Fig. 12, Plate 16b, c). These afferents enter the dorsal accessory from its most rostral border to about the level of the rostral extent of the ventral-lateral-outgrowth; this also coincides with an increase in the internal thin fibrous matrix of the dorsal accessory. Similar fibers, however, can also be seen within the tegmental region slightly lateral to the olivary complex, some of which appear to become incorporated in the maze of fibers of the lateral reticular nucleus.

Thick, darkly staining bundles of olive-cerebellar efferents are now present in the rostral olive, arising primarily from the rostral dorsal lamella and, to a lesser extent, from the ventral lamella (Fig 12, Plate 16d). The graduation of efferents from rostral to caudal corresponds with the distribution of rubro-olivary afferents, that is, the greatest number of efferents arises from the area where the afferents are most numerous, i.e., the rostral dorsal lamella. Most of the efferents from the principal olive project into the well-formed hilus and then project through the corresponding contralateral divisions of the olive and emerge to form bundles that pass through the tegmentum to the restiform body. In addition, in the caudal regions, many of the efferents pass among the diffuse neurons of the lateral reticular nucleus.

In the caudal section of the olivary complex, most of the efferents

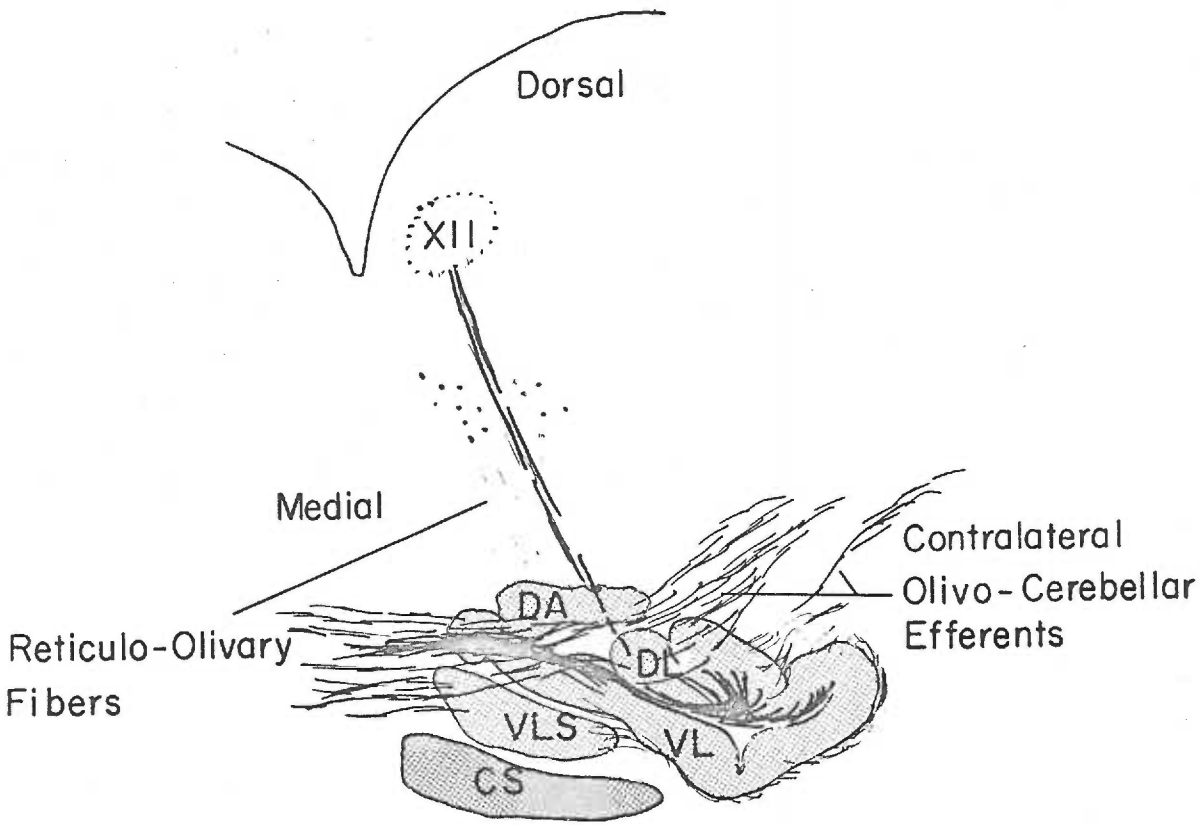


Fig. 12 Coronal section of the inferior olivary complex of a 129-day fetus. Reticulo-olivary fibers project mainly to the ventral-medial segment of the dorsal accessory nucleus (DA), parallel with the hypoglossal nerve rootlets. Also illustrated are the ipsilateral and contralateral olivary efferents. Most of the olivocerebellar fibers of this level originate from the dorsal lamella (DL), whereas only a few come from the ventral lamella (VL) or the ventral-lateral segment (VLS) of the medial accessory nucleus. The contralateral efferents project through the medial extension of the ventral lamella and then mainly through the dorsal lamella. A few, however, course around the lateral border of the principal nucleus. CS: corticospinal tract.

arise from the ventral-lateral segment and follow the general pattern seen in younger fetuses except that the pyramidal tract now occupies a more medial triangular position. Consequently, the efferents from the caudal region run in a dorsomedial direction over the pyramid, then on the contralateral side down in a ventrolateral orientation between the dorsal surface of the pyramidal tract and the ventral border of the medial accessory nucleus. Most of these fibers project along the lateral boundary of the brain stem to the restiform body; a few, however, become associated with the cells and fibers of the lateral reticular nucleus. At more rostral levels, efferents of the medial accessory must either project through the contralateral principal olive or become part of the capsule surrounding the lateral border of the principal olive. In fact, in the middle part of the olivary complex, the capsule has three distinct layers: an inner group of rubro-olivary afferents that project into the lamella, middle longitudinally oriented rubro-olivary fibers that project to the more caudal part of the principal olive, and outer transversely running efferent fibers of the medial accessory olive.

134- and 137-day fetuses. The general structure of the lower brain stem at this stage of development is approximately the same as in the previous fetuses. The pons is a little larger, the hemisphere and the posterior lobe of the cerebellum have increased in size, the medullary pyramid may be a little more distinct, but the maturational changes are rather subtle. Consequently, only a few observations will be made on the general brain stem; major focus will be on the maturational changes in the cerebellum and the inferior olive.

One of the more obvious characteristics of this stage of development is the continued buildup of fibers of all sizes throughout the whole lower

brain stem. For example, the latticework of the fine fibers that traverses the tegmentum in all directions has a greater density. The continuous addition of fibers to previously indistinct tracts makes them easier to trace. For example, the dorsal longitudinal fasciculus can now be followed in the ventral portion of the periventricular gray matter. Likewise, the complete boundaries of the fiber systems become apparent, e.g., the MLF, which was already identifiable in the 60-day fetus, changes shape from rostral to caudal. Its larger diameter at the rostral level in the mesencephalon is due to lateral extensions; in the caudal region, the field does not extend far laterally but is rather extensive in its dorsoventral diameter.

The hemisphere and the posterior lobe of the cerebellum have increased in size and manifest secondary and tertiary lobules. Concomitantly, the three peduncles and the internal white matter have also increased, although most of the lateral or caudal tertiary lobules have few, if any, fibers. The external granular layer is thinner than in previous stages of development. The molecular layer is wider than before and consists of a few, small, darkly staining cells and many lightly stained dendritic arbourizations of Purkinje cells (Plate 22). Most apparent in the molecular layer are the distinct axons of the basket cells that connect with a number of Purkinje dendrites and, to a limited degree, with the cell bodies. The distinctly formed Purkinje cells, which have a much larger cell body than before and dendrites with secondary and tertiary branches, have axons that can be followed at right angles to the basket fibers for a short distance into the dense granular layer. The inner granular layer consists of a dense pack of uniform granular cells interspersed with an occasional medium-sized cell. This layer is traversed by many fibers, including many mossy fibers that end near the

granular cells.

The deep cerebellar nuclei show both structural and cytological changes. The dentate nucleus has increased considerably in total size and can be subdivided into a large ventrolateral group and a smaller wedge-shaped dorsomedial region; the two divisions are separated by a fibrous hilus. Around the lateral border of the dentate nucleus is a dense layer of fibers, many of which are spino-cerebellar afferents. The emboliform and globose nuclei, which are larger than in previous fetuses, are composed of large triangular-shaped multipolar neurons with long branching dendrites. There is a slightly higher density of cells in the emboliform nucleus as well as a compact mass of fibers. A large number of efferents can be seen leaving the ventral region of these nuclei. The fastigial nucleus, which is still relatively small and surrounded by many long and short fibers, consists of many small undifferentiated and a few large triangular cells similar to those of the globose and emboliform nuclei.

Structurally, the inferior olivary nucleus is extremely complex since more and more fibers surround and course through it. In fact, there are so many additional fibers, particularly olivocerebellar efferents, that many previously identifiable relationships are no longer observable (Plate 17a). The general appearance of the accessory nuclei is most affected. The dorsal accessory nucleus is totally inundated with more and larger internal arcuate fibers and many additional olivocerebellar efferents, so numerous that the boundaries of the nucleus are in many places very diffuse, especially in the region of the rostral connection with the ventral lamella. However, a large number of olivocerebellar efferents from both the accessory nucleus itself and from the contralateral olivary complex clearly separate the ventrolateral surface of the dorsal accessory from the dorsal lamella of

the principal nucleus. In addition, the vertically running fibers from the reticular formation into the rostral portions of the dorsal accessory nucleus are much harder to identify because of the increasing number of fibers around the olive (Plate 17b). Likewise, the dense matrix of fibers obscures the relationships of the rubro-olivary and spino-olivary terminations within the dorsal accessory nucleus.

The medial accessory nucleus, on the other hand, has variable degrees of fibers (Plates 17c, 18b). The caudal portion has more spino-olivary afferents than ever before as well as a slight increase in the number of efferents. In addition, the caudal-most part has a better developed thick capsule of fibers, which are primarily spino-olivary afferents, plus a few efferents from the caudal region of the opposite nucleus.

As in younger fetuses, the ventral-lateral segment has various concentrations of fibers along its rostral-caudal axis and a gradual diminution of spino-olivary fibers, most of which enter the more caudal region and become less dense rostrally (Plate 18a). However, with the addition of the rubro-olivary afferents to lateral surface, which are particularly well demonstrated on horizontal sections, the rostral third has become heavily infiltrated with fibers, except for the most rostral pole. Consequently, the ventral-lateral segment receives afferents from two major sources: rubro-olivary afferents predominate in the rostrolateral regions and spino-olivary affererents in the caudal portion. Strangely, as in younger fetuses, the most rostral pole is still relatively free of afferents although it is totally encapsulated with fibers. In addition, a few single afferents can occasionally be seen arising from the corticospinal tract and projecting through the medial lemniscus and surrounding capsule into the ventrolateral segment. However, a number of this same type of single strands from the

corticospinal tract project into the diffuse reticular formation, especially in the area just rostral to the inferior olivary complex.

The dorsal-medial segment of the medial accessory olive is most unlike the rest of the olivary complex. First, it appears to be clearly separated from the ventral-lateral segment by large bundles of olivo-cerebellar efferents from both the ipsilateral and the contralateral principal olive. Second, aside from a few olivocerebellar fibers traversing it and a few internal arcuate fibers penetrating it at a slightly different angle, the region contains relatively few large neurons or fine fibrous matrix (Plate 18b). Although spino-olivary fibers completely surround it, very few project into it; and those that do appear to be primarily from the lateral part of the spinal cord.

The dorsal cap has the highest concentration per area of large cells of any part of the olivary complex. The cells are so dense that many touch other cells, a relatively rare occurrence in other parts of the olive. Concentration of the cells in the center with only a few along the periphery results in a rather vague boundary for the nucleus. Most of the few fibers associated with this region are internal arcuate fibers and a few spino-olivary afferents. In addition, the ventral-lateral-outgrowth, like the dorsal-medial region, is relatively free of either large cells or afferents, although a few spino-olivary fibers can be observed entering this region. At this stage of development, its connection with the ventral lamella consists of only a few sections (about 300μ) with almost no cells of any type at the junction.

The distinction between the dorsal and ventral lamella is still apparent, but the internal matrix of afferents in the ventral lamella more closely resembles that of the dorsal area (Plates 17a, 18d, 18e). Just

slightly rostral to the olive, the descending CTF can be seen emerging from the intermingling fibers medial to the superior olive and bifurcating; as in younger fetuses, most of them project to the dorsal-lateral border of the principal olive, and a smaller group descends to the medial sides of the olive. Horizontal sections clearly demonstrate a thick amiculum olivae in the rostral lateral border of the dorsal lamella (Plate 18f), where many afferents enter the olive via the two large dorsal sulci. The capsule diminishes in thickness caudally, although many rubro-olivary fibers can be traced to almost the caudal pole of the principal olive, which corresponds with an increase in the internal matrix of caudal area of the principal olive. The small bundle of the medial rubro-olivary fibers projects to the region between the medial extensions of the principal olive and the lateral border of the medial accessory nucleus. Here afferents enter either the ventro-medial surface of the ventral lamella, or as noted above, the rostral third of the ventral-medial segment.

Efferents of the principal olive still originate predominately from the dorsal lamella, although at this age many more efferents arise from the ventral lamella, particularly the lateral portions (Plate 17a). As before, most of them project into the hilus, then exit through the medial extension of the ventral lamella and across the medial accessory olive and finally across the midline.

Another group of fibers, which can be observed on both sagittal and horizontal sections, goes between either the hilus or rostral principal olive and the diffuse reticular zone. Because neither their exact origin nor their termination can be determined, it is impossible to say whether these fibers are afferents or efferents of the olive; however, their size suggests the latter.

146- and 147-day fetuses. The overall structure of the cerebellum at this stage is similar to that of the 133- and 137-day fetuses. However, the internal morphology of the entire cerebellar cortex in all its sections is now consistent and pronounced and distinctly separated from the underlying white matter. The external granular layer and the molecular layer are about the same thickness as before. Although the Purkinje cells are about the same overall size and have approximately the same dendritic pattern, they now display climbing fibers on their main dendritic processes, and in a few random areas, show climbing fibers on the secondary and tertiary dendritic processes (Plate 22). Concomitantly, the internal granular layer manifests a greater number of long fibers among its cells than previously; however, the increased density of fibers makes it difficult to distinguish between mossy and climbing fibers. As for the medullary layer itself, it now forms a distinct bundle within all lobules and fewer cells are located within it.

The deep nuclei of the cerebellum show considerable rarefaction of cellular elements, but an increase in the fibrous matrix. The dentate nucleus is lobe-shaped and clearly demarcated. The globose and emboliform nuclei are located at the level of the median and rostral sections of the dentate nucleus. At the caudal levels, they are not yet totally isolated from each other, but have diffuse boundaries (Plate 19f), whereas at the rostral levels they are becoming distinct. Here the emboliform nucleus is triangular; the globose nucleus is situated more ventromedially than its emboliform counterpart, its boundaries are not very distinct, and its shape is convoluted. The fastigial nucleus, which lies in the area of the vermis, has an irregular ovoid shape that is elongated medioventrally. The fiber development is particularly heavy around the fastigial nucleus

and thus contributes to the vague boundaries of the nucleus. At this age, the cellular differentiation in the nuclei is well pronounced, especially in the fastigial nucleus (Plate 19d).

The myelencephalon, like the rest of the lower brain stem, shows a marked increase in all types of fibers. Especially noticeable is the medial lemniscus, which appears in saggital section like a broad midline band of longitudinally oriented fibers that course mainly between the midline and the medial border of the olivary complex, and to a lesser degree, between the dorsal-medial segment and the medial extensions of the principal olive and between the medial area of the ventral-lateral segment and the pyramidal tract. Correspondingly, there are so many internal arcuate fibers that in the caudal half of the olivary complex they form artificial divisions, particularly between the dorsal-medial segment. The reticular formation has a proliferation of both short and long fibers as well as a clear assemblage of large reticular cells along the midline, which contribute to the increased number of vertically running fibers that enter the ventral-medial part of the dorsal accessory nucleus.

The number of ascending fibers from the spinal cord has likewise increased, particularly the spino-olivary fibers (Plates 19a, c, e). As in younger fetuses, these fibers pass around the caudal segment of the medial accessory nucleus to form a thick capsule around the ventral-lateral segment. With the increased number of spinal afferents, more and more of the accessory nucleus becomes infiltrated with the fine fibers. The spino-olivary fibers now project more rostrally on the ventral-lateral segment, so that now even the rostral pole is no longer free of fibers. In addition, more afferents project over the dorsal surface of the caudal region and enter the caudal fourth of the dorsal accessory nucleus. On the other hand,

the dorsal cap, although surrounded by spino-olivary fibers as well as medial lemniscal and olivocerebellar fibers, is relatively free of fine fibers.

The greatest change, however, is the increase in rubro-olivary afferents which now project through the gradual diffuse area of the metencephalon until they become obscured by the superior olivary complex. They then form a darkly staining bundle just before entering the inferior olivary nucleus, where they form a thick capsule around the rostral half of the principal nucleus, which gradually becomes thinner toward the caudal pole (Plate 20a, b). The majority of afferents enter the dorsal lamella via the two large sulci, whereas most of the remaining fibers enter directly into either the rostral pole or the ventral lamella in a ventrolateral direction. An increasing number of rubro-olivary fibers, however, project to both the rostrolateral region of the dorsal accessory nucleus and the rostrolateral portion of the ventral-lateral segment of the medial accessory nucleus.

More and more efferents leave the medial accessory nucleus, especially from the ventral-lateral segment. They follow the same pattern as before, that is, most of them cross the midline and traverse around the ventral surface of the medial accessory nucleus, along the lateral border of the principal nucleus, and then along the lateral edge of the tegmentum before entering the ventrolateral corner of the restiform body. The largest number of efferents from the olivary complex, however, originates from the rostral half of the principal nucleus, corresponding to the distribution of the rubro-olivary fibers (Plate 20c). Unfortunately, the complexity of the fibers around the ventral-medial region of the dorsal accessory nucleus and around the dorsal-medial segment of the medial accessory makes it impossible to observe efferents leaving these nuclei.

153-, 154-, 155-, and 157-day fetuses. Only slight differences in size

and finer histologic maturity distinguish this stage from the previous stage of development. In fact, the external and internal configuration of the lower brain stem and cerebellum is nearly complete.

In the cerebellum the hemispheres have increased only slightly and have nearly reached the contour of the adult organ. The fissures have cut deeply into the hemisphere and vermis. The external granular layer is slightly reduced in thickness, whereas the molecular layer is thicker than before, except for the lateral-most border of the hemispheres where it is approximately the same thickness as before. The Purkinje cells are prominent and have a more complex dendritic arbourization, which also includes a continued increase in the number and distribution of climbing fibers. The internal granular layer has changed little from that of the previous stages. The medullary layer, however, shows a continued buildup of fibers which corresponds with the increased number of fibers both from the pontine nuclei and from the inferior olive.

The deep nuclear groups are more massive and distinct than at any previous time. They form areas that are completely surrounded by fibers of white matter; this relation gives them an appearance like that of adult. The fastigial nucleus is well defined dorsally and medially by the medullary matter, whereas ventrolaterally, the nucleus establishes continuity via a large fiber bundle with vestibular nuclear complex. Laterally, particularly in the rostral half of the nucleus, many fibers interconnect with the globose nucleus. Histologically, the structure consists of small, medium-sized, and large cells. These cells often form ill-defined clusters which, however, are too irregular to warrant definite subdivisions, except to note that there is a higher ratio of larger cells toward the rostral half of the nucleus. The distinction between the globose and emboliform nuclei is much

more apparent than in the previous stage; however, the globose nucleus has increased much more in size than has the emboliform. In addition, the cleft separating these nuclei from the dentate is wider than in earlier stages. The cells in the globose are larger and more densely packed; but both show an overall increase of internal fibrous matrix.

The dentate nucleus, as before, forms the largest component of the cerebellar nuclear complex. The most conspicuous changes are the increase in the fibrous capsule on the lateral and dorsal borders, which corresponds to the development of the hemispheres, and the increase of fine fibers within its hilus. The cells are prominent and well stained and, as in younger animals, are mainly large multipolar types. In some cases, the axons of the large cells can be followed into the hilus. Between the cells are large numbers of fine interlaced fibers with a heavier concentration in the rostroventral region.

The inferior olivary complex displays a continued maturation of its cells and a gradual buildup of its already established pattern of afferent and efferent fibers. The large cells of the inferior olivary nucleus have approximately the same diameter as those first seen in the 127- and 129-day fetuses, but their cytoplasm has changed so that now almost all the neurons have rather star-shaped contours (Plate 21). In addition, except for the dorsal cap with its high density of cells, there is a fairly uniform distribution of the cells throughout the whole olivary complex, although there is a slight tendency for more cells to be packed more densely along the outer periphery, especially in the principal nucleus. The cells appear to be regularly arranged in three to five somewhat discontinuous rows. Whenever a cell is located well within the olive, however, its nucleus tends to be equally surrounded by cytoplasm, whereas when the cell is

located peripherally, the cell body takes on a pearshaped contour, with its nucleus close to the peripheral border and its dendrites fanning out centrally.

The distribution of afferents closely resembles that in earlier fetuses, except that the rubro-olivary system shows a marked increase (Plate 21). There is now a massive fibrous capsule around the anterior three-fourths of the principal nucleus, particularly along its lateral border. Correspondingly, the internal fibrous texture is much more dense, especially within the rostral part of the dorsal lamella. More rubro-olivary fibers can also be observed entering the rostral third of the dorsal accessory nucleus, where they contribute to the dense matrix of fibers. Although they are becoming exceedingly difficult to trace, more rubro-olivary fibers are also projecting to the rostral third of the ventral-lateral segment.

Caudally, the percentage of fibers that enter the olivary complex from the spinal cord appear to be about the same as at the previous stage, but the increase of fibers of all types within this region of the complex makes it difficult to directly follow the spino-olivary fibers to the various parts of the complex. However, there is a continued increase of fine preterminal fibers within the caudal regions of the dorsal accessory nucleus and the caudal segment of the medial accessory nucleus, particularly its lateral parts.

The dispersion of olivary efferents follows the same course observed in younger fetuses; however, there is a marked increase from the rostral half of the principal nucleus, which closely corresponds to the enlargement of the capsule. Likewise, the hilus has a greater density of fibers and more large bundles of efferents pass through the ventral-lateral-outgrowth and the dorsal medial segment.

DISCUSSION AND SUMMARY

Although no species is an exact biological replica of man, some approximate him more closely than others. The rhesus monkey has been chosen by many investigators as a suitable model of the human nervous system. Valuable information is already available on the composition and some of the dimensions of the brain and other organs in this species (Kerr et al., 1969; Portman et al., 1972; Schultz, 1937; van Wagenen and Catchpole, 1965). But, much of the published information, like that on the human fetus, has been collected from autopsy material and from fetuses of uncertain gestational age born to mothers of uncertain health and undetermined parity. The data reported here, however, are from normal fetuses of precisely known gestational age, born to normal mothers maintained under standard conditions. To provide the most complete morphological picture possible, the brain stems were serially sectioned and carefully stained with a silver reduction method that gives detailed information on many parts of the neuron, e.g., the cell body, larger dendrites, axons of all sizes, and some preterminal and terminal processes (Stotler, 1958).

Some inherent limitations in the method and some unavoidable sources of error are bound to be reflected in the interpretations of the data. For example, since the fetuses used in this study were obtained as by-products of other research projects, fetuses of the desired age were not always available. Consequently, we were not able to examine the lower brain stem of fetuses younger than 60 days gestation nor to obtain fetuses at the precise ages needed to record the changes that had occurred between those ages and the one just studied. For example, we could not study a fetus between 60 and 80 days of age although many important changes take place in that period. Finally, sufficient material to obtain different angles

of section and to examine the degree of variation among individuals was not always available at each stage of fetal development. To fill in some of the gaps between different ages, a few fetuses that were involved in experimental manipulations had to be used.

However, some compensatory features of this study help to balance these limitations. Most of the developmental changes of interest to this study occurred between 60 days and parturition; moreover, from observations of fetuses of the same age, we concluded that there was not a great deal of individual variability. In fact, these data indicate that morphological development proceeds along a gradual continuum and that even during periods of most rapid growth three or four days must elapse between ages before morphological differences can be detected with silver stained material.

A constant problem in studying morphological development is the difficulty of correctly identifying a structure at any given time. Has this structure (e.g. the Purkinje cells in this study) not developed or is it simply not visible because of inadequate staining? Despite the precision that can be achieved with the silver impregnation method in identifying and describing neuronal elements, variability in staining remains a problem not only between brain stems but sometimes within the serial sections. In addition, it is sometimes especially difficult to completely visualize neuronal connections between structures in normal material, especially since the number and size of the fibers increase with development. Consequently, in this study, various afferent connections were interpreted only after verification of more than one slide and of subsequent ages.

Having stated the advantages and limitations of this investigation, we shall discuss the results from two different angles. The morphological

structure of the inferior olivary complex at different periods will be considered first. Then the regions of origin and termination of demonstrated olivary connections will be discussed.

Morphology. Our data indicate that the inferior olive of primates is more detailed structurally than has been assumed by previous investigators. One of the outstanding features of its development is its consistency, which is best illustrated by the relative areas occupied by its three main divisions. At 60 days gestation, when the three main divisions can be clearly delineated, each division occupies about the same area in relation to the others as it does at 80, 100, 121, 129, 147, and 257 days. This ratio maintains despite some notable changes in the surrounding brain stem, such as the enlargement of the corticospinal tract or the medial lemniscus; despite structural changes within each division, variations in cellular density, differences in the distribution of afferents, and a constant increase in efferents.

The inferior olivary complex is one of the earliest parts of the lower brain stem to mature in the monkey. In fact, at 60 days gestation, it has already become one of the characteristic landmarks of the lower brain stem, along with the cranial nuclei, red nucleus, superior olivary nucleus, pons, and cerebellum. It develops rapidly from 60 to 121 days when, for the first time, it changes its external structure, shows internal fiber development, and displays cellular differentiation (92 to 100 days). Thereafter, like the surrounding brain stem, it changes much more gradually; the chief changes are an enlarging of the fiber system and an organized distribution of the cellular elements. This developmental sequence closely parallels that of the rest of the lower brain stem, although the first onset of cellular differentiation begins at different times in the various

neuronal groups, the cranial nuclei being among the first to differentiate and the diffuse, large reticular neurons among the last. Most of the fiber systems appear to be firmly established by about 130 days, after which enlargement, development of the collaterals and terminal processes, and myelination occur. In other words, the basic organization of the lower brain stem of the rhesus monkey occurs between 60 and 130 days gestation.

This pattern of the rapid growth of most of the cellular elements and the establishment of most pathways of the brain stem between 60 and 130 days, which is followed by the gradual enlargement of the fibers, differs radically from that suggested by the weight curves of Portman et al. (1972). The reason for this difference is not apparent in the present data, but one of the more obvious explanations is that myelination of the various fibers had not yet been completed. Although the rhesus monkey brain is probably more completely myelinated than the human brain at birth, myelin concentration in the brain stem of both species continues to increase for a long time after birth. For example, Yakovlev and Lecours (1967) have demonstrated histochemically that the myelination of the reticular formation, particularly in intrinsic fibers, continues past puberty. The protracted development of myelination in the primate brain was demonstrated in 1919 by measurements of myelin lipids (MacArthur and Doisy, 1919). Another explanation is the possible enlargement of dentrites, which were not stained completely in this study. Using Golgi stain, Noback and Purpura (1961) observed a sizable increase in the dendritic patterns of cells in the neocortex of kittens just before and continuing after birth.

Although the three major divisions of the inferior olive can be identified at 60 days gestation, it undergoes numerous structural changes during the period of rapid growth. In the 60- and 80-day fetus, the

medial border of the dorsal accessory nucleus is connected with the caudal, medial extension of the dorsal lamella, and the rostral pole of the ventral-lateral segment contacts the rostral, medial part of the ventral lamella. These two connections are evident only during the 60- and 80-day periods, but they may exist before the 60-day stage. According to Harkmark (1954a), the deep migration from the rhombic lip in the chick gives rise to the entire dorsal lamella, which, according to Kooy (1917), is homologous with the mammalian accessory olivary nuclei. A superficial migration of cells in the chick gives rise to the ventral lamella, which, according to Kooy (1917), is homologous with the principal olivary nucleus in the mammal. This suggests that the connections between the dorsal accessory and the dorsal lamella on the one hand, and the rostral ventral-lateral segment and the ventral lamella on the other are formed sometime before 60 days and then for some reason become separated before 92 days of gestation. The reason for this dissociation is not apparent in the present data, but it could be caused by a mechanical influence from the surrounding enlarging fiber systems. Both the pons and the medial lemniscus show considerable growth between the 80- and 92-day period; the increase in total size of the corticospinal tract especially changes the position of the medial accessory nucleus. In the 60- and 80-day fetuses, the dorsal-medial segment is situated more ventrolaterally, almost on the floor of the myelencephalon; but as the corticospinal tract assumes its characteristic adult pyramid shape, this segment assumes a more upright, medial position. Another possible factor, especially with the dorsal accessory-dorsal lamella connection, is the continued increase in the number and distribution of olivocerebellar efferents. Many contralateral olivocerebellar fibers passing between the dorsal accessory nucleus and the dorsal lamella would

help to clearly delimit their boundaries. Finally, a corresponding increase in the size of the principal nucleus may exert a mechanical influence that would simply break these connections.

The ventral-lateral-outgrowth also changes in size, being proportionally largest at 80 days, when there is very little difference between it and the ventral lamella in diameter or distribution of neuroblasts. As it continues to develop, the ventral-lateral-outgrowth becomes proportionally smaller until by 129 days it extends for only a few sections and its cell population is extremely low.

In general, the morphology of younger fetuses more clearly resembles that of the more phylogenetically primitive forms. However, the increase in the dorsal lamella, the decrease in the size of the ventral-lateral-outgrowth, and the loss of connections between the dorsal accessory and the dorsal lamella, and between the rostral, ventral-lateral segment and the ventral lamella resemble the structure of phylogenetically newer species. For example, Kooy (1917) noted that in lower mammals, the ventral lamella is connected with the medial accessory nucleus almost over its total length, whereas in higher mammals this connection is chiefly confined to the caudal sections. As it develops, the principal nucleus becomes independent of other divisions, and the caudal connection is via the ventral-lateral-outgrowth alone until, in higher primates, there is no connection at all.

Afferents. When these observations are compared with those of previous investigators, only minor discrepancies in the origin and distribution of afferents to the inferior olivary complex are found. Ontogenetically, the first distinctly evident fibers are from the spinal cord; these are followed by those from the red nucleus, from the corticospinal tract, and finally from the different parts of the reticular formation. These afferents all

have discrete regions of termination within the olivary complex where they, in turn, increase and overlap with age.

The spino-olivary afferents, the only ones to be identifiable at 60 days gestation, are limited to the caudal-most regions of the medial accessory nucleus. Even at later stages of development when the pathway is much larger, it is extremely difficult to follow them for any length into the spinal cord, mostly because of the decussation of the corticospinal tract. However, most of the spino-olivary fibers, in conjunction with a number of other ascending fibers, appear to traverse the spinal cord in the ventral, and to a limited degree, in the ventrolateral funiculus. After entering the brain stem, most of the ascending spinal fibers course over the dorsal and lateral surfaces of the inferior olivary nucleus (as well as a few under the ventral border) to more rostral areas of the brain; only a few, probably about a sixth of the bundle, project onto the inferior olive. These findings are in complete agreement with those of Brodal et al. (1950), Mizuno (1966), and Kusama (1961).

As to the nucleus of origin of the spino-olivary afferents, some investigators, using electrophysiological techniques, suggested that in the cat it was the lateral cervical nucleus (Grundfest and Carter, 1954; DiBiagio and Grundfest, 1955, 1956; Krieger and Grundfest, 1956). These investigators did not deny the possibility of other spino-olivary pathways but suggested that the lateral cervical nucleus is the main source of spinal afferents to the inferior olive. However, Mizuno's (1966) morphological study indicated that the lateral cervical nucleus is not the main source of spino-olivary fibers, since a large number of these fibers were observed to originate below the level of the lateral cervical nucleus. Moreover, lesions of the lateral cervical nucleus produced very little degeneration

in the inferior olive, no more, at least, than in those cases where the lesion did not involve the lateral cervical nucleus. Mizuno (1966) suggested instead that the spino-olivary fibers originate in the posterior horn at all levels of the spinal cord and ascend chiefly through the ventral funiculus.

The terminal distribution of spino-olivary fibers in the fetal monkey is initially to the caudal part of the olivary complex, mainly the dorsal and medial accessory nuclei. Using the Golgi technique, Scheibel and Scheibel (1955) likewise described a large number of afferents terminating directly in the caudal pole. However, in the present study, by 80 days gestation, most of the fibers were coursing around the most caudal region of the medial accessory nucleus and entering its caudal ventral-lateral segment. With increasing age, more and more spino-olivary fibers projected more rostrally along the ventral-lateral segment, although its rostral pole was always relatively free of any fibers. The distribution of afferents correspond closely to the extremely dense internal fibrous matrix, which always made the ventral-lateral segment distinctive from the other divisions of the inferior olive. Likewise, with age, there were more spinal afferents in the rostral regions of the dorsal-lateral part of the dorsal accessory nucleus, but at no time could they be identified in the rostral ventral-medial region. In addition, a limited number of spino-olivary fibers entered the dorsal-medial segment, the dorsal cap, and the ventral-lateral-outgrowth.

In several morphological investigations of the spino-olivary pathway in the cat, much degeneration was observed in the caudal regions of the dorsal accessory nucleus (Anderson and Berry, 1959; Brodal et al., 1950; Johnson, 1954; Mizuno, 1966; Nauta and Kuypers, 1958; Wilson and Magoun, 1945). On the other hand, the medial accessory nucleus was reported to

receive a more limited number of spinal afferents (Anderson and Berry, 1959; Brodal et al., 1950; Mizuno, 1966; Nauta and Kuypers, 1958) or none at all (Wilson and Magoun, 1945). However, the fact that all these investigations involved either cats or rabbits may account for the discrepancy between these findings and ours of a higher proportion of spinal afferents terminating within the medial accessory nucleus. In a study of spinal afferents in the adult monkey, Mehler et al. (1960) also noted a large number of spinal fibers terminating within the caudal medial accessory nucleus as well as in the dorsal accessory nucleus. They caution, however, that there may be no homologous connection in man, since they were unable to demonstrate them in either the chimpanzee or man after a complete ventrolateral cordotomy (Mehler et al., 1960).

In younger fetuses, a few spinal afferents could be seen entering the dorsal cap and the ventral-lateral-outgrowth. Only Brodal et al. (1950) and Mizuno (1966) mention finding degeneration in both these areas after lesions of the spinal cord. The dorsal cap and the dorsal-medial segment have also been suggested as an area of termination of fibers from the spinal trigeminal nucleus (Stewart and King, 1963). However, we were unable to separate out any trigeminal afferents from the mass of spinal afferents that surround this region of the inferior olive. Moreover, in younger fetuses, a few spinal fibers were observed to enter the caudal-most region of the principal nucleus. However, the use of several different species, including primates, has resulted in a great deal of contradictory evidence for the projection of spinal fibers to the principal nucleus (Anderson and Berry, 1959; Bowsher, 1962; Mehler et al., 1960; Mizuno, 1966).

The other major afferent system to the inferior olive is the rubro-olivary tract, which was first observed at the rostral pole of the principal

nucleus of the 80-day fetus. The red nucleus of the monkey differs from that of the cat in that in the latter the parvicellular region and the magnocellular part cannot easily be distinguished (Pompeiano and Brodal, 1957). Although the magnocellular region is present at 60 days gestation in the fetal monkey, the parvicellular portion cannot be identified until 92 days, after which the transition between the two parts becomes more apparent, especially on sagittal sections. In addition to the distinction between small and large cells, each portion received afferents and has distinctly separate efferents. The parvicellular region receives projections from the precentral gyrus, the rostrally adjacent frontal areas, and to some degree from the postcentral gyrus and from the dentate nucleus; the magnocellular region, on the other hand, receives afferents from the precentral gyrus and from the interpositus nucleus (Kuypers and Lawrence, 1967; Massion, 1967; Papez and Stotler, 1940). In addition, many fibers surround and course through both regions of the red nucleus, such as those from the subthalamic, reticular, tectal, and substantia nigra, to name a few. In fact, the close association between these fibers suggests functional connections at least. However, lesions to these adjacent nuclear regions do not result in degeneration within the inferior olive (Walberg, 1956; Rasmussen, 1936).

There is no universal agreement about the distribution of the efferents of the red nucleus, particularly of the parvicellular portion. Classically, this nucleus was thought to send fibers to the ventral-lateral nucleus of the thalamus (Crosby et al., 1962). In fact, Carpenter (1956), using the silver technique, reported finding homolateral ascending degeneration in the arcuate and reticular thalamic nuclei, in the globus pallidus and subthalamic nucleus, and descending, in the homolateral spinal trigeminal nucleus, lateral reticular nucleus, and the principal nucleus of the

inferior olive. The most convincing evidence, however, was presented by Poirier and Bouvier (1966), who described a single descending pathway from the parvicellular portion to the dorsal lamella via the CTF. However, in the fetal monkey, the organization of this pathway is highly diffuse. On selected sections, a small number of fibers can be traced from the parivcellular region, straight through the magnocellular parts and the descending brachium conjunctivum, where it mingles with the many longitudinal fibers of the tegmentum of the metencephalon. This close association with other fibers helps to explain some of the confusions in the literature about the components of the CTF. Just caudal to the superior olivary nucleus, the CTF can again easily be distinguished and followed to the rostral end of the principal nucleus where it forms a capsule of fibers that go both dorsally and ventrally around the nucleus. Unfortunately, even in the younger fetuses, it was never completely possible in the metencephalon to isolate the rubro-olivary fibers from the adjacent longitudinal fibers, an indication that the CTF probably incorporates afferents to the inferior olive from other sources besides the red nucleus.

The orientation of the termination of the rubro-olivary fibers was mainly toward the dorsal lamella via the large dorsal sulci. With continued development numerous fibers were seen to project onto the rostral ventral lamella and a small number onto the most rostrolateral regions of the dorsal and medial accessory nuclei. At almost every stage of development, the rubro-olivary fibers were seen to progress more and more caudally in all areas of termination, so that there were areas, especially in the medial accessory nucleus, where an overlap with other afferents occurred. However, these findings are somewhat at variance with the pattern of distribution described for the cat (Stotler, 1954; Walberg, 1956) and the monkey (Poirier

and Bouvier, 1966) which restricts the distribution mainly to the dorsal lamella. This difference is not too surprising since in the cat the relatively small principal nucleus is mainly made up of the dorsal lamella (Brodal, 1954), whereas the accessory nuclei in both the cat and monkey are heavily infiltrated with fine fibers which could make indentification of degeneration very difficult. Another possible explanation is that in the present material it was not possible to identify other afferents that may project to the olive in the CTF; the ventral lamella has been suggested as the area of termination of fibers from the central gray of the mesencephalon (Mettler, 1944; Poirier and Bouvier, 1966; Walberg, 1954) and for cortical projections (Snider and Barnard, 1949).

Two smaller groups of afferents were also observed, the cortico-olivary and the reticulo-olivary. In the 92-day fetus a few fibers from the corticospinal tract were observed projecting at about a 45° angle into the caudal region of the medial accessory nucleus and the caudal-most part of the ventral-lateral segment. During the next few stages of development, we observed a continued increase in the number of fibers entering the olive from the corticospinal tract. However, because of the increased density of the spinal afferents, both within and surrounding the medial accessory nucleus, continued identification of the cortico-olivary became extremely difficult after 121 days gestation. Several other investigators have reported cortico-olivary afferents, but with a much greater terminal distribution within the inferior olive (Mettler, 1935, 1947; Sousa-Pinto, 1969; Sousa-Pinto and Brodal, 1969; Walberg, 1956). Walberg (1956) reported that in the cat most of the cortico-olivary fibers project upon the caudal medial accessory nucleus and the medial region of the ventral lamella. An even greater distribution, which included the dorsal accessory nucleus, dorsal

lamella, dorsal-medial segment, and to a lesser extent, the ventral lamella, was observed in the cat by Sousa-Pinto and Brodal (1969). In addition, within the inferior olive they noted a somatotopical organization of fibers from the somatomotor cortex. Numerous correlative electrophysiological studies also demonstrate evoked potentials in the inferior olive after cortical stimulation (Armstrong and Harvey, 1966; Crill and Kennedy, 1967; Sedgwick and Williams, 1967; Snider and Barnard, 1949). Although these electrophysiological studies are in general agreement with the morphological findings, they do not pinpoint precisely the areas of termination.

The large region of termination of cortical fibers within the inferior olive appears to be a contradiction of the small number of fibers observed leaving the corticospinal tract. Although the corticospinal tract has been suggested as the primary pathway for the cortico-olivary fibers (Mettler, 1935, 1947; Walberg, 1956), other pathways may be involved, including the CTF (Snider and Barnard, 1949). The variation of latency between the evoked potential in the inferior olive and cortical stimulation also suggests more than one pathway. Thus, Armstrong and Harvey (1966) reported a latency of 8 to 9 msec., whereas Sedgwick and Willaims (1967) obtained latencies of 10.0 to 24.9 msec., and Crill and Kennedy (1967) reported a mean latency of 12.8±3.6 msec. However, the anatomy of the alternative connection is insufficiently known.

The reticulo-olivary fibers were the last observed to develop. Faintly in the 121-day fetus and more distinctly in the 127- and 129-day fetuses, fibers with a vertical course could be traced from the diffuse medial tegmental region into the rostral dorsal accessory nucleus, especially the ventral-medial segment. In a few instances, fibers could also be followed through the dorsal accessory nucleus to become incorporated

within the hilus. Similar reticulo-olivary fibers have been described in Golgi-stained material (rats, cats, and dogs), which originated from the large reticular cells that overlie the inferior olive (Valverde, 1961). Scheibel and Scheibel (1955) also observed in Golgi material reticulo-olivary fibers which originate from the medial diffuse reticular area and project to the dorsal parts of the inferior olive. In addition, they suggest that some of the fibers are contributions from the medial longitudinal fasciculus, which has also been suggested as the pathway for reticulo-olivary fibers that have their cell bodies within the mesencephalon, usually just dorsal and lateral to the red nucleus (Mabuchi and Kusama, 1970; Ogawa, 1938; Walberg, 1960). Scheibel and Scheibel (1955) have described in the monkey and in man another group of reticulo-olivary fibers which originate from the reticular cells just dorsolateral to the olive and project to the principal and medial accessory nuclei. In our study, a few thin fibers were observed between the principal nucleus and laterally-located reticular cells, but it was impossible to identify them as reticular afferents rather than the credible olivo-cerebellar efferents.

Efferents. The inferior olivary complex is generally believed to project via a distinct bundle within the restiform body to the cerebellar cortex (Brodal, 1954; Holmes and Stewart, 1908) where it then terminates upon the large Purkinje cells as climbing fibers (Eccles et al., 1964, 1966; Hamori and Szentagothai, 1966; Stotler, 1973). This raises the question whether there is any relationship between the morphological development of the inferior olivary nucleus and the differentiation of the cerebellum.

Data from the present study show that cellular differentiation of the inferior olivary complex precedes that of the cerebellum by about 20 days. In fact, cellular maturation of the inferior olive rather closely

corresponds to a similar development of the deep cerebellar nuclei. Fiber development also occurs earlier in the inferior olivary nucleus than in the cerebellum. Olivary efferents can be seen leaving the nucleus long before they can be observed around the Purkinje cells. A number of olivocerebellar fibers can be seen exiting from the ventral-lateral segment as early as 80 days, but the only fibers seen within the cerebellum at this time are vestibulocerebellar and, by 92 days, a few spino-olivary afferents. It is not, however, until 100 days that a definite medullary layer is clearly visible within the vermian component of the cerebellum. At 100 days, there is also the first hint of differentiated Purkinje cells, but it is not until 107 days that immature Purkinje cells can be clearly identified. At this time, the inferior olivary complex displays a great deal of cellular differentiation and a large number of olivocerebellar efferents, which leave not only the ventral-lateral segment but the dorsal-medial region and the principal nucleus as well. At about 127 days, the Purkinje cells have an adult appearance, but another 20 days must elapse before any climbing fibers can be identified around their dendritic tree. Meanwhile, the olivary efferents can be seen as a profusion of thick, dark-staining bundles that arise from all parts of the olive, especially the dorsal lamella. Consequently, over 65 days elapse between the time efferents are first observed to leave the olive and their identification upon the Purkinje cells.

It may also be inappropriate to state that the climbing fibers observed on the Purkinje cells at 146 days are from the inferior olive since there is still some question whether all climbing fibers originate from the inferior olivary nucleus (Batini and Pumain, 1968; Llinas et al., 1967). Older experimental work (Carrea, Reiseig, and Mettler, 1947) suggests that climbing fibers are collaterals of the deep cerebellar nuclei and that the inferior

olivary efferents end as mossy fibers. The deep cerebellar nuclei seem unlikely sources of climbing fibers since their cellular differentiation and fiber development are well established by 100 days; in many cases, their cells are located almost adjacent to the Purkinje cells, yet no climbing fibers are seen until 146 days. More recently, Dunsker et al. (1970) reported finding in cats and rats mainly degenerated mossy fibers and only a few degenerated climbing fibers after bilateral lesions of the inferior olivary nucleus. These results may be explained by the fact that their lesions also involved many reticulo- and arcuatocerebellar fibers, which terminate in the cerebellum as mossy fibers (Brodal, 1954). Moreover, it is extremely difficult to stain axonal degeneration fragments in the molecular layer of the cerebellar cortex (Hamori and Szentagothai, 1966; Stotler, 1973).

Function. Although the main objective of this study is morphological, it seems appropriate, however, to discuss some of the inferior olive's functional relationships. Since the only output from the inferior olive is to the cerebellum, it must be permissible to conclude that the organization of the fiber connections established via the inferior olive between separate regions of the nervous system and the cerebellum reflects functional differences within various subdivisions of the cerebellum. That is, the various afferent fibers to the inferior olivary complex have distinct areas of termination; the inferior olivary efferents, in turn, project to very discrete locations within the contralateral cerebellum (Brodal, 1954; Holmes & Stewart, 1908). The spino-olivary fibers project mainly upon the caudal and ventral-lateral segments of the medial accessory nucleus and upon the caudal third of the dorsal accessory nucleus. These areas send their efferents only to the cerebellar vermis, which is the approximate same location as the termination of the bulk of the dorsal and ventral spinocerebellar tracts (Brodal, 1954). In addition,

both the dorsal spinocerebellar tract and the spino-olivocerebellar pathway in the cat are mainly involved in mediating impulses from the hind limb and tail (Brodal et al., 1950). The functional significance of multiple pathways from the spinal cord to the cerebellum are not known.

Another distinct olivocerebellar connection is between the rostral part of the ventral-lateral segment, which receives afferents from both the CTF and the spinal cord, and the flocculonodular lobe. The dorsal-medial segment of the dorsal accessory nucleus, which receives reticulo-olivary fibers, sends efferents solely to the more lateral regions of the anterior lobe. Finally, the principal nucleus, which has rubro-olivary fibers as its major source of afferents, is the origin of fibers to the cerebellar hemispheres. The possible relative importance of each of these olivocerebellar connections upon the final cerebellar output is not, at present, known.

The similarity between the sequence of development of the inferior olivary nucleus and the cerebellum parallels their similar phylogenetic development, which is also suggestive of a functional relationship. The phylogenetically older parts of the inferior olive, the accessory nuclei (Ariens Kappers et al., 1936), are the first regions to receive afferents and the first area from which efferents can be identified. In the cerebellum, on the other hand, the vermian component is phylogenetically older than the hemispheres (Larsell, 1947) and it is the region where lobulation, cellular differentiation, and fiber development (including climbing fibers) first occurs. At different periods, the vermian component is as much as 10 days earlier in the developmental sequence than the hemispheres, which agrees with data for the human and monkey fetus (Verbitskay, 1969). Conversely, the phylogenetic newer parts, the principal nucleus of the inferior olive and hemispheres of the cerebellum, are the last to morphologically develop.

The inferior olive has also been related to the cerebellum on the basis of changes in behaviour after lesions of the nucleus. In general, disturbances in equilibrium, ataxia, intention tremors of the neck, hypotonia of the trunk, and nystagmus have been observed to varying degrees after damage to the inferior olivary complex or to the olivocerebellar fibers in cats (Wilson & Magnoun, 1945; Carrea et al., 1947; Murphy & O'Leary, 1971), in rabbits (King, 1948), and in monkeys (Orioli & Mettler, 1956), which are very similar to the behaviour changes seen after lesions of the cerebellum (Crosby et al., 1962). However, there are very little data on the effects of selective damage to subdivisions of the olivary complex. Carrea et al. (1947), in one cat, observed that truncal ataxia was associated with lesions placed in the posterior part of the olivary complex, whereas in four other cats lesions of the anterior region of the olivary complex resulted in a gait disturbance where the animals walked with their hindlimbs fully extended and only the tips of footpads touching the floor. They concluded that the effect of the former resembled lesions placed in the posterior vermis and of the latter in the cerebellar hemispheres. These data are in somewhat agreement with the present study, since, in general, the pattern of the afferents to the inferior olive is suggestive of a rostrocaudal difference. However, a scrutiny of Carrea et al.'s (1947) diagrams of the lesions shows in every case damage to the spinal parts of the accessory nuclei. In another study, Murphy and O'Leary (1971) reported that lesions confined to the rostral principal nucleus with only minor involvement of the accessory nuclei produced, as the only behavioural change, a broadbased stance upon landing after being dropped a few feet. The meaning of this deficit of a rather artificial, narrow behaviour in relation to the wide range of posture and movements that are characteristic of a normal is not known, nor, consequently, is the relationship between the behavioural deficit and the anatomical con-

nection: rubro-olivoneocerebellum (hemispheres). The interpretations of the lesion data are also confounded by the fact that in all the above reports there was damage to several tracts that lie at the circumference of the inferior olivary complex (e.g.: corticospinal, vestibulospinal, rubrospinal, medial lemniscus, spinothalamic, spinocerebellar, and reticulocerebellar), all of which have known effects upon motor activity (Crosby et al., 1962). There has also been very little attempt to control for species differences, maturity of the animals, or the processes of a lesion becoming chronic.

Although the present data provides for a more complete understanding of the relationship between various parts of the nervous system and the cerebellum, at present neither the functional role of the afferents to the inferior olive, the subdivisions within the inferior olive, nor the cerebellar organization and its efferents are sufficiently known to warrant more than the general conclusion that the inferior olive provides a relay for descending fiber systems and spinal afferents to the cerebellum, which are somehow related to normal motor activity. Further studies of the physiology of the inferior olivary nucleus may be expected to yield decisive information only when consideration is given to its anatomical organization.

CONCLUSIONS

1. Cellular differentiation and the establishment of fiber pathways in the lower brain stem occur between 60 and 130 days gestation in the rhesus monkey. This does not correspond to the 130-days-to-parturition period of rapid weight gain, which may be due to myelination.
2. The inferior olivary nucleus, like the cranial nerves, shows considerable structural organization by 60 days gestation. At this time, the three major divisions of the inferior olive can be delineated: the principal nucleus and the dorsal and medial accessory nuclei. Although they undergo considerable structural change with age, their areas remain fairly proportional to each other throughout fetal development.
3. The shape of the inferior olive in younger fetuses rather closely resembles that of phylogenetically lower species. Associated with development is the loss of contact between the dorsal accessory nucleus and the caudal part of the dorsal lamella of the principal nucleus and between the ventral-lateral segment of the medial accessory nucleus and the ventral lamella of the principal nucleus.
5. The inferior olivary nucleus receives afferents from a number of sources, each of which has a distinctive distribution within the complex:
   a. Spinal afferents, first identified in the 60-day fetus, ascend the spinal cord in the ventral and ventrolateral funiculus. They project primarily upon the caudal and ventral-lateral segments of the medial accessory nucleus and upon the caudal dorsal accessory nucleus.
   b. Rubro-olivary fibers, first identified at 80 days, originate from the parvicellular region of the red nucleus and descend to the rostral principal nucleus via the CTF. Their distribution is

mainly to the dorsal lamella and, to a lesser extent, to the ventral lamella, although a few fibers also project to the rostral dorsal accessory and medial accessory nuclei. In the medial accessory nucleus, there is some overlap with spinal afferents.

c. Cortico-olivary fibers can be clearly identified only between 92 and 121 days of gestation, before they are obscured by the density of spinal afferents. They project off the corticospinal tract to the caudal and ventral-lateral segments of the medial accessory nucleus.

d. Reticulo-olivary fibers are the last to be identified and look like thin vertically oriented fibers, which originate from the medial diffuse reticular region of the myelencephalon and project mainly to the ventral-medial segment of the dorsal accessory nucleus.

5. Maturation of the inferior olive occurs as much as 20 days before that of the cerebellum. Likewise, olivocerebellar efferents leave the inferior olive more than 65 days before they can be identified as climbing fibers around the Purkinje cells.

6. The fact that most of the olivocerebellar fibers originate from the dorsal lamella corresponds with the distribution of rubro-olivary fibers.

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APPENDICES

APPENDIX I

Dehydration, Clearing, Infiltration

1. Formalin-fixed tissue (blocks of about 4-8 mm.)

2. Dehydration:

a.	50% Ethyl alcohol	-	1 to 2 hours
b.	50% Ethyl alcohol	-	2 hours to overnight
c.	70% Ethyl alcohol	-	2 to 3 hours
d.	70% Ethyl alcohol	-	2 hours
e.	80% Ethyl alcohol	-	2 hours
f.	95% Ethyl alcohol	-	2 hours
g.	Butyl alcohol	-	overnight

3. Clearing

a.	Toluene	-	2 hours
b.	Toluene	-	2 hours

4. Infiltration with paraffin

a.	Paraffin	-	1 hour
b.	Paraffin	-	1 hour
c.	Paraffin	-	1 hour

APPENDIX II

A. Procedure for INTENSIFIED PROTARGOL STAIN FOR NERVE TISSUE
modification of Stotler (1951)

1. Tissue embedded in paraffin, sectioned at 15μ and mounted on glass slides

2. Deparaffinized and hydrated in successive graduate alcohols, washed in water:

a.	Xylene	-	10 min.
b.	Xylene	-	10 min.
c.	100% ETOH	-	10 min.
d.	95% ETOH	-	10 min.
e.	80% ETOH	-	10 min.
f.	70% ETOH	-	10 min.
g.	Water		

3. Place sections in dish of 200cc of water, add copper shot to cover the bottom of staining dish

4. Add 4 drops of pyridine and .25 gm of protargol (protargol is sifted onto the solution).

5. Leave on 37° C heating plate for 12 hours.

6. Reduce in following solution:

Sol. I	6% silver nitrate
Sol. II	25 gm sodium sulfite, 400 ml water
Sol. III	30 gm sodium thiosulfate, 400 ml water
Sol. IV	20 gm sodium sulfite, 3.2 gm Elon (p-Methylamino-phenol sulfate), 400 ml water

(combine one part of solutions I, II, and III, and three parts of solution IV. Slides are flooded with reducing solution, optimum reduction obtained by direct microscopic observation.)

7. Sections washed briefly in water, dehydrated in graded alcohols, cleared with toluene, and covered-slipped.

a.	70% ETOH	-	10 min.
b.	80% ETOH	-	10 min.
c.	95% ETOH	-	10 min.
d.	Butyl	-	10 min.
e.	Toluene	-	10 min.
f.	Toluene	-	10 min.

B. Procedure for CRESYL FAST VIOLET FOR NERVE TISSUE
modification by Stotler (1973)

1. Steps 1 and 2 above

2. Stain in 0.1% Cresyl Fast Violet - 5 min.

3. Dehydrate in successive graduated alcohols (70%, 80%, 95%, 100%)

4. Differentiate by direct microscopic observation in:
   a. 100 ml 100% ETOH
   b. 100 ml chloroform

5. Clear and covered-slipped in:
   a. Butyl - 10 min.
   b. Toluene - 10 min.
   c. Toluene - 10 min.

ABBREVIATIONS FOR ALL PLATES

aq	cerebral aqueduct
bc	brachium conjunctivum
bp	brachium pontis (middle cerebellar peduncle)
C	caudal
cb	cerebellum
cg	central gray
chp	choroid plexus
ctf	central tegmental fasciculus
cn	cuneate nucleus
cp	cerebral peduncle
cs	corticospinal tract
cv	cerebellar vermis
D	dorsal
dc	dorsal cap
den	dentate nucleus (lateral cerebellar nucleus)
dio	dorsal accessory nucleus
dl	dorsal lamella
dt	descending root of trigeminal nerve
egl	external granular layer
emb	emboliform nucleus
fas	fastigial nucleus
fc	fasciculus cuneatus
fg	fasciculus gracilis
fl	flocculus
fv	fourth ventricle
glb	globose nucleus
hip	habenulointerpeduncular tract
ic	inferior colliculus
igl	internal granular layer
ion	inferior olivary nucleus
j	juxtarestiform body
L	lateral
lcn	lateral (external) cuneate nucleus
loc	locus coeruleus
lrn	lateral reticular nucleus
M	medial
mes	mesencephalic root of the trigeminal
mio	medial accessory inferior olive
ml	medial lemniscus
mlf	medial longitudinal fasciculus
mol	molecular layer
oc	olivocerebellar efferents
Pc	Purkinje cells
pf	primary fissure
plf	posterior-lateral fissure
pn	pontine nuclei
R	rostral

rb	restiform body
rn	red nucleus
ro	rubro-olivary tract
rs	rubro-spinal tract
rtn	reticular nuclei
sc	superior colliculus
sf	solitary fasciculus
sn	substantia nigra
son	superior olivary nucleus
tb	trapezoid body
ts	tectospinal tract
vn	vestibular nerve

III(N)	oculomotor nucleus (nerve)
IV (N)	trochlear nucleus (nerve)
V (N)	trigeminal nucleus (nerve)
VI (N)	abducens nucleus (nerve)
VII (N)	facial nucleus (nerve)
VIII (N)	statoacoustic nerve
X (N)	vagal complex (nerve)
XII (N)	hypoglossal nucleus (nerve)

PLATES

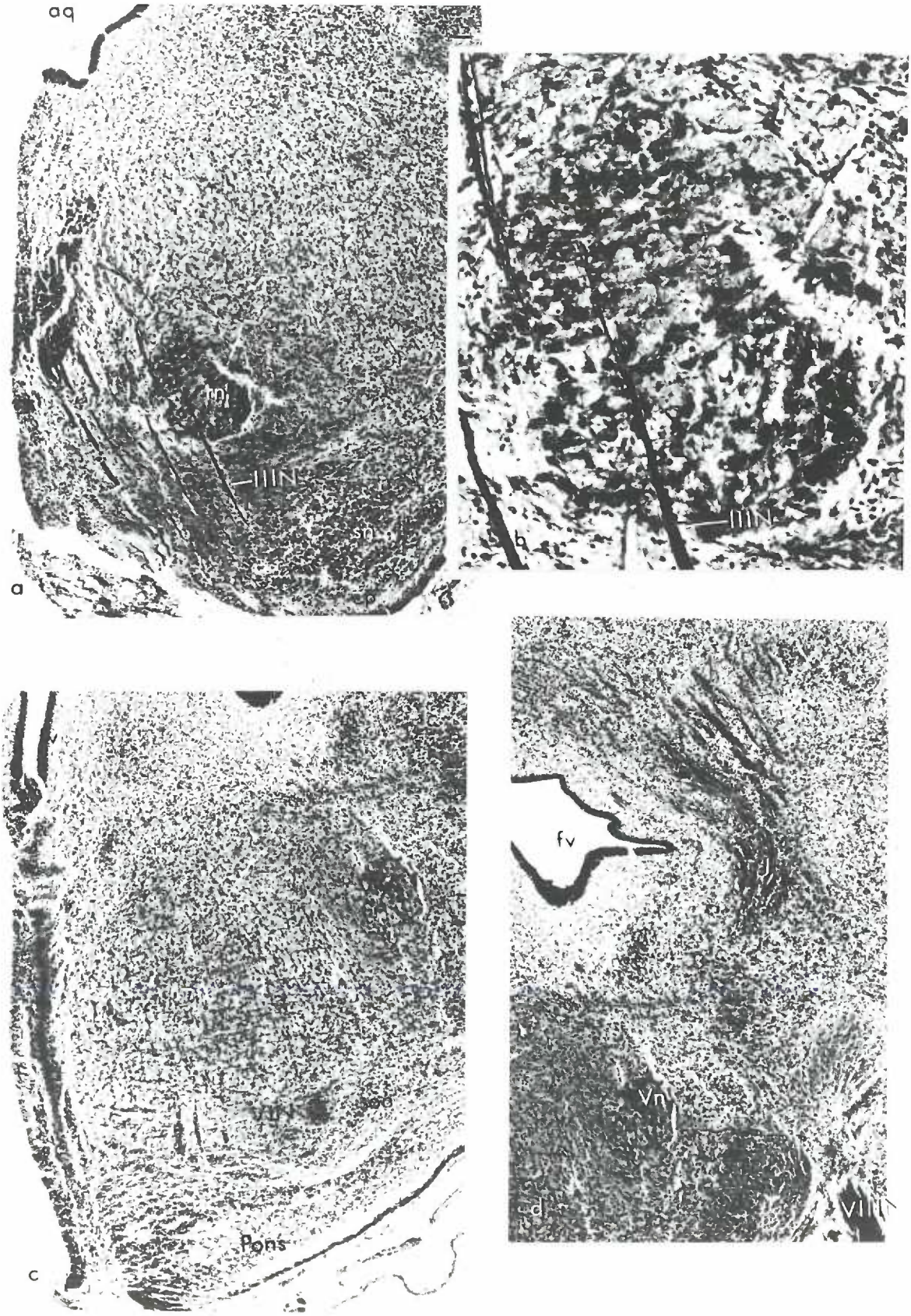
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PLATE 2

a. Cerebellum of the 60-day fetus.
Evident are the external granular layer, which surrounds the whole structure, and the primary and posterior-lateral fissures.
(sagittal, X 60)

b. Deep nuclei of the cerebellum of the 60-day fetus.
Some of the fibers of the juxtarestiform body are evident.
(coronal, X 100)

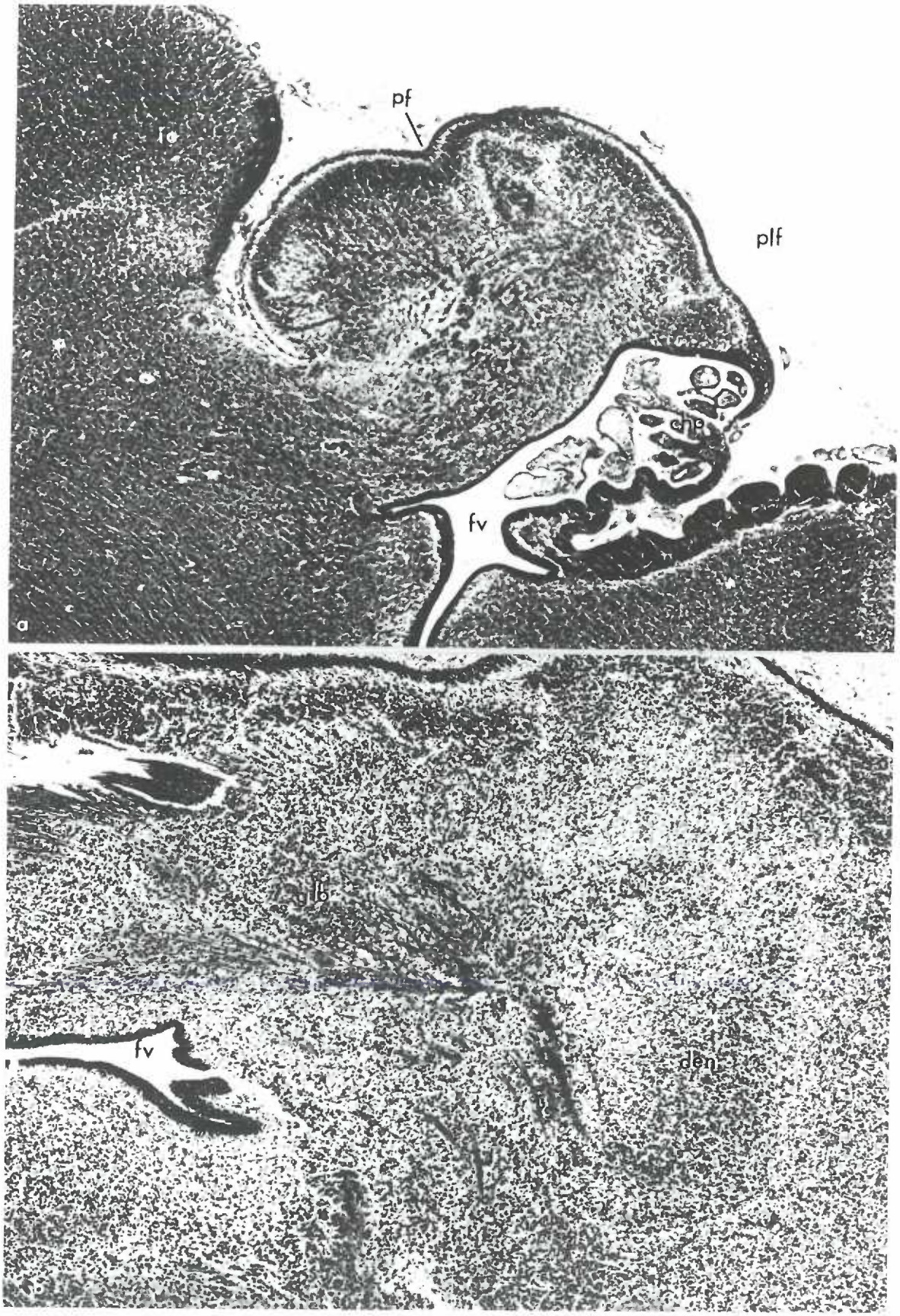
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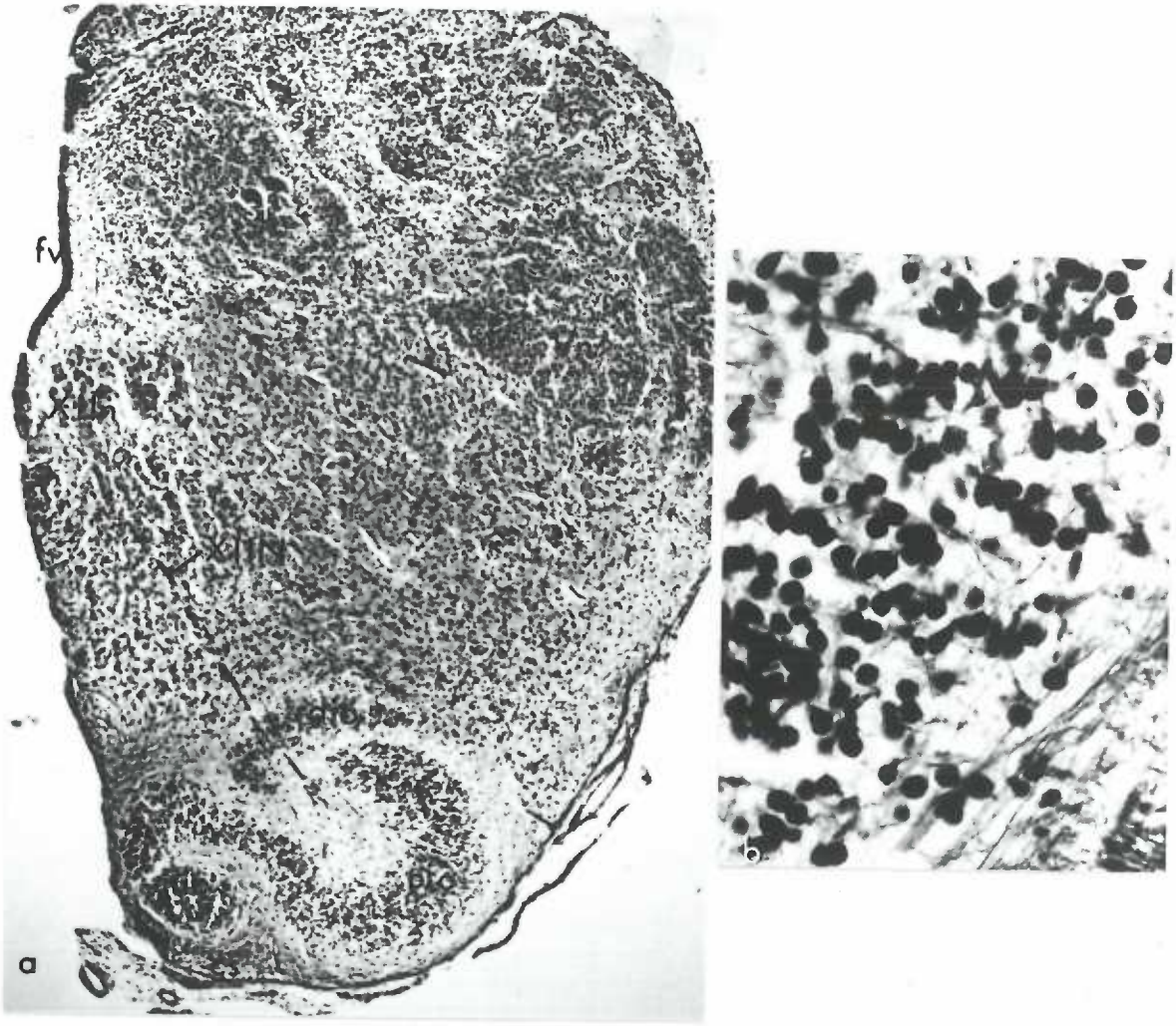
PLATE 3

a. Rostral myelencephalon of the 60-day fetus.
Most prominent are the cranial nuclei and the inferior olivary complex, whereas the pyramids and other longitudinally oriented fiber systems are difficult to distinguish. At this age the olivary complex can be clearly divided into three regions. (coronal, X 35)

b. Undifferentiated neuroblasts of the medial accessory nucleus of the 60-day fetus. (coronal, X 350)

c. Rostral inferior olivary nucleus.
The complex displays undifferentiated neuroblasts, absent hilus, and direct contact between the principal and dorsal accessory nuclei. Parts of the hypoglossal nerve and internal arcuate fibers can be identified. (coronal, X 135)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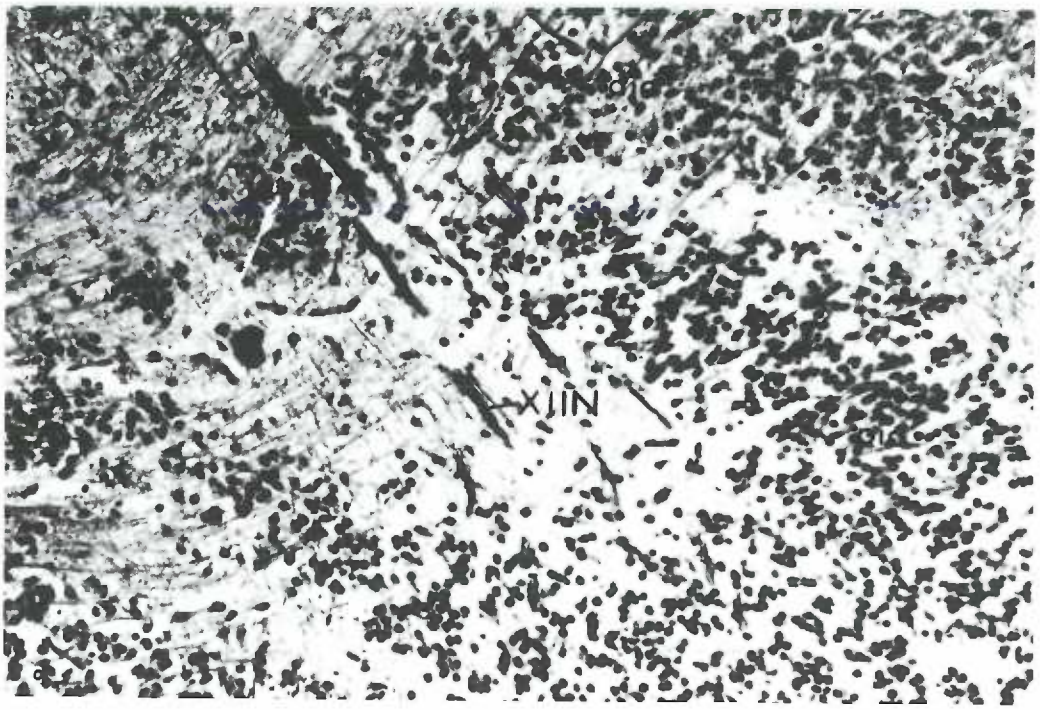
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PLATE 4

a. Caudal myelencephalon of the 60-day fetus. (coronal, X 35)

b. Enlargement of the medial accessory nucleus shown in *a*. Three regions of the nucleus can be identified: dorsal cap, dorsal-medial segment, and ventral-lateral segment. A few thin spino-olivary fibers are located laterally. (coronal, X 135)

c. Caudal region of a sagittal section of the medial accessory nucleus of the 60-day fetus. A few spino-olivary fibers form a thin capsule around its caudal end, although most of the spinal fibers project dorsal to the olivary nucleus. (sagittal, X 135)

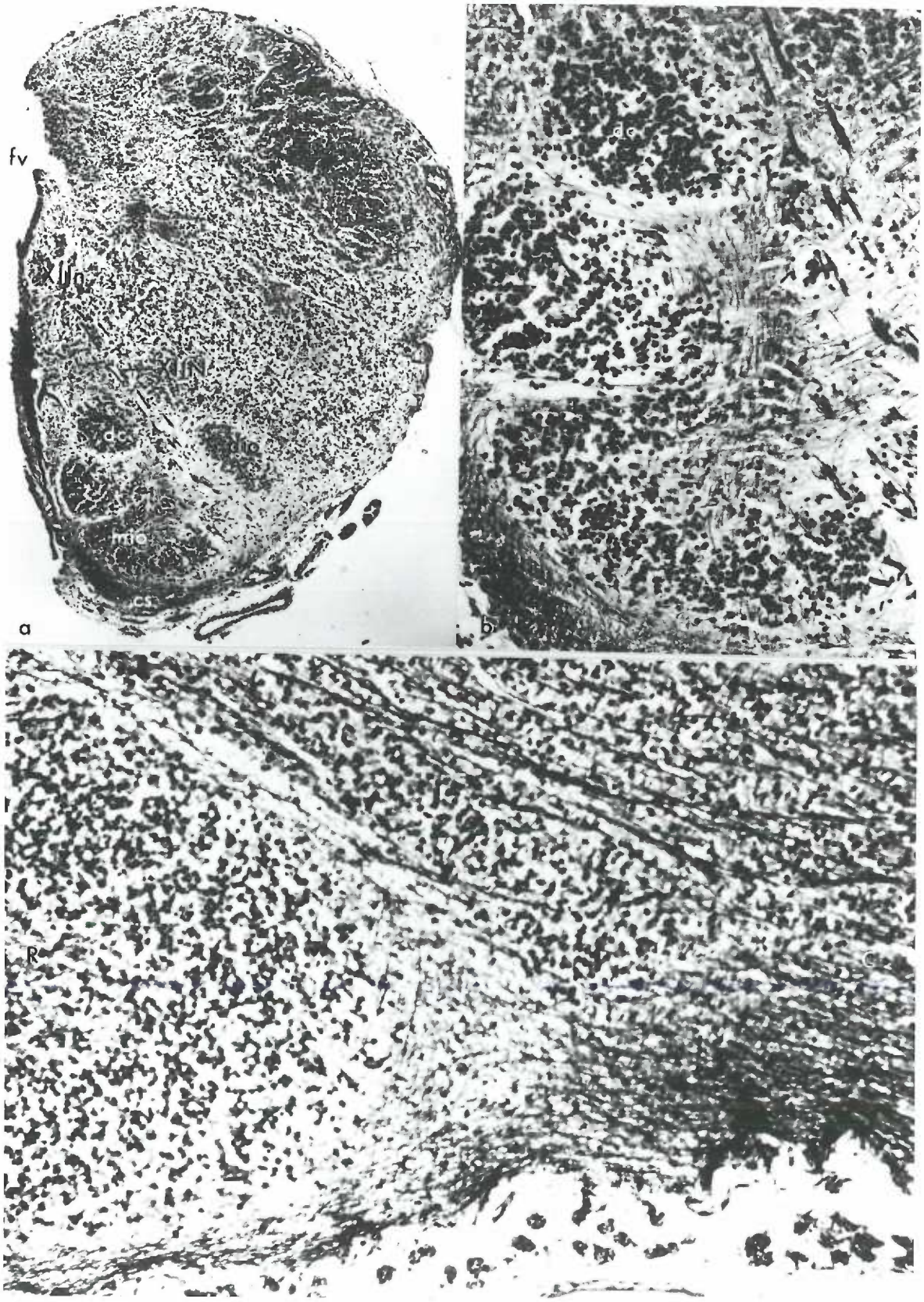
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PLATE 5

a. Cerebellum of the 80-day fetus, showing the vermis to the right and the rudimentary hemispheres to the left. The external granular layer forms a distinct border around the whole structure. (coronal, X 35)

b. Deep cerebellar nuclei of the 80-day fetus. (coronal, X 35)

c. Undifferentiated neuroblasts of the dentate nucleus. (coronal, X 135)

d. Early cellular differentiation of the globose and fastigial nuclei of the 80-day fetus. Also note the increased number of fibers around the fastigial nucleus. (coronal, X 135)

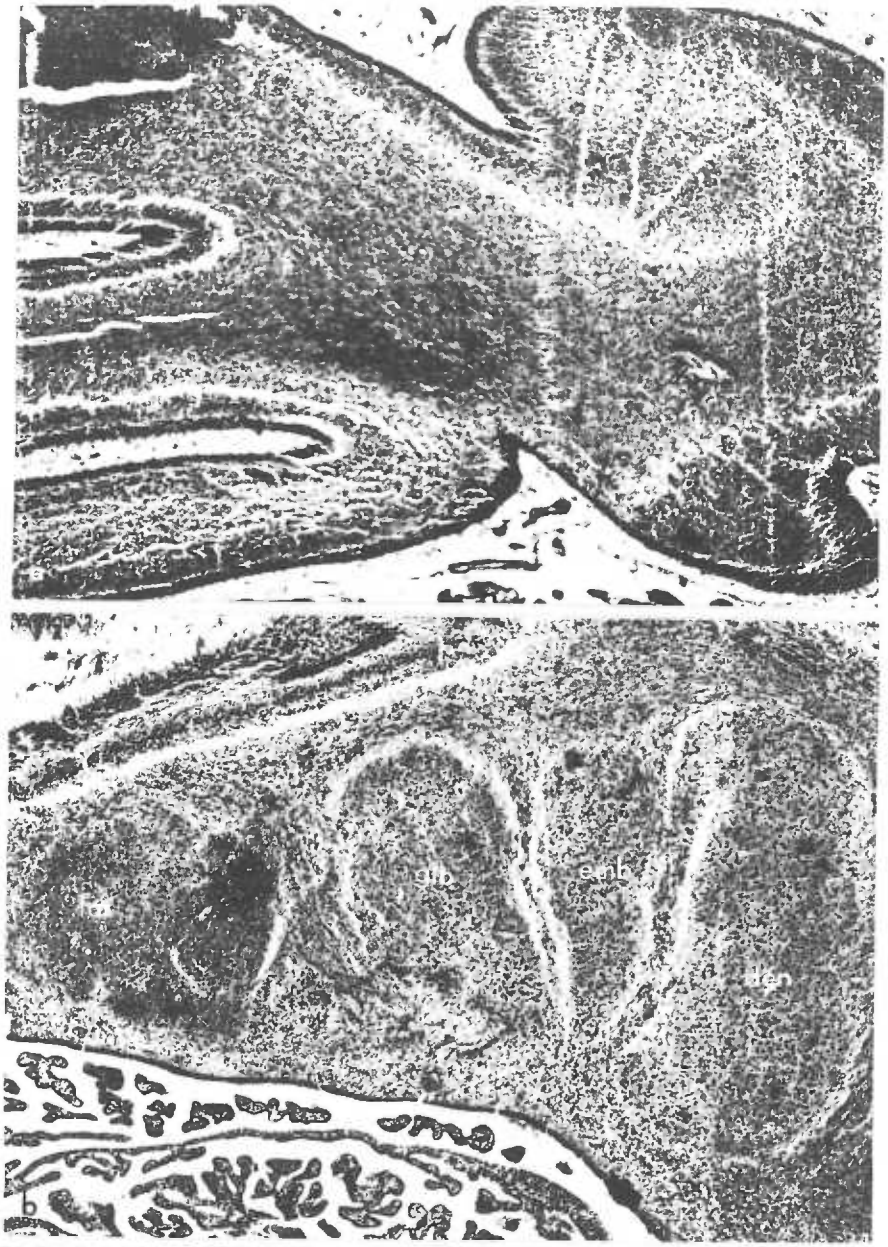


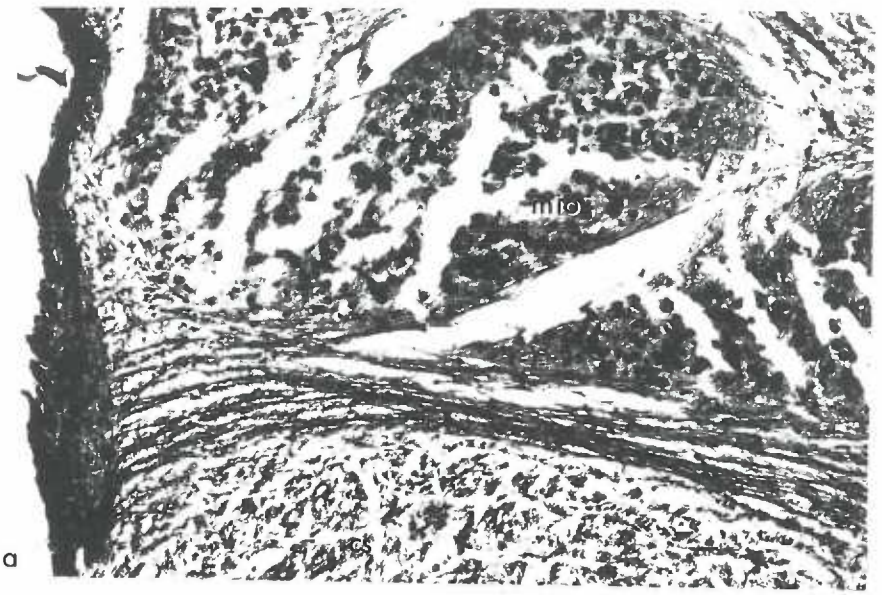
PLATE 6*

a. The ventrocaudal region of the medial accessory nucleus of the 80-day fetus (the whole section is shown in *c* below). Fibers can be identified that project between the area of the arcuate nucleus on the left and the ventral border of the accessory nucleus. (coronal, X 135)

b. Caudal metencephalon of the 80-day fetus. Particularly evident are the enlarged corticospinal tract, the well-developed superior olivary nucleus and the trapezoid body, and the abducens nucleus and nerve. (coronal, X 135)

c. Caudal myelencephalon of the 80-day fetus. Particularly evident are the corticospinal tract, the cranial nuclei, and internal arcuate fibers that originate from the cuneate and gracilus nuclei. (coronal, X 135)

* The artifactual separations within all these sections occurred during the final stages of processing and do not change the morphological interpretations.



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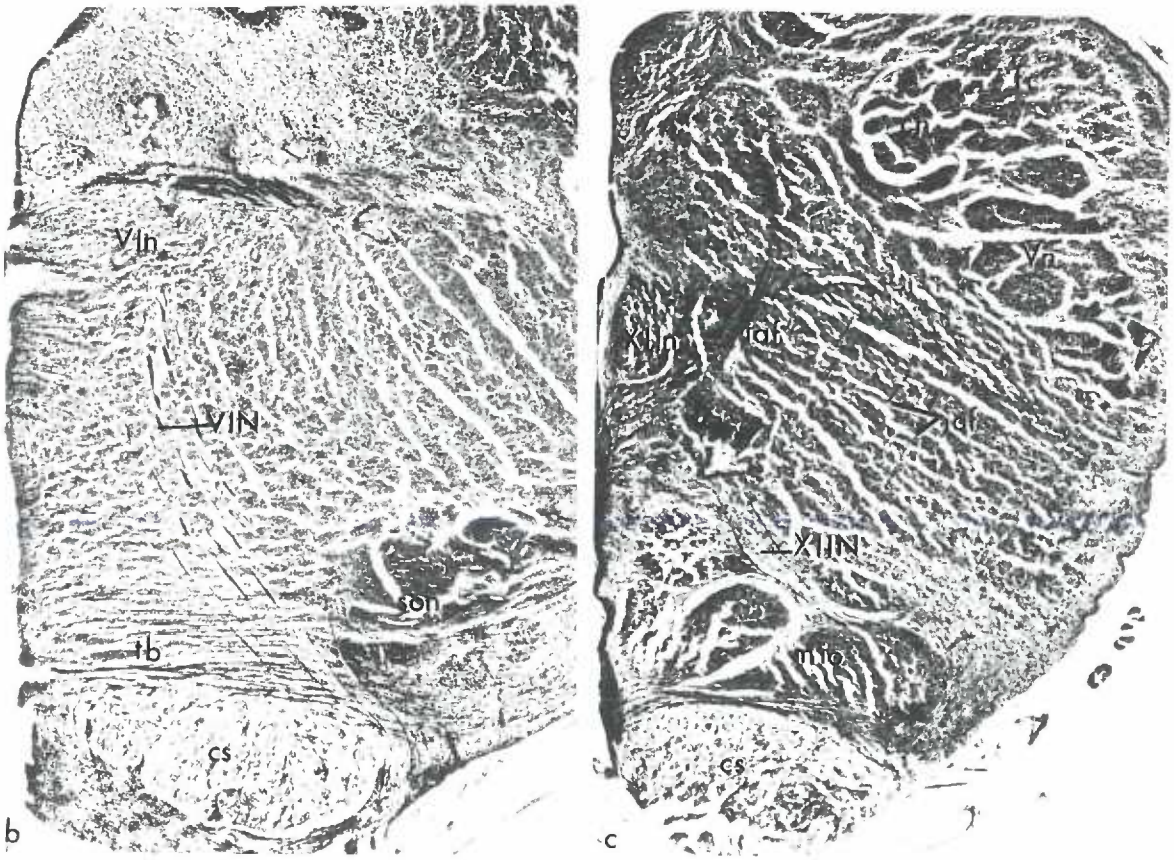

b c

PLATE 7

a. Rostral pole of the principal nucleus of the 80-day fetus. Rubro-olivary fibers are seen coursing both dorsally and ventrally around the nucleus and contributing to its internal fibrous matrix. (sagittal, X 135)

b. Caudal region of the medial accessory nucleus of the 80-day fetus. Most spino-olivary fibers project ventrally, but a few can be followed to the dorsal surface. (sagittal, X 135)

c. Caudal part of the principal nucleus of the 80-day fetus. Note the high concentration of cells within the ventral-lateral-outgrowth (VLO). The early formation of the hilus is evident as a few olivary efferents project medially. (coronal, X 135)

d. The inferior olivary complex of the 80-day fetus, including the principal nucleus shown in *c*. (coronal, X 35)

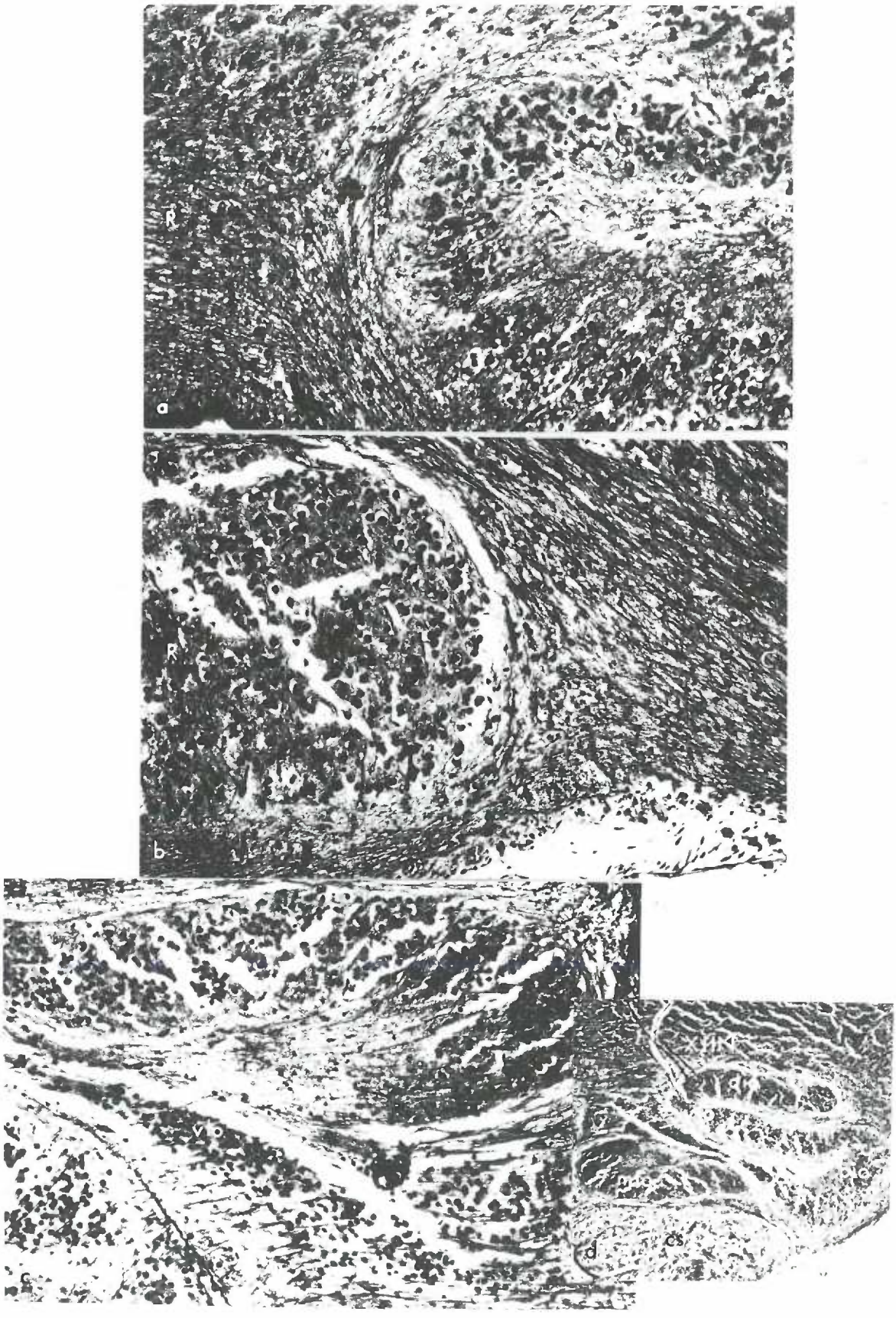
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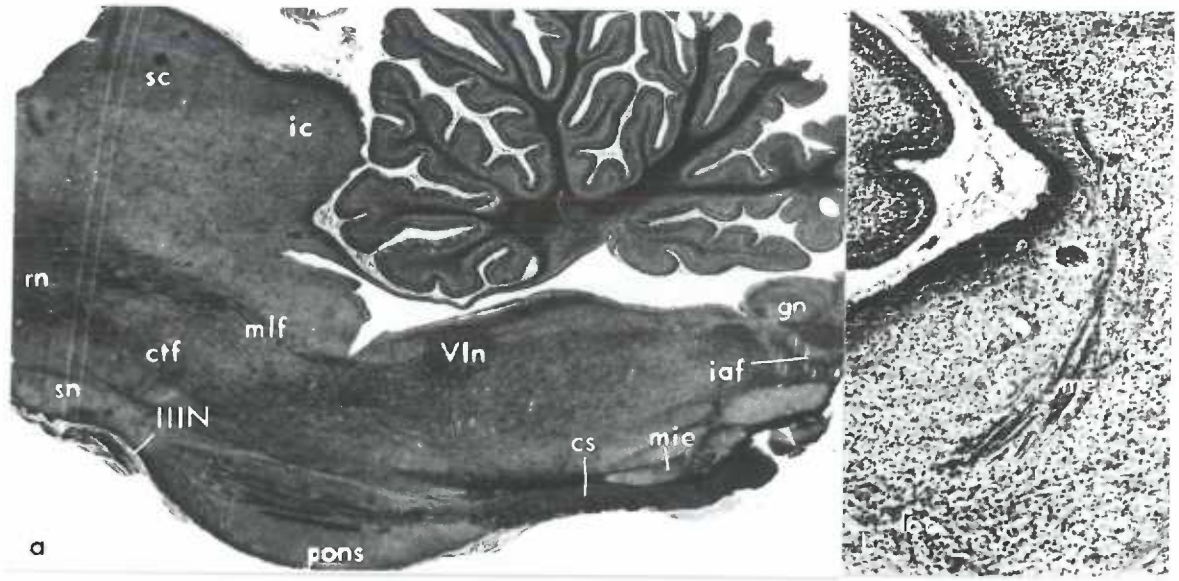
PLATE 8

a. Whole brain section of 92-day fetus.
An increase in various fiber systems, such as the cortico-spinal tract, are evident, as well as an increase in the size of the inferior olivary complex, cranial nuclei, pons, and the superior and inferior colliculi. (sagittal, X 12)

b. The mesencephalic root of the trigeminal is an example of small fibers that are now evident. (sagittal, X 35)

c. Mesencephalon of the 92-day fetus.
Note the increase in fibers around the red nucleus.
(sagittal, X 35)

d. Magnocellular region of the red nucleus of the 92-day fetus. Fibers of the brachium conjunctivum can be seen surrounding the large, early differentiating cells. (sagittal, X 135)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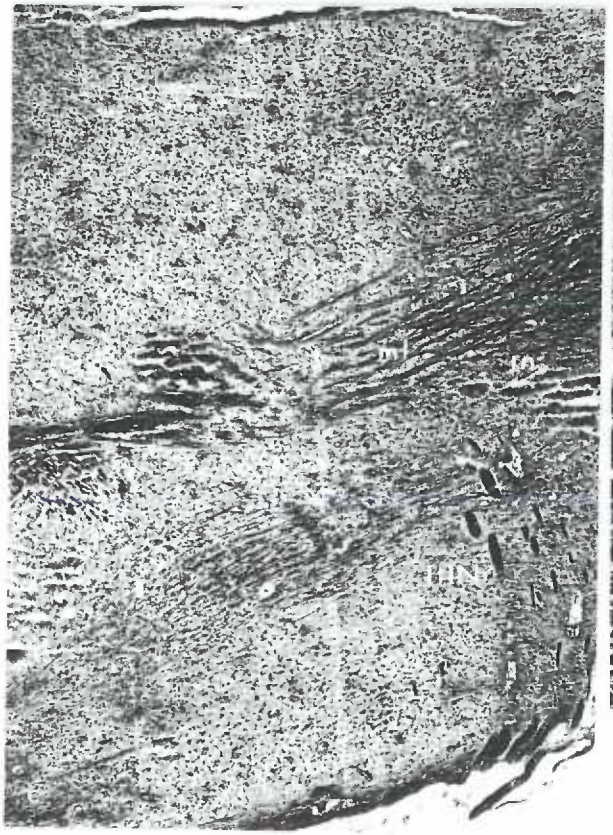


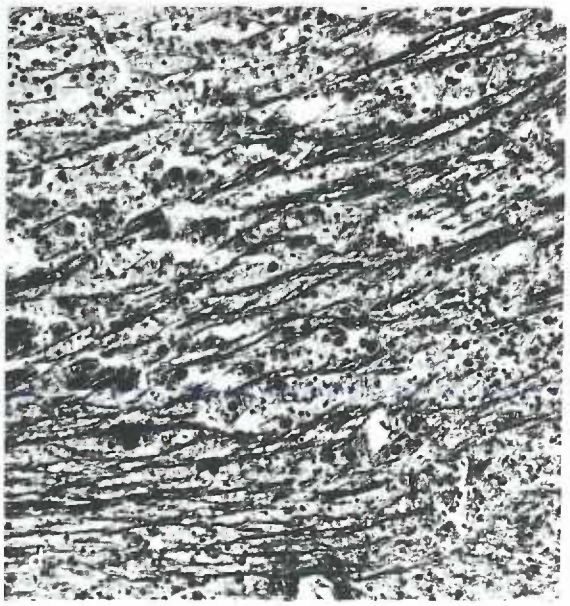


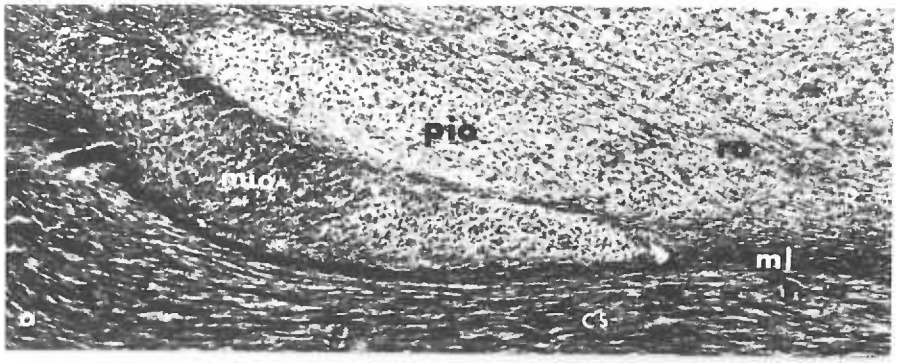
PLATE 9

a. The ventral-lateral segment of the medial accessory nucleus of the 92-day fetus. A gradation of spino-olivary fibers can be seen; the rostral pole is almost void of fibers. A few rubro-olivary fibers can be identified entering the rostromedial part of the ventral lamella. (sagittal, X 35)

b. Caudal pole of the dorsal accessory nucleus of the 92-day fetus. Spino-olivary fibers can be traced over the caudal portion of the medial accessory nucleus and then in and around the dorsal accessory nucleus; however, most spinal fibers pass dorsal to the olivary complex. (sagittal, X 135)

c. Rostral pole of the principal nucleus of the 92-day fetus. At this age, the principal nucleus has an indistinct rostral border, which is lightly encapsulated with rubro-olivary afferents. (sagittal, X 135)

d. Cells and small fibers of the rostral dorsal lamella. (sagittal, X 350)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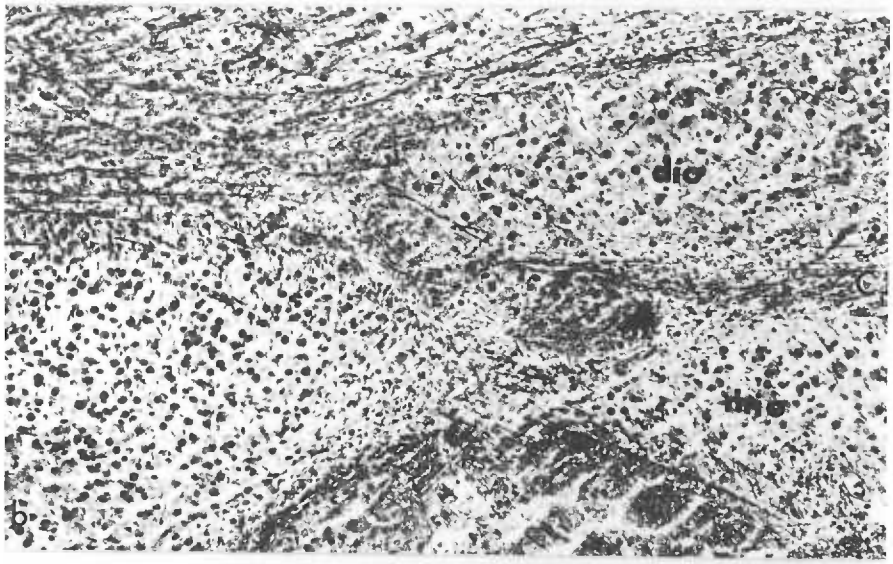
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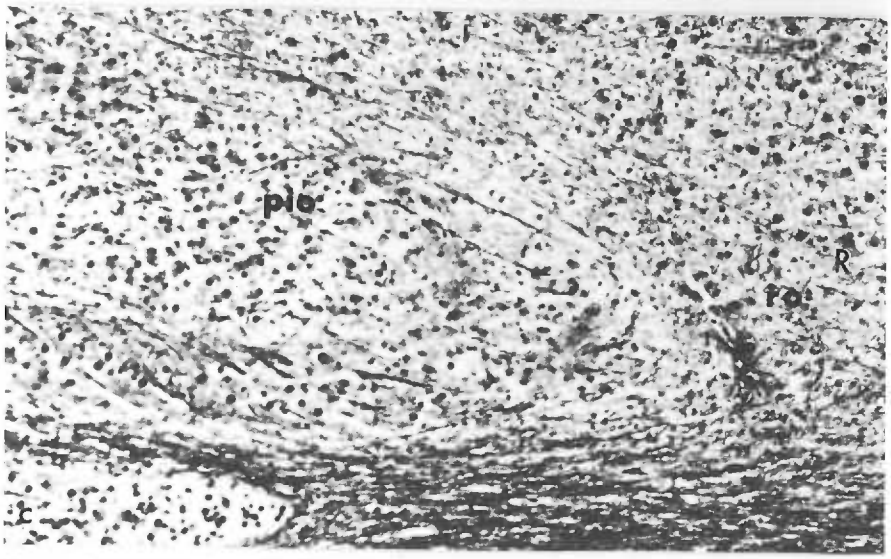
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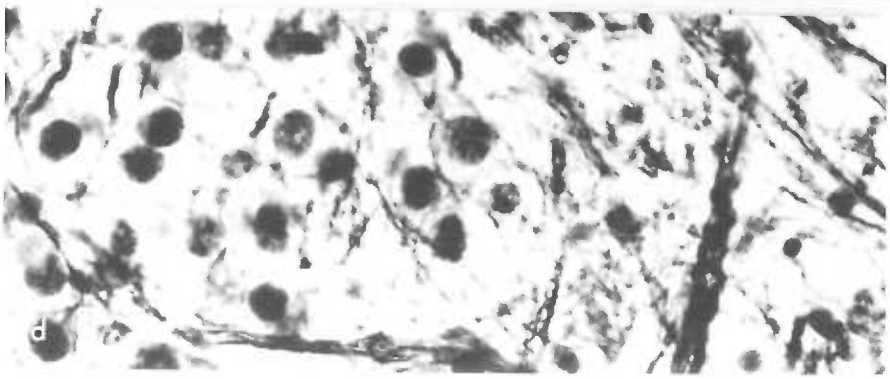
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PLATE 10

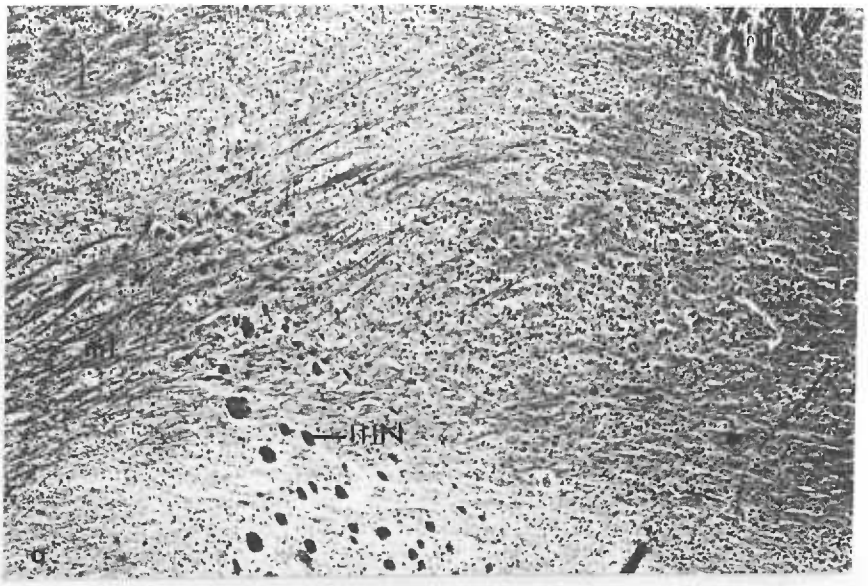
a. Mesencephalon of the 102-day fetus.
The two divisions of the red nucleus are evident; i.e., the oval region slightly rostral to the oculomotor nerve rootlet is the parvocellularis section, whereas caudally, it is the magnocellular region. (sagittal, X 35)

b. Cells of the magnocellular region of the red nucleus and fibers of the brachium conjunctivum of the 100-day fetus. (horizontal, X 135)

c. Horizontal section of the mesencephalon of the 100-day fetus. See Fig. 9 for complete identification. (horizontal, X 35)

d. Coronal section through the region of the red nucleus of the 100-day fetus. Tectospinal fibers, both large and small, can be identified projecting between the large cells of the magnocellular region. (coronal, X 135)

e. Sagittal section through the rostral portion of the red nucleus of the 102-day fetus. Fine rubro-olivary fibers can be seen arising from the dorsolateral region of the nucleus. (sagittal, X 135)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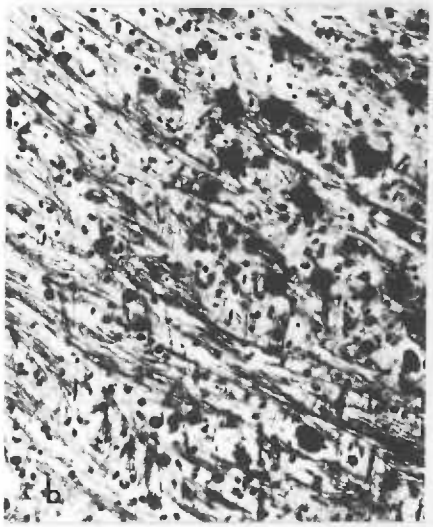
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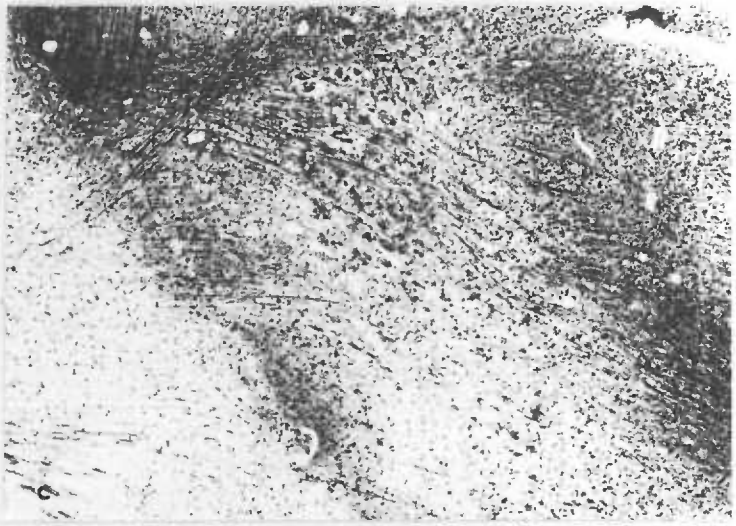
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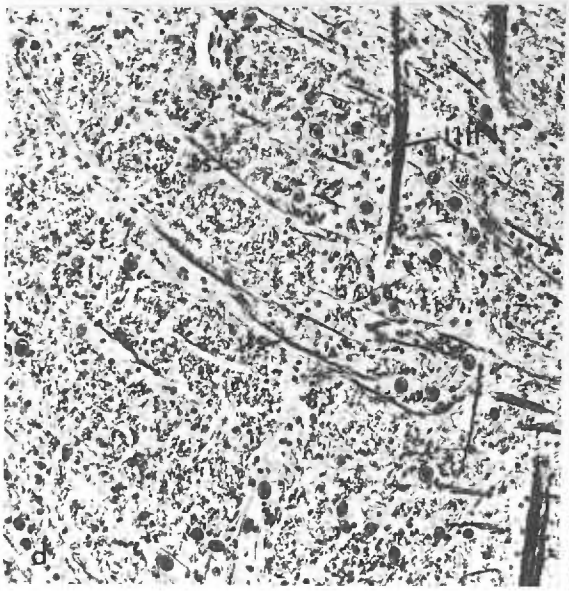
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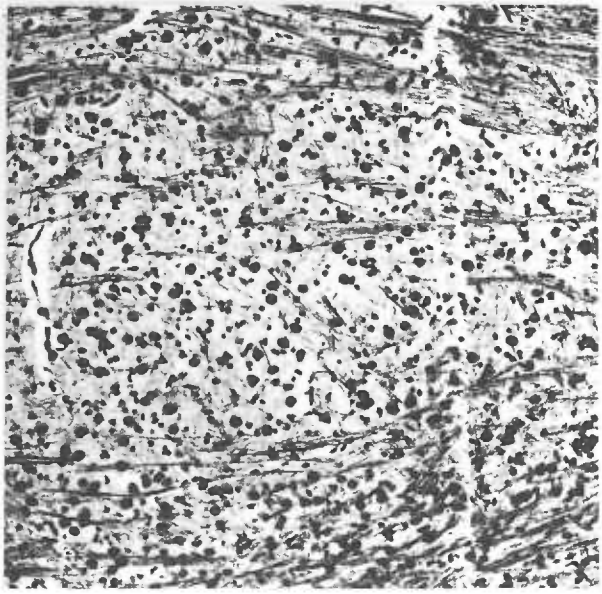


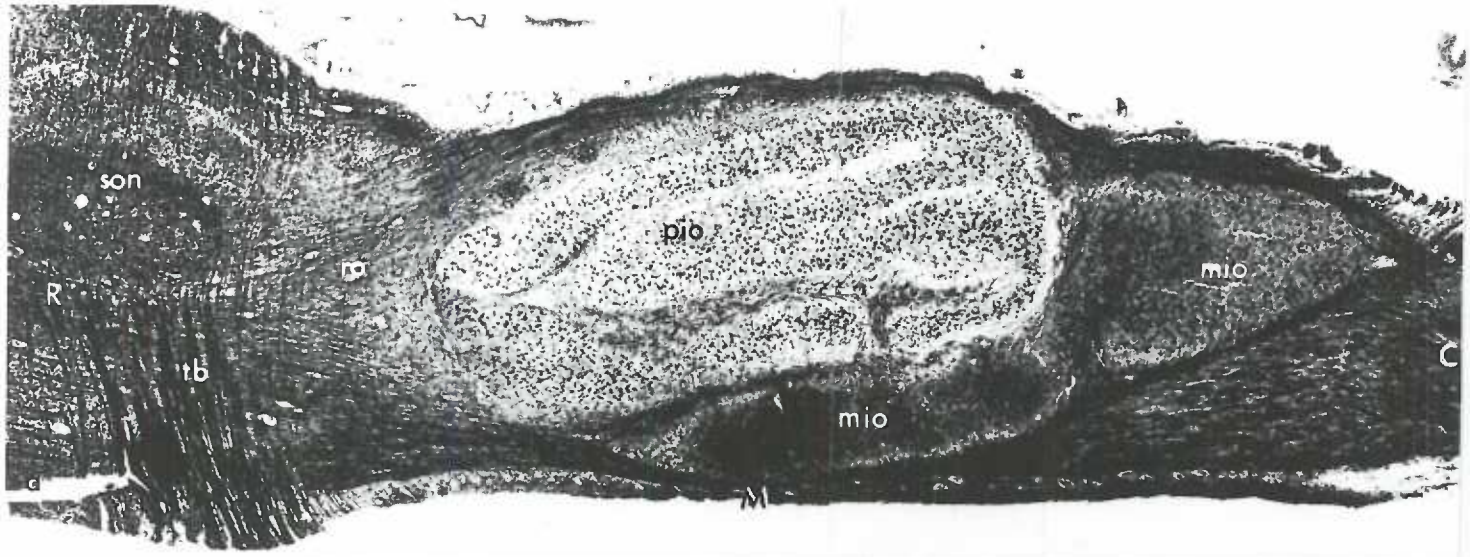
PLATE 11

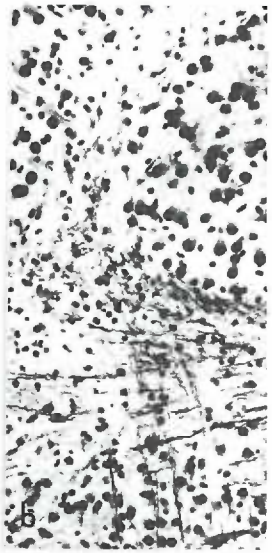
a. Horizontal section of the inferior olivary complex of the 100-day fetus. Rubro-olivary fibers can be traced through the medial region of the superior olivary nucleus where they form a capsule around the principal nucleus. Spino-olivary fibers can be seen projecting around the caudal segment of the medial accessory nucleus and then almost inundating the dorsal part of the ventral-lateral segment. Fine fibers of the hilus are also present, with a greater density in the rostral part of the principal nucleus. (horizontal, X 35)

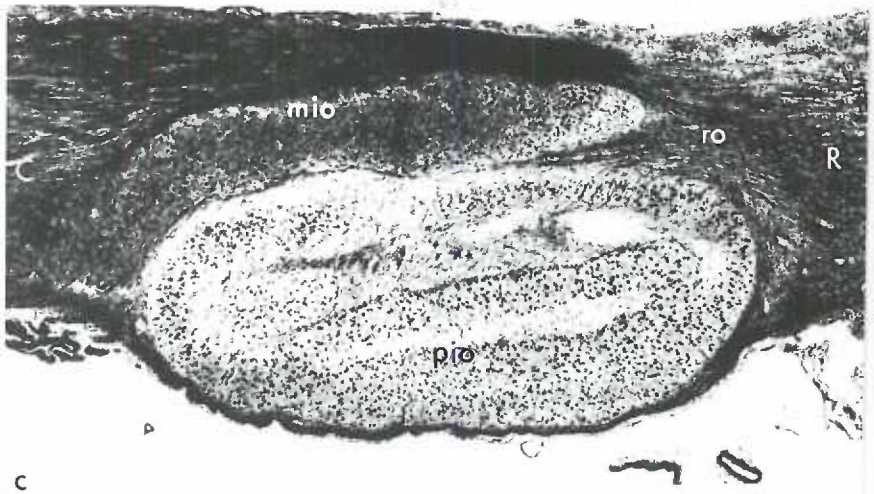
b. Cells of the dorsal lamella of the 100-day fetus. (coronal, X 135)

c. A slightly more ventral section than *a* of the inferior olivary complex of the 100-day fetus. A clear demonstration of the absence of fibers within the most rostral pole of the medial accessory nucleus. (horizontal, X 35)

d. A sagittal view of the inferior olivary complex of the 102-day fetus. Again very evident is the distribution of spino-olivary fibers within the ventral-lateral segment, the small rubro-olivary fibers around the rostral pole of the principal nucleus, and the internal arcuate fibers traversing the whole complex. (sagittal, X 35)







c

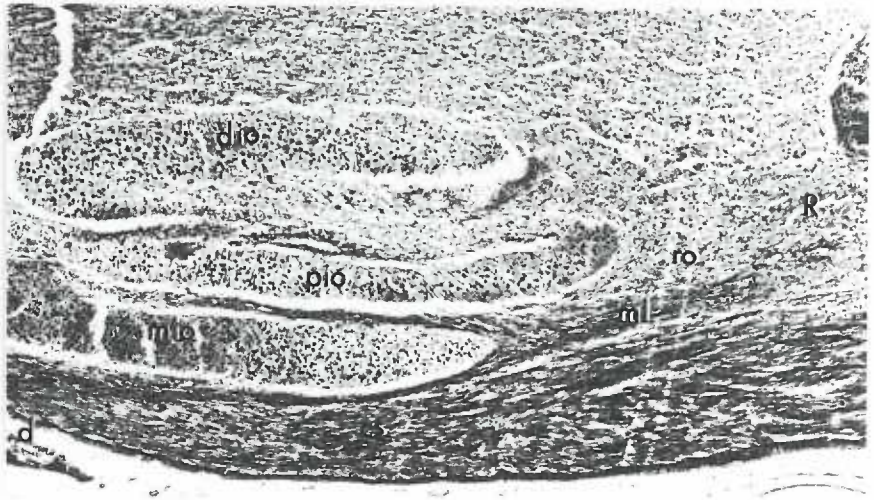


PLATE 12

a. The rostral mesencephalon of the 107-day fetus. Easy to identify are the parvocellular region of the red nucleus and the oculomotor nuclear complex. There are numerous small fibers that course between the red nucleus and the adjacent fibers. (coronal, X 35)

b. The mesencephalon through the caudal part of the red nucleus in the 107-day fetus. (coronal, X 35)

c. The rostral metencephalon of the 107-day fetus. There is a large increase in the size of the pons, which now shows a number of transverse pontine fibers. The tegmentum consists primarily of longitudinal fibers with the medial lemniscus and, laterally, the spinothalamic and lateral lemniscus being the most obvious. (coronal, X 12)

d. Caudal region of the metencephalon of the 107-day fetus. There is an enlargement of the cranial nuclei and their nerve rootlets, the superior olivary nucleus, trapezoid body, and the pons. (coronal, X 12)

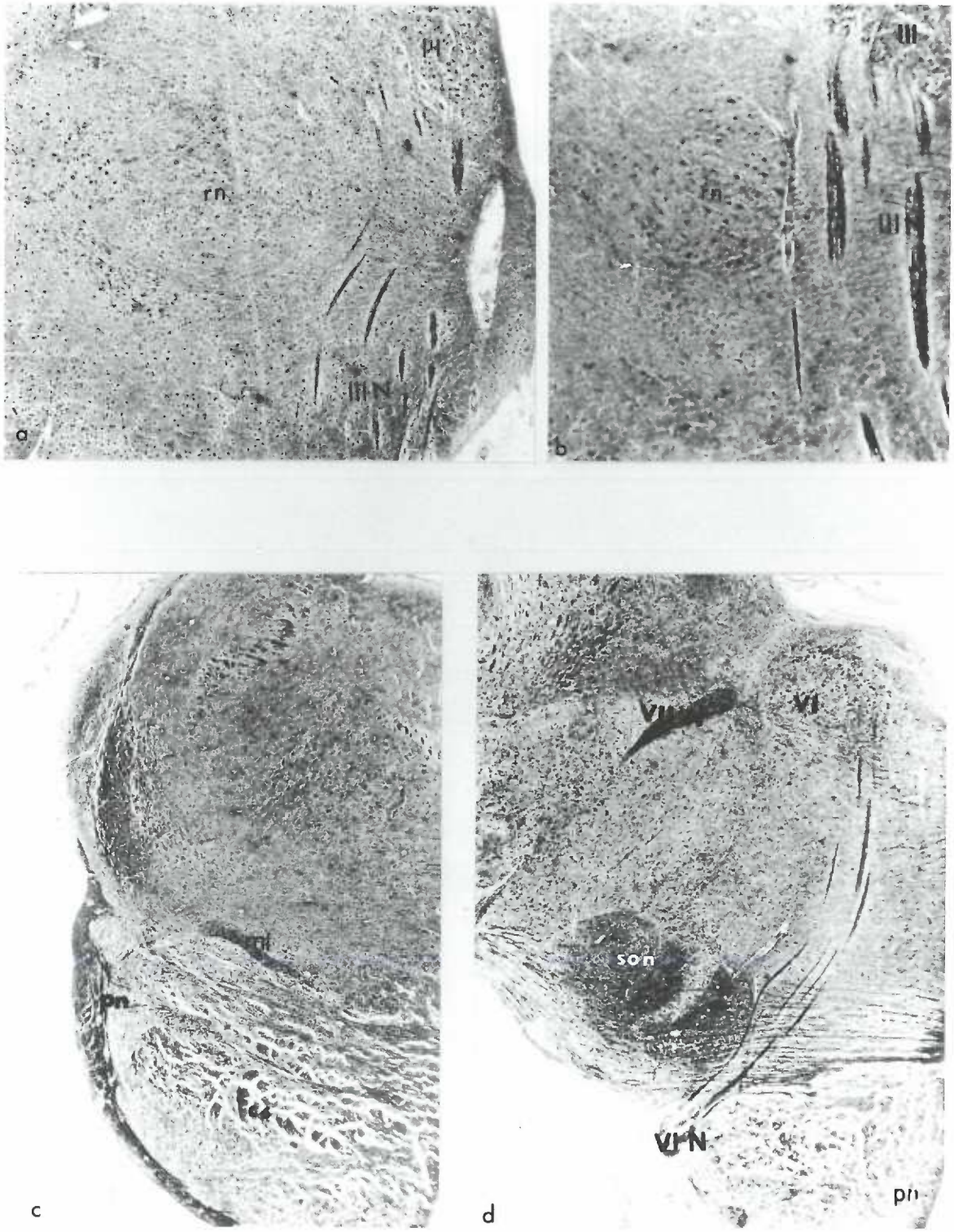

III
rn
IIIN
a
III
rn
III
b
VII
VI
son
VIN
pn
c
d

PLATE 13

a. The caudal third of the inferior olivary complex of the 109-day fetus. Spinal afferents can be seen projecting dorsally to the inferior olive, as well as forming a thin capsule around the posterior dorsal accessory and principal nuclei. (sagittal, X 35)

b. Caudal region of the dorsal accessory nucleus of the 109-day fetus. Spino-olivary fibers can be observed entering the nucleus from a dorsocaudal direction. (sagittal, X 135)

c. Rostral pole of the inferior olivary complex in the 109-day fetus. Rubro-olivary fibers form a capsule around the rostral pole of the principal nucleus. (sagittal, X 35)

d. Rostral pole of the principal nucleus (slightly more lateral than *c* above) in the 109-day fetus. (sagittal, X 35)

e. Deep nuclei of the cerebellum of the 107-day fetus. There is both cellular differentiation, especially in the fastigial nucleus, and an increased fiber development. (coronal, X 35)

f. Reticular nuclei located in the rostromedial tegmentum of the myelencephalon in the 107-day fetus. (coronal, X 135)

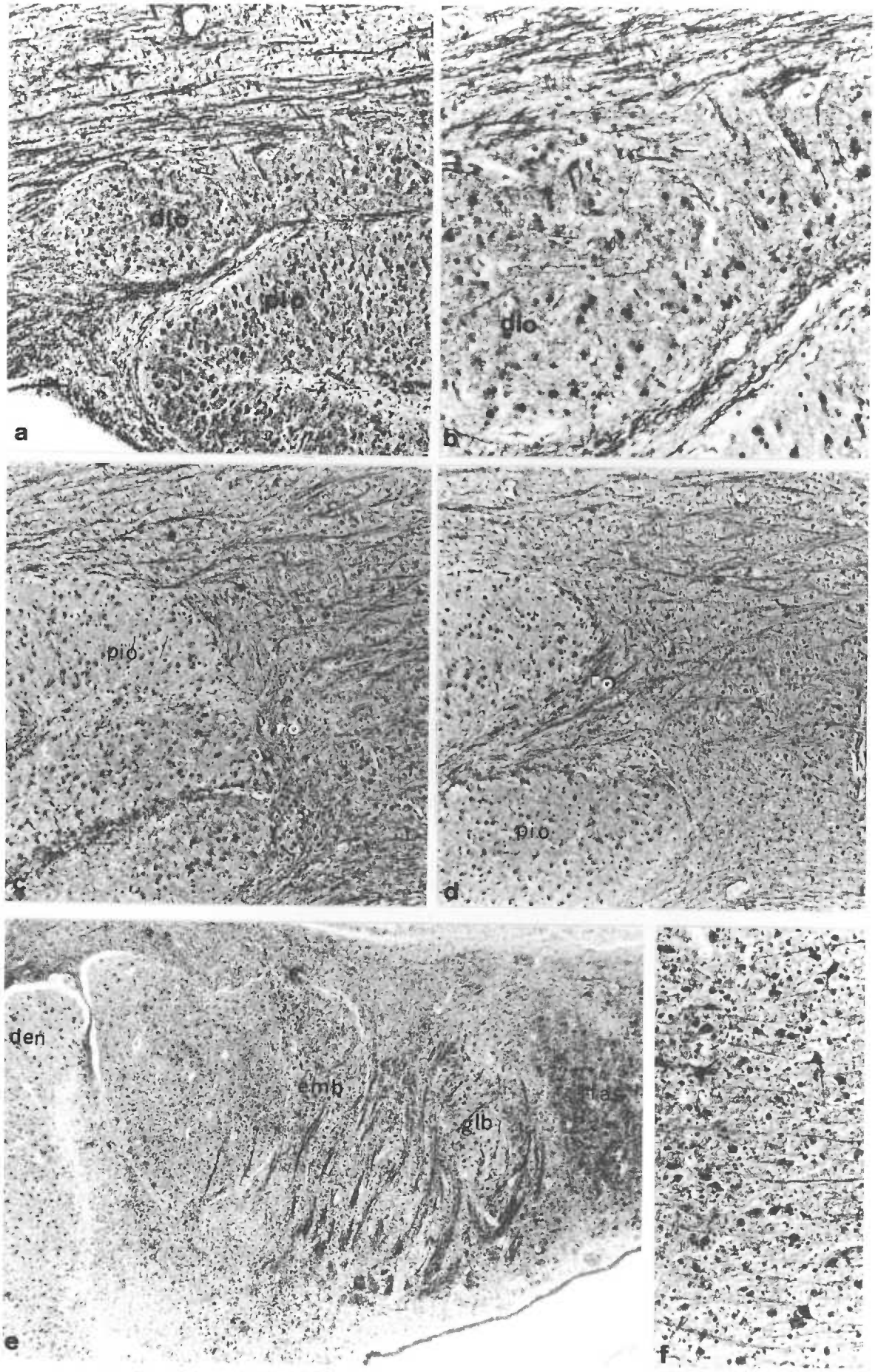

dlo
plo
a
dlo
b
plo
c
pio
d
den
emb
glb
e
f

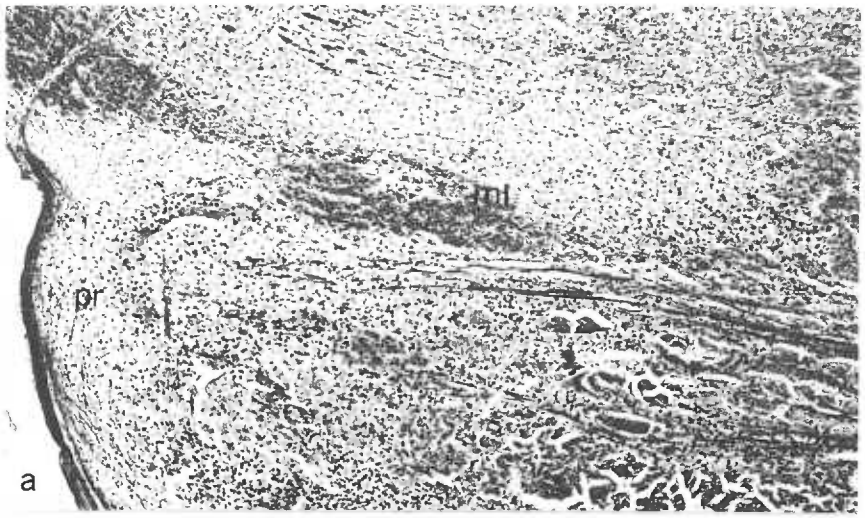
PLATE 14

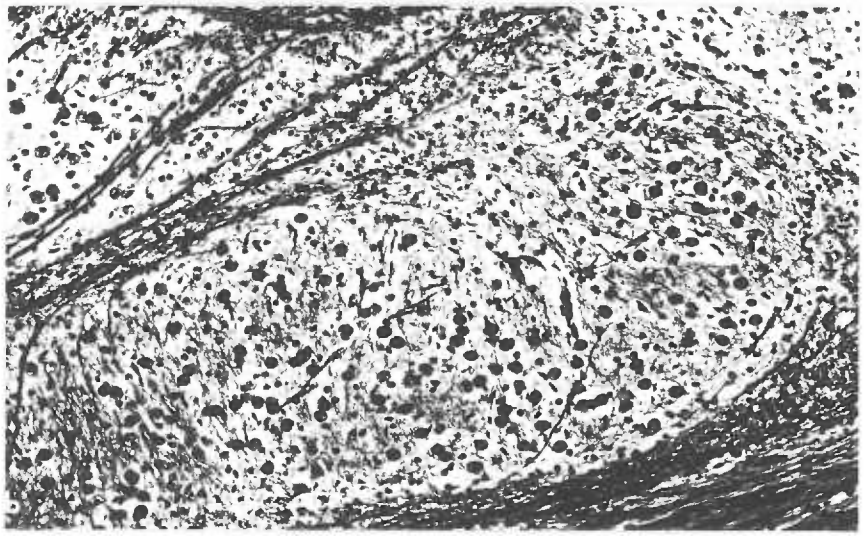
a. Junction between the metencephalic tegmentum and the pons of the 121-day fetus. There is an increase in the number of pontine nuclei. The medial lemniscus, which is dorsal to the pons, is very distinct, but it is difficult to distinguish the CTF. (coronal, X 35)

b. Ventral-lateral segment of the medial accessory nucleus of the 121-day fetus. (sagittal, X 135)

c. The rostral region of the inferior olivary complex of the 121-day fetus. (coronal, X 35)

d. The ventral-medial segment of the dorsal accessory nucleus (an enlargement of the same area as shown in *c*) of the 121-day fetus. Its boundaries are very diffuse because of the large number of fibers that traverse this region. (coronal, X 135)


rml
pr
a



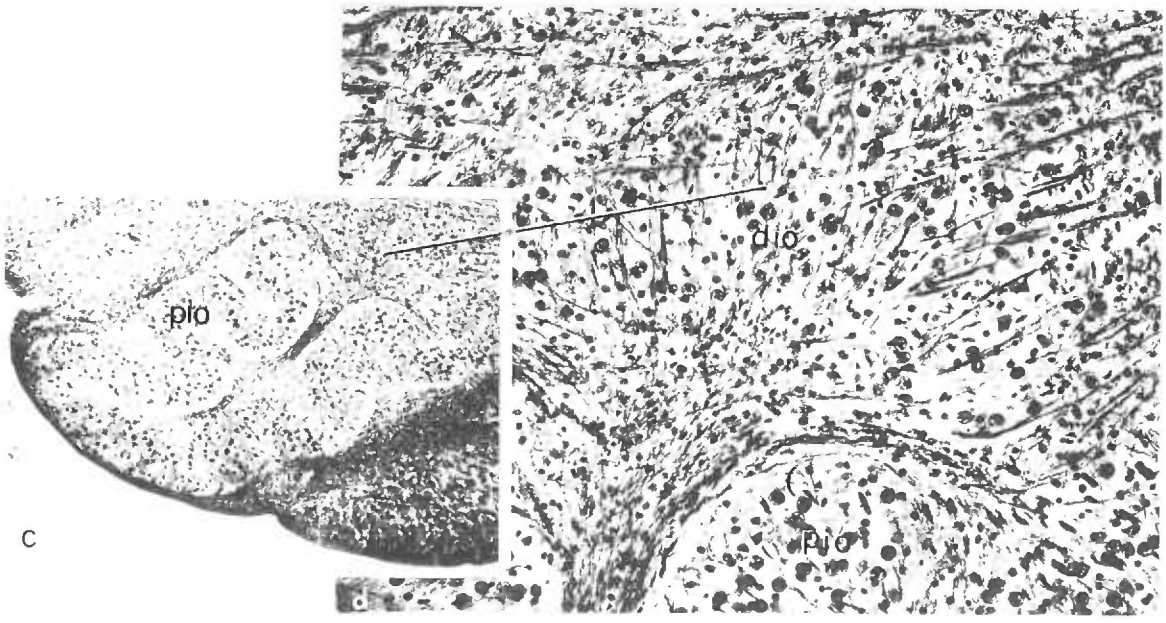

dlo
plo
c
Plo

PLATE 15

a. Dorsal-lateral segment of the dorsal accessory nucleus of the 121-day fetus. Note the variation in the density of the large cells within the nucleus. The thick fibers projecting through the nucleus are olivocerebellar fibers from the contralateral inferior olivary complex. (coronal, X 135)

b. Section through the caudal inferior olivary complex of the 121-day fetus, from which enlargements of different divisions have been made: *a*, *c*, *d*, and *e*. (coronal, X 35)

c. The cellular organization within the dorsal cap. (coronal, X 350)

d. The ventral-lateral segment of the medial accessory nucleus. Note the high density of internal fibrous matrix, which corresponds with the distribution of the spino-olivary afferents. (coronal, X 350)

e. The dorsal-medial segment of the medial accessory nucleus. There is a low density of cells and fibers in comparison to other parts of the olivary complex. Numerous ipsilateral and contralateral olivo-cerebellar efferents project through this area. (coronal, X 135)

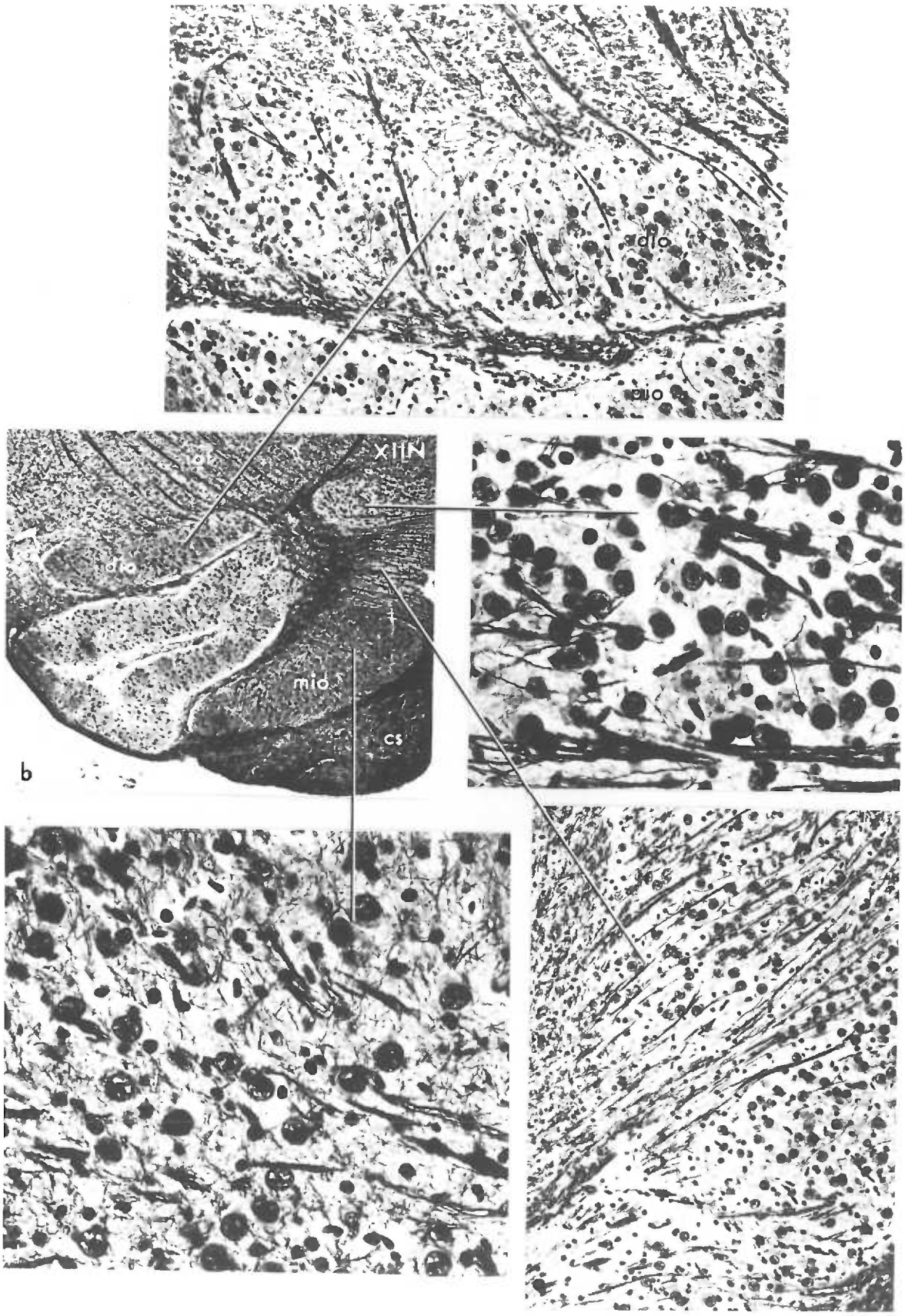

dlo
mlo
XIIN
mlo
mlo
cs
b

PLATE 16

a. Medial accessory nucleus of the 129-day fetus. There is a high concentration of cells within the dorsal cap, whereas the dorsal-medial segment is almost void of cells of any size. There is also an increase in the olivo-cerebellar fibers, most of which are from the ipsilateral dorsal lamella, that cross through the dorsal-medial segment. (coronal, X 135)

b. The ventral-medial segment of the dorsal accessory nucleus of the 129-day fetus. Evident are a few thin reticulo-olivary fibers that parallel the hypoglossal nerve rootlets. (coronal, X 135)

c. Cells of the dorsal lamella of the 129-day fetus. (coronal, X 350)

d, e, f. Serial sections (150μ between sections) of the region of the ventral-lateral-outgrowth of the 129-day fetus. (coronal, X 135)

g. A section through the middle region of the inferior olivary complex of the 129-day fetus. (coronal, X 35)

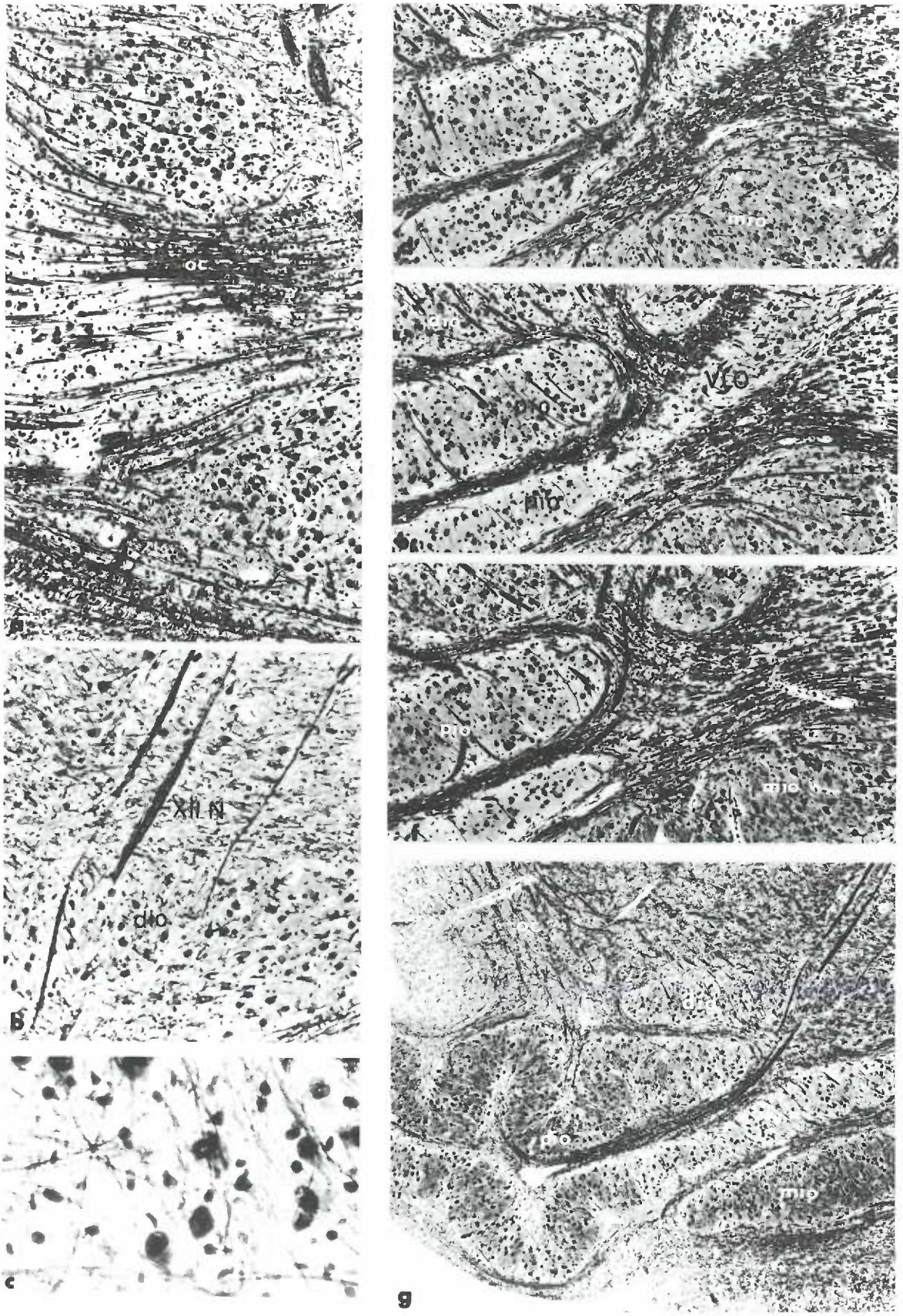
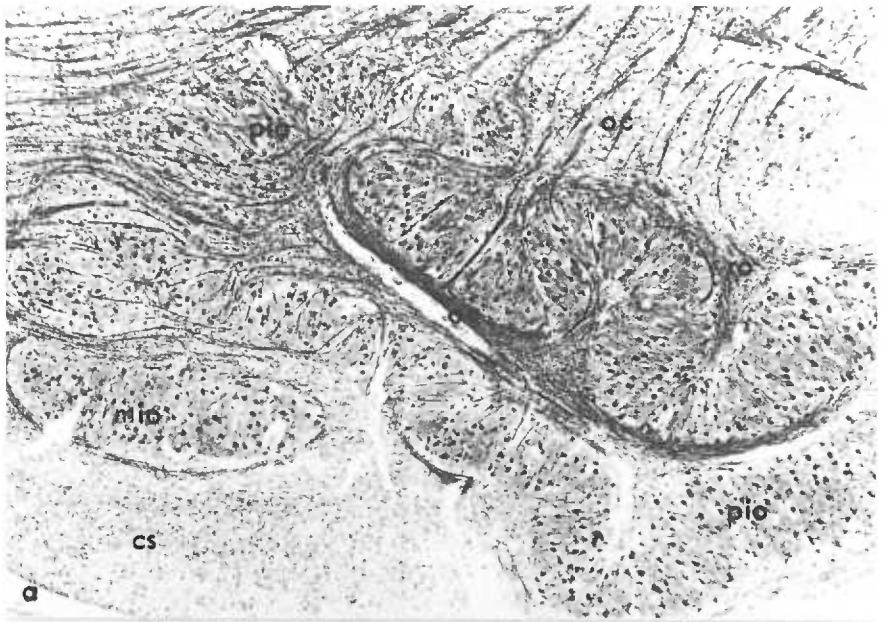

oc
XII N
dio
vio
mio
b
c
g

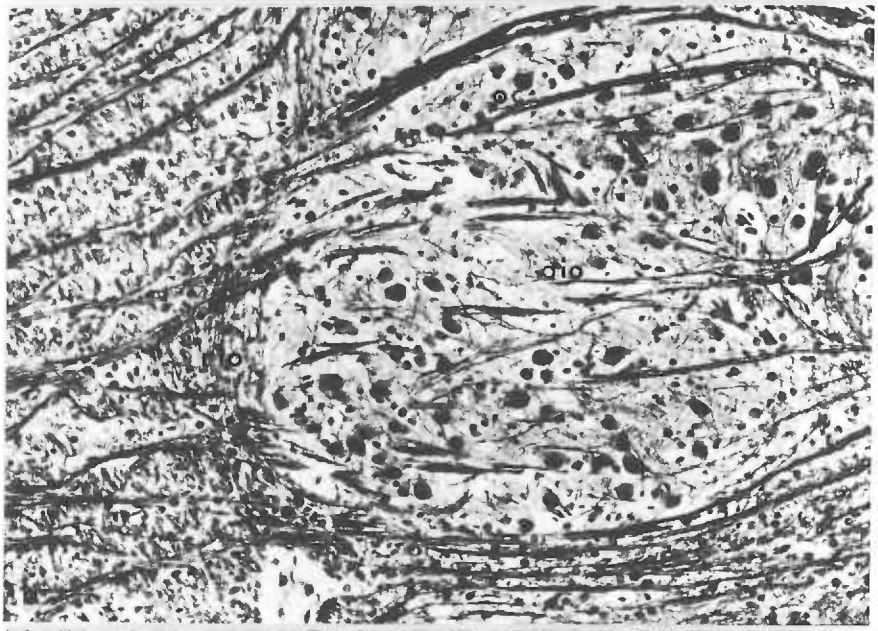
PLATE 17

a. Coronal section of the rostral inferior olivary complex of the 137-day fetus. Particularly evident are the variations of the fiber density between the different divisions of the complex, the large proportion of olivo-cerebellar efferent exiting from the dorsal lamella of the principal nucleus, and the large number of rubro-olivary fibers entering the dorsal sulcus of the dorsal lamella. (coronal, X 35)

b. The ventral-medial section of the dorsal accessory nucleus of the 137-day fetus. Large olivo-cerebellar fibers from the contralateral side project through in a horizontal plane, whereas in the dorsal to ventral direction are the thin reticulo-olivary fibers, some of which curve around the medial border of the nucleus. (coronal, X 350)

c. Coronal section through the ventral-lateral segment of the medial accessory nucleus of the 137-day fetus, which demonstrates the high density of the spino-olivary afferents. (coronal, X 135)


plo
oc
mlo
cs
plo
a


oc
plo
mlo

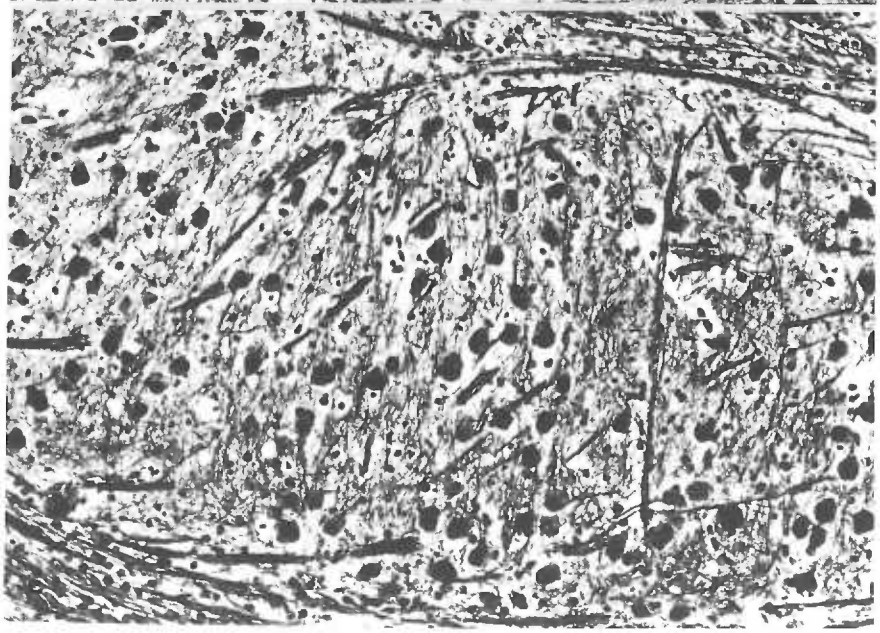


PLATE 18

a. Sagittal section of the rostral half of the inferior olivary complex of the 134-day fetus. Note the absence of fibers in the most rostral pole of the medial accessory nucleus, whereas its caudal region has many spino-olivary fibers. (sagittal, X 35)

b. The three divisions of the medial accessory nucleus of the 137-day fetus. There is a high concentration of cells within the dorsal cap, but the dorsal-medial segment is almost void of cells. (coronal, X 35)

c. Cells within the dorsal lamella of the 137-day fetus. (coronal, X 550)

d. The rostral half of the principal nucleus of the 134-day fetus. The rubro-olivary fibers can be seen to form a capsule around the rostrodorsal region and in the region of the dorsal sulcus. (sagittal, X 35)

e. Rostral pole of the principal nucleus (slightly more lateral than *d*), which illustrates the density of rubro-olivary afferents rostrodorsal region, as well as some of which project caudally under the ventral lamella. (sagittal, X 135)

f. Horizontal section through the lateral edge of the dorsal lamella in the 133-day fetus. The outer part of the capsule contains rubro-olivary fibers that run in rostral-caudal direction, whereas the inner part has rubro-olivary fibers that run in a dorsal-ventral direction. (horizontal, X 350)

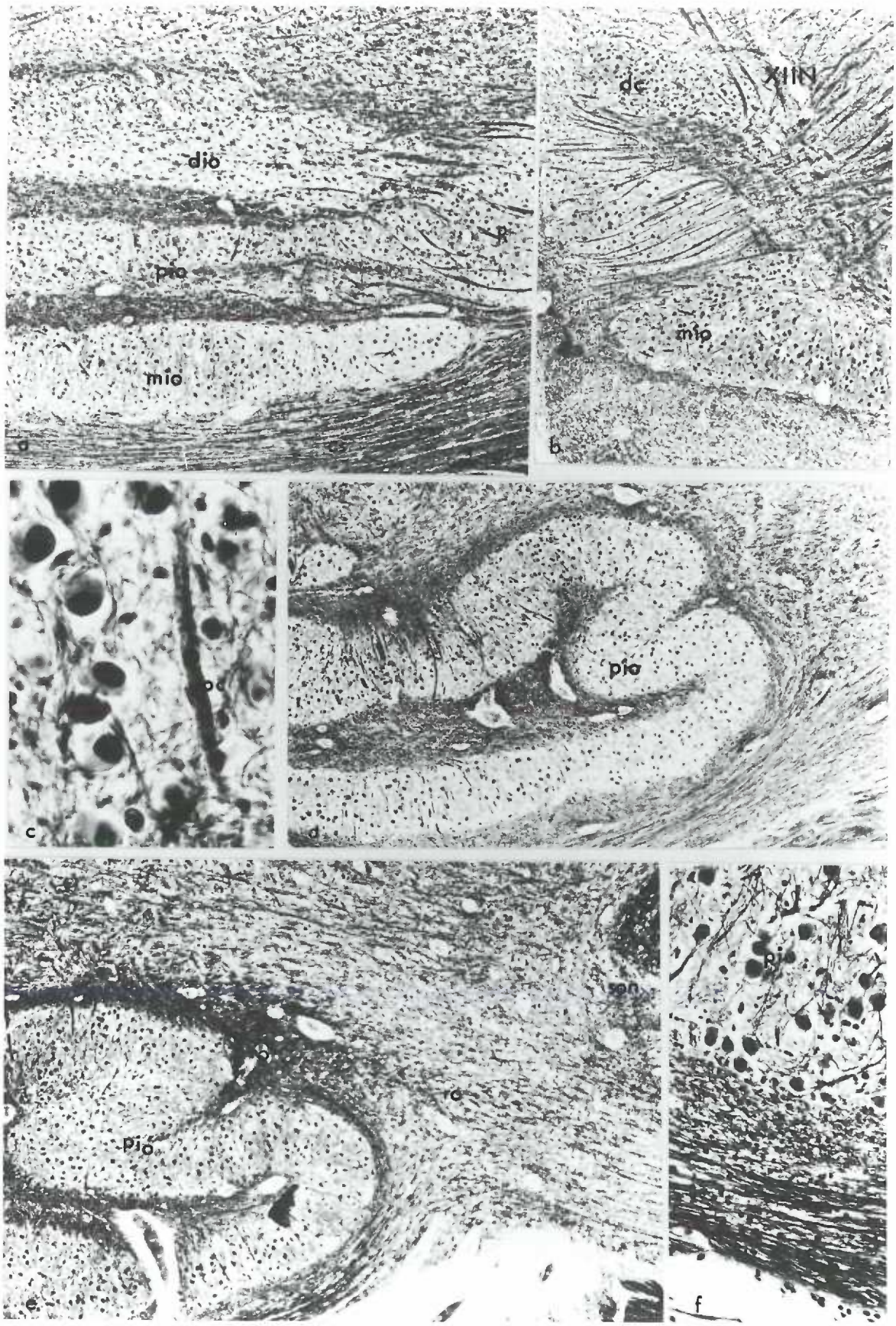

dio
pio
mio
a
dc
XIIN
mio
b
pio
d
c
pio
sen
pio
e
pi
f

PLATE 19

a. Sagittal section of the inferior olivary complex of the 147-day fetus. Particularly evident is the graduation of the internal fibrous matrix along the ventral-lateral segment of the medial accessory nucleus. (sagittal, X 35)

b. Ventral-medial segment of the dorsal accessory nucleus of the 147-day fetus. (coronal, X 135)

c. Rostral part of the ventral-lateral segment of the 147-day fetus. (sagittal, X 135)

d. Cellular differentiation within the fastigial nucleus of the 147-day fetus. (coronal, X 135)

e. Caudal region of the inferior olivary complex of the 147-day fetus. Evident is the large number of spino-olivary fibers that course ventral to the medial accessory nucleus. (sagittal, X 35)

f. The caudal junction of the emboliform (left) and the globose (right) cerebellar nuclei. (coronal, X 35)

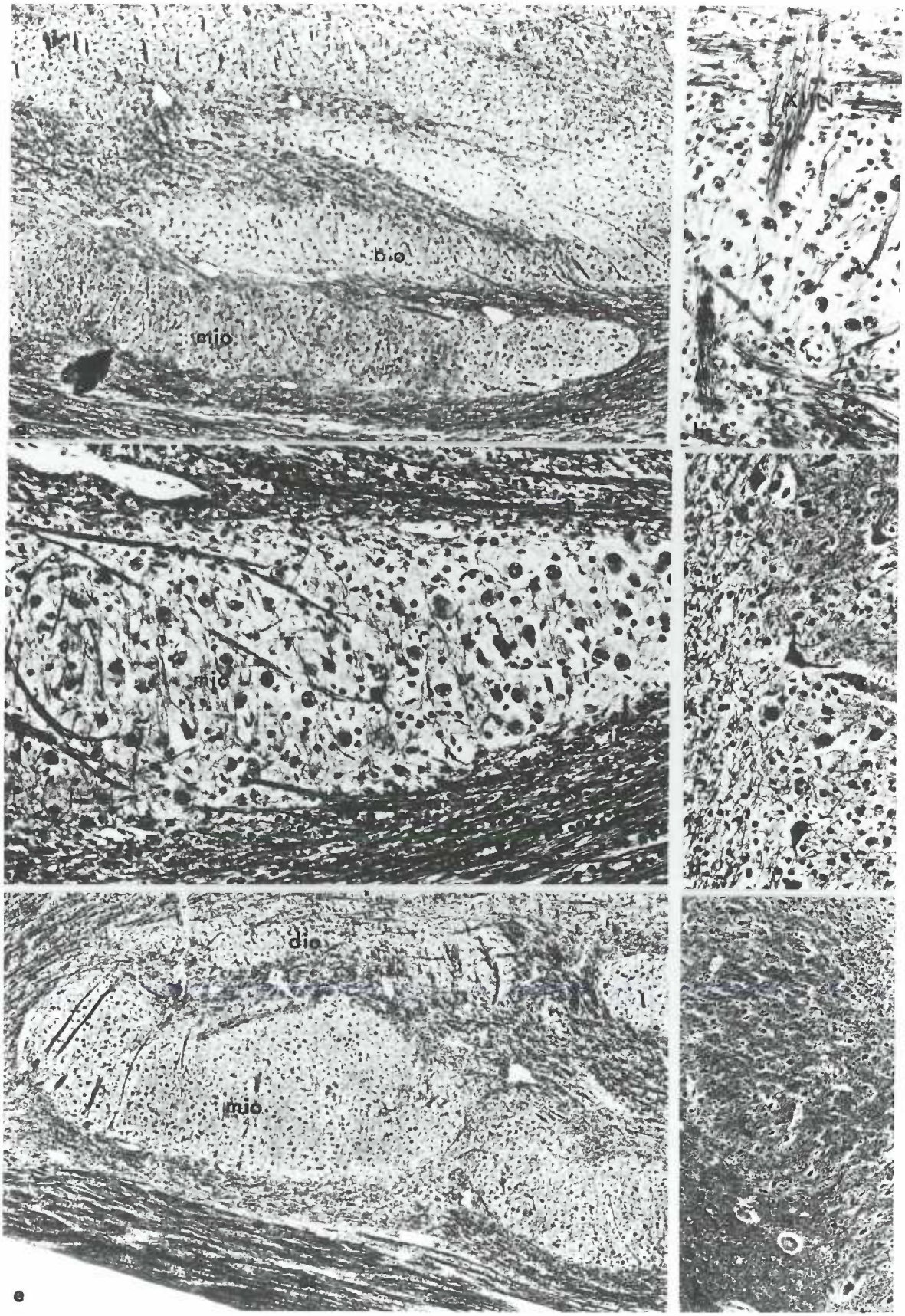

b.o.
mio.
mio.
dio.
mio.

PLATE 20

a. Rostral half of the inferior olivary complex of the 147-day fetus. Rubro-olivary fibers can be identified entering the rostral pole of the principal nucleus, as well as projecting caudally under the ventral lamella. A few rubro-olivary fibers enter the rostral region of the medial accessory nucleus. (sagittal, X 35)

b. Most rostral pole of the principal nucleus (slightly more lateral than *a*) of the 147-day fetus. A few rubro-olivary fibers can be traced through the superior olivary nucleus and into and around the principal nucleus. (sagittal, X 35)

c. Coronal section of the inferior olivary nucleus of the 147-day fetus. There is an increased number of olivo-cerebellar fibers, particularly from the dorsal lamella, in comparison to younger stages. (coronal, X 35)

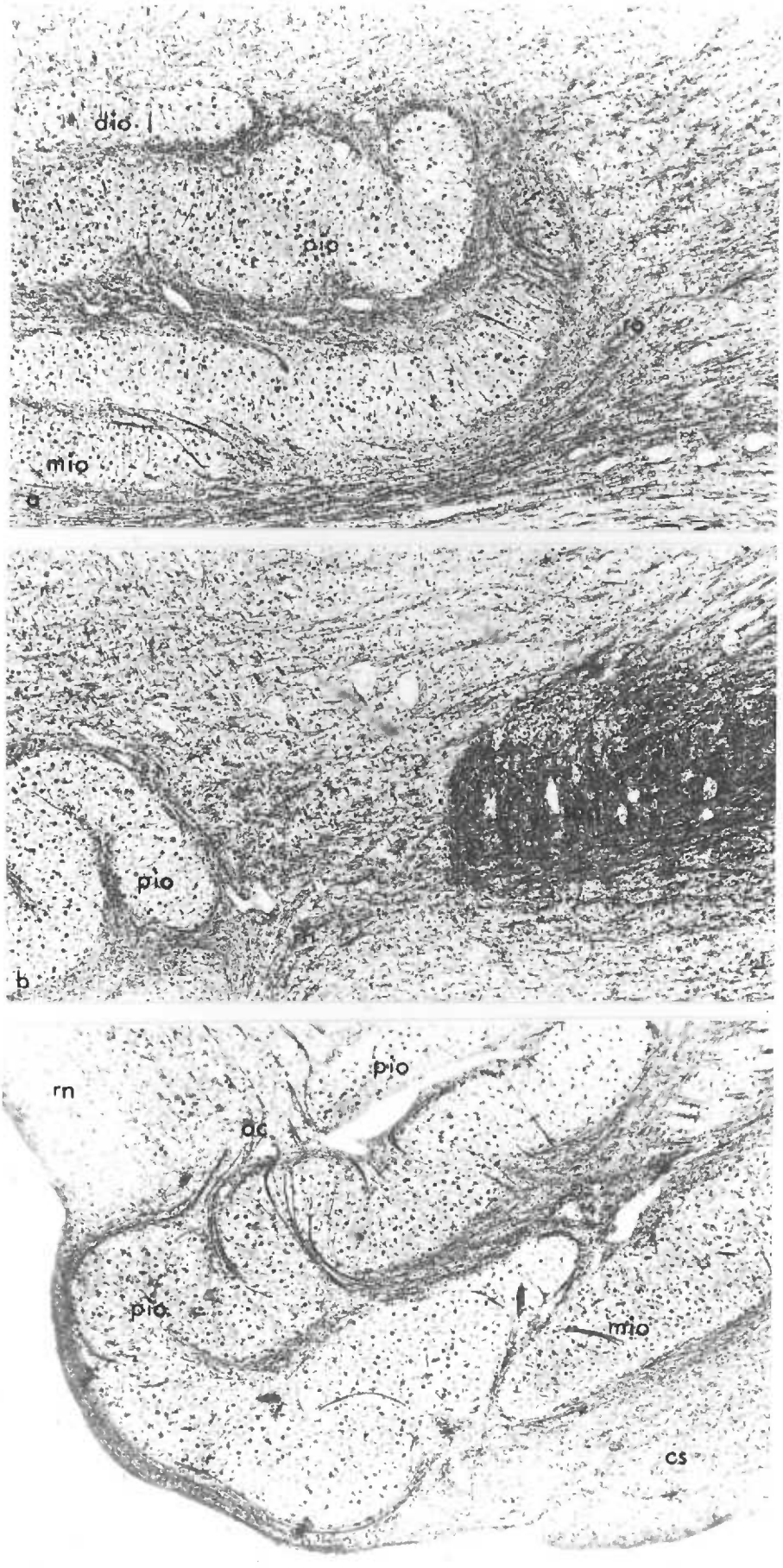

dio
pio
ro
mio
a
pio
b
rn
pio
ac
pio
mio
cs
c

PLATE 21

a. Section of the dorsal lamella of the 154-day fetus. Note that the cells form rather uniform rows; those at the periphery have more pearshaped contours. (coronal, X350)

b. Cells within the dorsal lamella of the 155-day fetus. (sagittal, X 550)

c. Sagittal section of the whole inferior olivary complex (the rostral pole is to the right of the picture) of the 155-day fetus. Note that the caudal two-thirds of the medial accessory nucleus has a higher density of fibers than that of the rostral third, which is mainly attributed to the distribution of spinal afferents. The rostral third of the medial accessory receives both spino-olivary and rubro-olivary fibers. Spinal afferents can also be traced to the caudal region of the dorsal accessory nucleus. Most of the rubro-olivary fibers project to the more lateral regions of the principal nucleus but a small dense bundle can be identified at the rostral pole of the dorsal lamella. (sagittal, X 35)

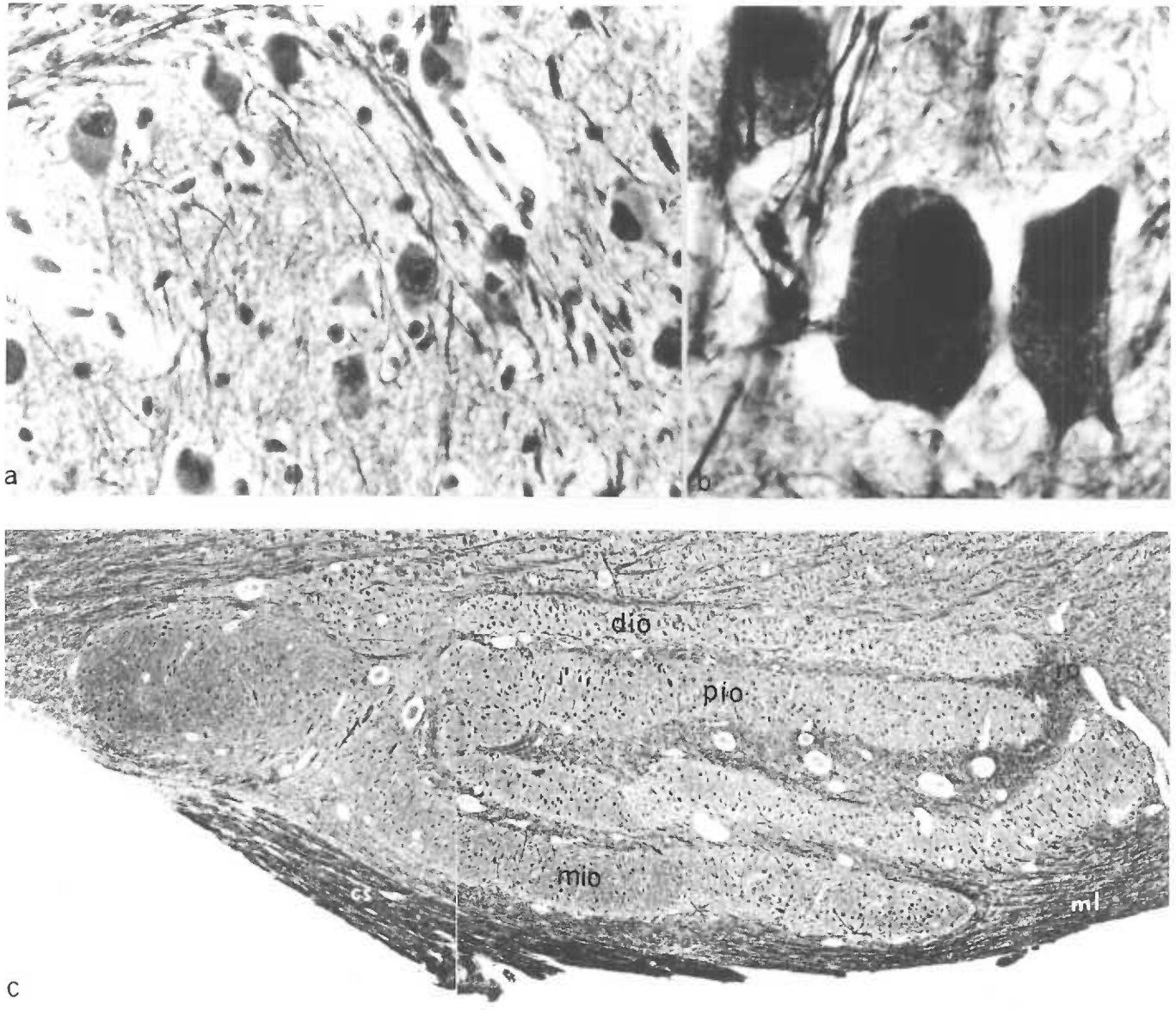

a
b
dio
pio
mio
ml
c

PLATE 22

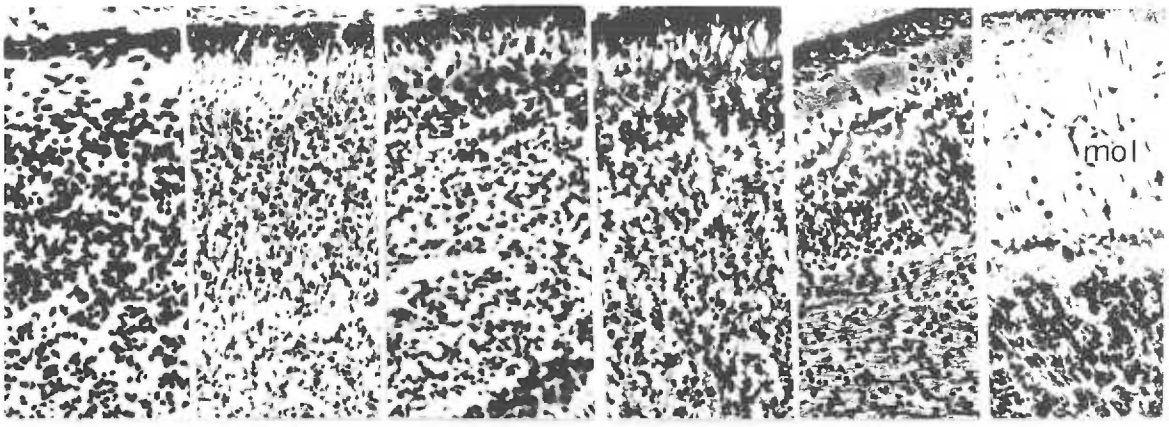
Representative sections of the vermian cerebellar cortex of the 60-, 80-, 100-, 107-, 121-, and 134-day fetuses. Demonstrated are the changes with age in the external granular layer, molecular layer, and internal granular layer. For a more complete description see the observations at each age. (sagittal, X 135)

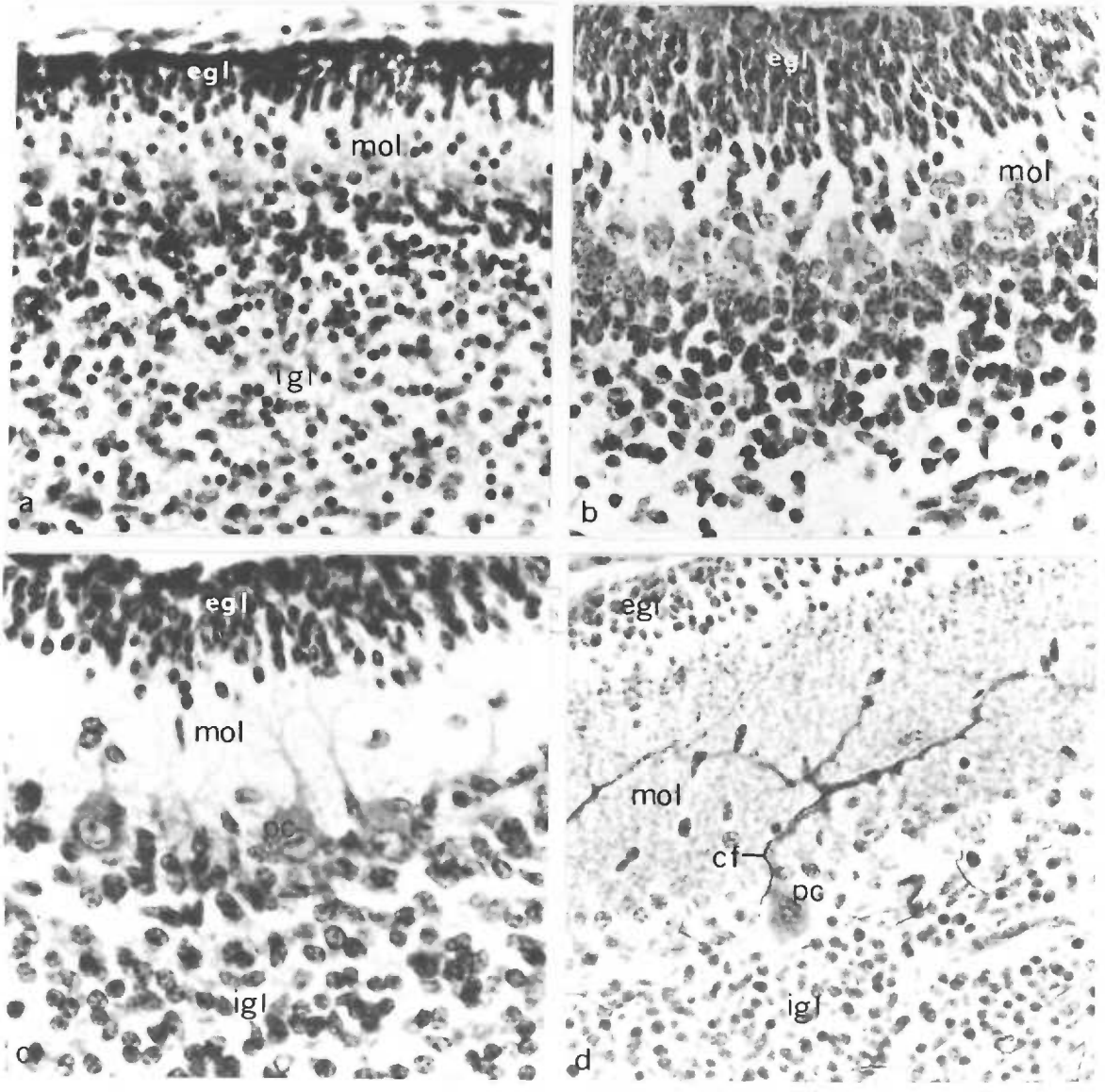
a. Cerebellar cortex of the 92-day fetus.
Cells can be identified migrating from the external granular layer to the rudimentary internal granular layer.
(sagittal, X 235)

b. Cerebellar cortex of the 102-day fetus.
There is an enlargement of the external granular layer and more cells are migrating to the internal granular layer.
(sagittal, X 235)

c. Cerebellar cortex of the 122-day fetus.
Well developed Purkinje cells with dendrites that project into the molecular layer are evident. (sagittal, X 235)

d. Cerebellar cortex of the 140-day fetus.
A large Purkinje cell with a large dendrite process is demonstrated; along its surface is a climbing fiber.
(sagittal, X 135)


mol
60
80
100
107
121
134


egl
mol
igl
a
egl
mol
b
egl
mol
pc
igl
c
egl
mol
cf
pc
igl
d

typed by Virginia Belknap