Pathogenesis of Central Nervous System Lesions Induced by Exposure to Hyperbaric Oxygen

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In a previous study selective and bilaterally symmetrical central nervous system (CNS) lesions were induced in rats paralyzed by intermittent exposure to hyperbaric oxygen.[1] The lesions consist of necrosis of specific nuclei in the brain and spinal cord, with the following areas being consistently involved: globus pallidus, substantia nigra, superior olivary complex, ventral cochlear nucleus, nucleus of the spinal tract of cranial nerve V, and anteromedial horn cells of the spinal cord. The neocortex and striatum were spared in contrast to their being the most susceptible areas of the rat brain to anoxic ischemia.[2] The contrast in distribution between the CNS lesions of hyperbaric oxygen exposure and those of anoxia suggests that the former are the result of a direct toxic effect of excess oxygen on specific neurons. This hypothesis is supported by the fact that high oxygen tension inhibits specific enzymes vital to cellular metabolism [3,4] and by the findings of Jamieson and Van Den Brenk that exposure to hyperbaric oxygen elevates cerebrocortical oxygen tension.[5] Nevertheless, high oxygen tension also causes significant cerebral vasoconstriction [6] and could cause regional alterations of blood flow in areas remote from the cerebral cortex. Fundamentally, therefore, the cerebral lesions induced by hyperbaric oxygen exposure result from either excessive tissue oxygenation or regional ischemia.

Levine has shown that unilateral carotid artery ligation (UCAL) does not produce lesions in the brains of rats.[2] This is explicable on the basis of the rapid collateral flow received from the other carotid artery.[7] If the rats are briefly exposed to pure nitrogen subsequent to UCAL, anoxic-ischemic lesions occur *unilaterally* in the hemisphere of the brain ipsilateral to the UCAL.[2] These observations indicate that UCAL pro-

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Supported by Research Training Grant 2T1-NB-5212 and by Research Grant 1 RO1 NB 07545-01 from the National Institute of Neurological Diseases and Blindness, U. S. Public Health Service.

Presented in part to the 64th Annual Meeting of The American Association of Pathologists and Bacteriologists, Mar. 10, 1967, Washington, D.C.

Accepted for publication Sept. 9, 1968.

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duces a significant, but not critical, reduction in cerebral blood flow in the ipsilateral brain hemisphere.

In order to test the alternate hypothesis concerning the pathogenesis of the CNS lesions of hyperbaric oxygen exposure—i.e., ischemia vs. excessive tissue oxygenation—unilateral common carotid artery ligations (UCAL's) were performed in rats subsequently exposed to the same conditions of hyperbaric oxygen employed in the previous study. The results of the present investigation support the thesis that excessive tissue oxygenation is responsible for the occurrence of the CNS lesions.

Materials and Methods

Chamber Operations

The same Vacudyne hyperbaric chamber (approximately 816 L.) previously described was employed utilizing similar operational procedures.[1] The ventilation rate of the chamber was increased to 20–30 L. per minute. No more than 60 animals were exposed at a time. Oxygen tensions were measured with an Instrumentation Laboratory gas analyzer from samples of the chamber outflow, after flushing and prior to compression. The values obtained (697–725 mm. Hg) always indicated the presence of 98–100% oxygen prior to compression. Carbon dioxide (CO_2) tensions were measured in a Van Slyke analyzer from outflow samples at the end of each exposure and prior to decompression. The values obtained (0–0.33%; average, 0.11%) indicated minimal CO_2 accumulation in the chamber during exposure.

Animals

Albino female rats of the Osborne-Mendel strain, weighing 150–200 gm., were divided into three groups.

Group I. Sixty animals were subjected to the same experimental conditions as in the previous study.[1] They were exposed for 1 hr. per day to oxygen at 57 lb. per square inch gauge (psig) on consecutive days until the onset of limb paralysis (19 animals) or death from the acute effects of hyperbaric oxygen exposure[1] (41 animals). Of the 19 paralyzed rats, 1 was omitted from the study because of incidental meningoencephalitis. Three animals from the previous investigation[1]—whose brains were studied in similar histologic detail (serial sections at every 10 μ) as employed in the present study—were added to this group, providing a total of 21 rats paralyzed by hyperbaric oxygen exposure and suitable for detailed histologic study.

Group II. Sixty rats were subjected to the same conditions of hyperbaric oxygen exposure as those in Group I. At 12–24 hr. prior to their first exposure, however, they underwent right UCAL's. The ligations were performed, under general anesthesia with pentobarbital sodium, utilizing a direct clear surgical technique. Clean 4-0 silk ligatures were placed around the common carotid artery 2.0 cm. from its origin; interruption of the major veins of the neck and the vagus nerve was carefully avoided. Ligation of the cervical sympathetic nerves was often unavoidable and resulted in an ipsilateral ptosis in many animals. No other signs or symptoms were noted subsequent to the ligation and prior to oxygen exposure. The success of the ligation and normality of the cerebral arteries were confirmed by postmortem contrast filling as described below. In this group, 28 animals became

paralyzed, the remaining 32 dying. Of the paralyzed rats, 5 had incidental meningoencephalitis and were omitted from the study, leaving a total of 23 rats suitable for histologic study.

Group III. To provide a control for the effects of ligation, 9 rats underwent right UCAL's and were sacrificed 7 days later. Postmortem contrast studies similar to those used in Group II were performed to confirm the effectiveness of ligation and the normality of the cerebral arteries. No signs and symptoms other than ptosis were observed. All 9 animals were suitable for histologic study.

Preparation of Tissues

Each animal was anesthetized with pentobarbital sodium and given heparin intraperitoneally. The thoracic cavity was opened, and an incision was made in the right ventricle. A 19-gauge scalp vein needle, attached to a glass syringe filled with a heparinized isotonic saline solution, was inserted into the left ventricle. Each animal was perfused with 20 ml. of the saline solution, immediately followed by 75–100 ml. of Susa's solution. At 10–20 min. after fixation, the vasculature of every animal was perfused through the left ventricle with a contrast medium (either a dyed micropaque or gelatin solution) in order to visualize the anatomy of the cerebral vessels in all groups and to examine the carotid artery ligations in Groups II and III. The animals were then placed in 10% phosphate-buffered (pH 7.0) formalin for 24–48 hr. The fixed brains were subsequently removed from the skull. A cut was made in the left side of each brain to facilitate left-right orientation of the histologic sections. Each whole brain was then dehydrated in a series of alcohols and embedded in paraffin. Serial 10-μ coronal sections were taken through each entire brain and mounted on a 35-mm. plastic film strip according to the technique of Pickett, Greene, and Sommer [8] (Fig. 1). The spinal cords were processed as previously described.[1] All sections of brain and spinal cord were stained with luxol fast blue-hematoxylin and eosin.

Results

The clinical responses to hyperbaric oxygen exposure of the rats in Groups I and II were similar to those previously described.[1] The mean onset of paralysis among survivors was at 4.4 exposures in Group I and 3.9 exposures in Group II (not significantly different statistically). The paralysis observed was bilateral in both groups.

The anatomy of the circle of Willis in every animal in this study conformed to the pattern diagrammed in Fig. 2. This pattern is similar to that reported by Zeman and Innes.[9] The basic differences between the cerebral arteries in the rat and in man are that (1) the anterior cerebral arteries in the rat fuse into a common vessel, and (2) the posterior communicating arteries are as large or larger than the first portion of the posterior cerebral arteries in the rat. This latter fact seems to indicate that the posterior cerebral artery blood flow in the rat is more dependent on the carotid artery than the basilar artery; however, the proximal posterior cerebral arteries are of significant size and often form multiple plexiform connections with the posterior communicating arteries as shown in Fig. 2.

The carotid artery ligations were found to be complete and intact upon direct inspection, after contrast filling of the vasculatures at necropsy, in all of the animals in Groups II and III.

Bilateral symmetrical selective necrosis of specific neurons identical to those previously reported [1] following hyperbaric oxygen exposure were found in Group I (Fig. 3 and 5).

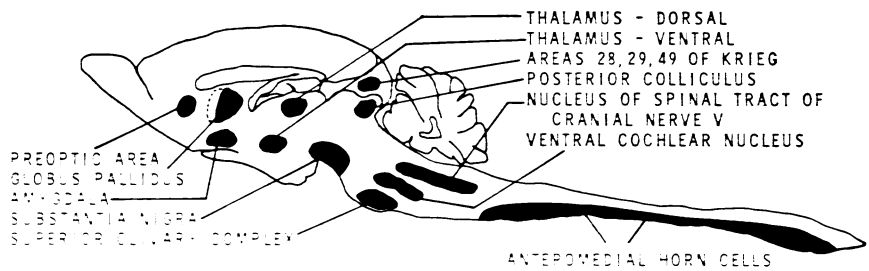
CNS lesions exactly like those found in Group I were observed in Group II; however, the lesions in the area of the brain supplied by the internal carotid artery (i.e., the forebrain and rostral midbrain in the rat) occurred, as a rule, *unilaterally* in the hemisphere *contralateral* to the carotid artery ligation, rather than bilaterally (Fig. 4 and 6). The area of the brain ipsilateral to the carotid artery ligation was protected from the occurrence of lesions.

The lesions in the area of the brain supplied by the basilar, vertebral, and spinal arteries were consistently bilateral in both Groups I and II.

No lesions of any nature were found in the brains of the rats in Group III.

Although the type and distribution of the lesions found in Groups I and II were identical to those reported in the previous study,[1] involvement of the preoptic area; amygdala; Areas 28, 29, and 49 of Krieg; and the posterior colliculus was more commonly encountered in the present study. Text-figure 1 illustrates the characteristic distribution of the oxygen-induced lesions, considering all of the studies compositely. Each region included was involved in 50% or more of the animals (the nucleus entopenducularis and dorsal thalamus separately are involved in less than 50%, but are considered together as parts of the thalamus).

An overall comparison of the findings in Groups I and II are presented in Table 1.



TEXT-FIG. 1. Rat brain model, sagittal plane, illustrating characteristic distribution of lesions produced by exposure to hyperbaric oxygen. Areas in black correspond to location of nuclei involved and not necessarily to shape of lesions.

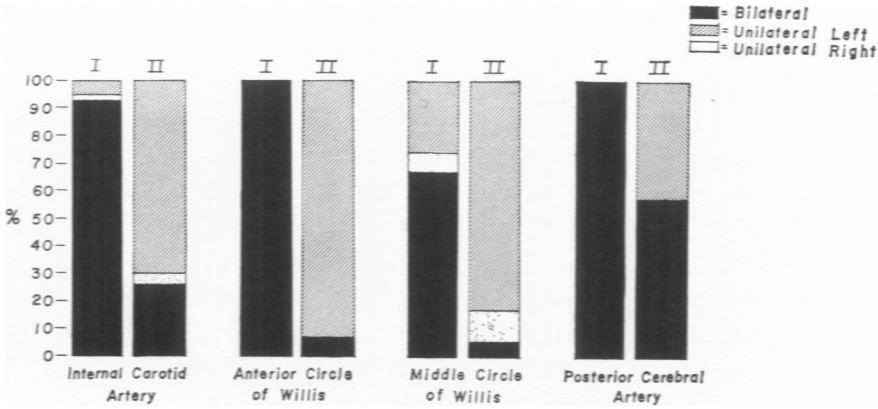
Table 1. Parameters Considered and Overall Findings*

Parameters	Group I	Group II
Experimental conditions	HO†	HO after right UCAL
No. of animals	21	23
No. of animals with lesions	19	17
No. of lesions	74	101
Bilateral	69	26
Unilateral	5	75
Left	4	71
Right	1	3

* Only those lesions in the area of rat brain supplied by the internal carotid artery (forebrain and rostral midbrain) are included in these data.
† HO, hyperbaric oxygen exposure.

Table 2. Data Pertaining to Lesions in Rats

Nucleus	Group	Lesions (No.)				
				Unilateral		
		Total	Bilat.	Total	Left	Right
Anterior circle of Willis						
Area preoptica	I	9	9	0	0	0
	II	13	2	11	11	0
Medial parolfactory area	I	1	1	0	0	0
	II	2	0	2	2	0
Amygdala	I	10	10	0	0	0
	II	12	0	12	12	0
Globus pallidus	I	10	10	0	0	0
	II	9	0	9	9	0
Middle circle of Willis						
Dorsal thalamus	I	6	3	3	2	1
	II	8	0	8	8	0
Nuc. entopeduncularis	I	7	6	1	1	0
	II	5	0	5	5	0
Hypothalamus	I	2	1	1	1	0
	II	5	1	4	3	1
Hippocampus	I	0	0	0	0	0
	II	6	0	6	4	2
Posterior cerebral artery						
Areas 28, 29, 49 of Krieg	I	14	14	0	0	0
	II	20	15	5	5	0
Substantia nigra	I	13	13	0	0	0
	II	17	4	13	13	0
Oculomotor nuc.	I	2	2	0	0	0
	II	2	2	0	0	0
Nuc. darkschevitch	I	0	0	0	0	0
	II	2	2	0	0	0



TEXT-FIG. 2. Bar graph representation of percentage of lesions bilateral and unilateral in Groups I and II compared by regions of arterial blood supply (see text for specific definitions of regions). The left unilateral lesions in Group II are contralateral to the UCAL's performed on the right.

The data pertaining to the number of lesions, are presented in detail in Table 2 in order to compare the two groups in terms of specific nuclei involved and of regional distribution of lesions within the overall area of internal carotid artery supply.

The information from Table 2 is presented graphically in Text-fig. 2 by comparing the percentages of lesions that are bilateral with those that are unilateral within each region of blood supply of the rat brain. The region of the internal carotid artery is defined as the forebrain and rostral midbrain in this study, and the data given in this category are based on the figures listed in the overall comparison in Table 1. Therefore, the lesions in the caudal midbrain, pons, medulla, cerebellum, and spinal cord (which are in the regions of the CNS supplied by the vertebral, basilar, and spinal artery systems) are not included in this graph. The region of the anterior circle of Willis is that area of the brain supplied by the anterior and middle cerebral tributaries of the internal carotid artery; the posterior cerebral artery region is the area of the brain supplied by the posterior cerebral artery; and the middle circle of Willis region is defined as those areas of the brain which receive portions of their blood supply from both the anterior and posterior arterial systems.

Discussion

The data from Text-fig. 2 in the region of the internal carotid artery reveal that, of the lesions in Group I—the animals receiving hyperbaric oxygen exposure alone—93.2% were bilateral. However, in Group II,

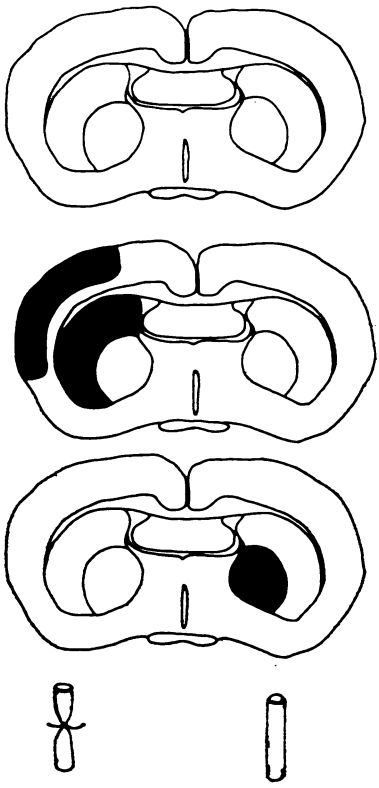
in which the rats underwent right UCAL prior to hyperbaric oxygen exposure, 71.3% of the lesions were unilaterally located contralateral (on the left) to the carotid artery ligation (on the right).

The differences between the number and percentage of the lesions that were bilateral in Group I compared to those that were unilateral and contralateral in Group II are highly significant statistically ($p<0.001$) in all of the categories of comparison made in Text-fig. 2. Only three lesions in Group II were unilateral and ipsilateral, all being located in the middle circle of Willis region in uncommonly involved nuclei. The occurrence of the three lesions is explicable by chance and compares with the one lesion occuring unilaterally on the right in Group I.

There is a significant difference ($p<0.001$) between the number of lesions in Group II that were bilateral vs. those that were unilateral and contralateral in the anterior circle of Willis region as compared to the posterior cerebral artery region. This comparison indicates that the anterior region was more altered in its response to hyperbaric oxygen exposure following UCAL than the posterior region. This is undoubtedly due to the fact that the anterior region is more dependent on carotid artery blood flow than the posterior region, which is near the collateral influence of the basilar artery.

The results of this study suggest that the CNS lesions induced by hyperbaric oxygen exposure are due to excessive tissue oxygenation, rather than to vasoconstriction producing ischemia. The reasoning behind this conclusion is recapitulated in Text-fig. 3. If one common carotid artery of a normal young adult rat is ligated, no structural changes are produced in the CNS. However, if subsequent to UCAL, the animal is placed briefly in a pure nitrogen atmosphere, anoxic-ischemic lesions in the cerebral cortex and striatum are induced ipsilateral to the ligation. These findings indicate that UCAL produces a significant reduction in ipsilateral cerebral blood flow, but it is not critical enough to itself produce ischemic necrosis.

Repeated exposure to hyperbaric oxygen consistently produces bilateral selective neuronal necrosis of a distribution distinctly different from that of anoxic-ischemic encephalopathy. UCAL prior to excessive oxygen exposure protects the ipsilateral hemisphere from lesions, while in the contralateral hemisphere selective neuronal necrosis characteristic of hyperbaric oxygen exposure is produced regularly. Since these unilateral lesions occur in the brain hemisphere with the greatest expected blood flow, and since the hemisphere with the least expected blood flow remains protected, it is concluded that the oxygen-induced neuronal



TEXT-FIG. 3. Diagram of coronal sections of rat forebrains comparing the effects of UCAL on normal, anoxic, and hyperoxic animals. Areas in black represent necrosis of specific regions of gray matter (nuclei). Ligation alone (*top*) induces no lesions; ligation plus anoxia (*middle*) produces ipsilateral necrosis of the neocortex and striatum; ligation plus hyperbaric oxygen (*bottom*) results in contralateral necrosis of the globus pallidus. Drawings below coronal sections indicate carotid artery, ligated (*left*) and not ligated (*right*).

necrosis results from excessive tissue oxygenation, rather than from regional ischemia. The expected results, if the latter mechanism were operational, would have been the reverse—i.e., lesions ipsilateral to the carotid artery ligation.

Although this study tends to support the biochemical data indicating that excessive oxygen has a direct toxic effect on neuronal metabolism [3,4] the observation may need to be supported by other approaches, such as direct measurements of regional oxygen tensions and regional biochemical events under appropriate experimental conditions. An evaluation of the effects of hyperbaric oxygen on the blood brain barrier is also necessary for understanding the precise mechanism of the lesions.

Summary

Rats paralyzed by repeated exposure to hyperbaric oxygen are found consistently to develop bilateral symmetrical selective CNS lesions of a distinct distribution. In the present investigation unilateral carotid artery ligations were performed in one group of rats prior to their being

paralyzed by repeated hyperbaric oxygen exposure; their brains were studied in detail histologically by serial sections. The lesions in the region of the brain supplied by the internal carotid artery were characteristic of those of hyperbaric oxygen exposure, but they occurred unilateral and contralateral to the ligation in a statistically significant number, as compared to the number of similar lesions occurring bilaterally in rats exposed to hyperbaric oxygen without prior carotid artery ligation studied in comparable histologic detail. The results of this investigation indicate that the CNS lesions of hyperbaric oxygen exposure are the result of excessive tissue oxygenation, rather than of regional ischemia.

References

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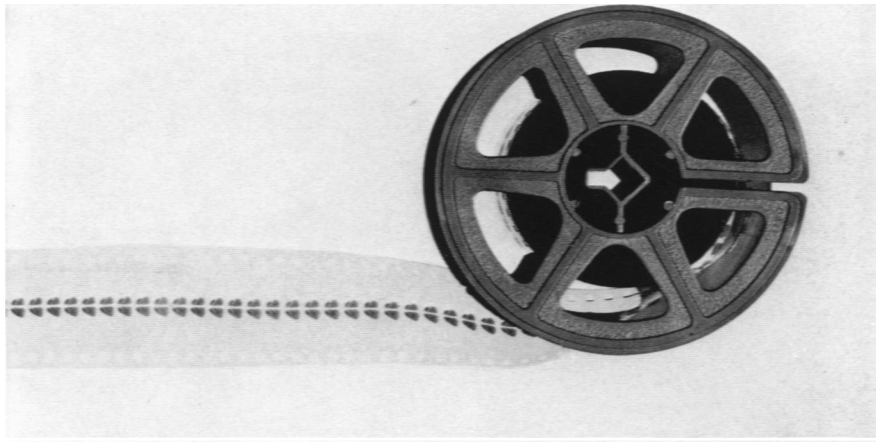
[*Illustrations follow*]

Legends for Figures

Fig. 1. Serial sections of rat brain mounted on 35-mm. plastic film strip wound on a reel for handling and storage. Sections were stained with luxol fast blue-hematoxylin and eosin. Special devices were used to place the film flatly on a light-microscope stage for viewing of sections through standard objectives and eyepieces.

Fig. 2. Diagram of cerebral arteries of rat based on postmortem contrast filling in animals in this study.



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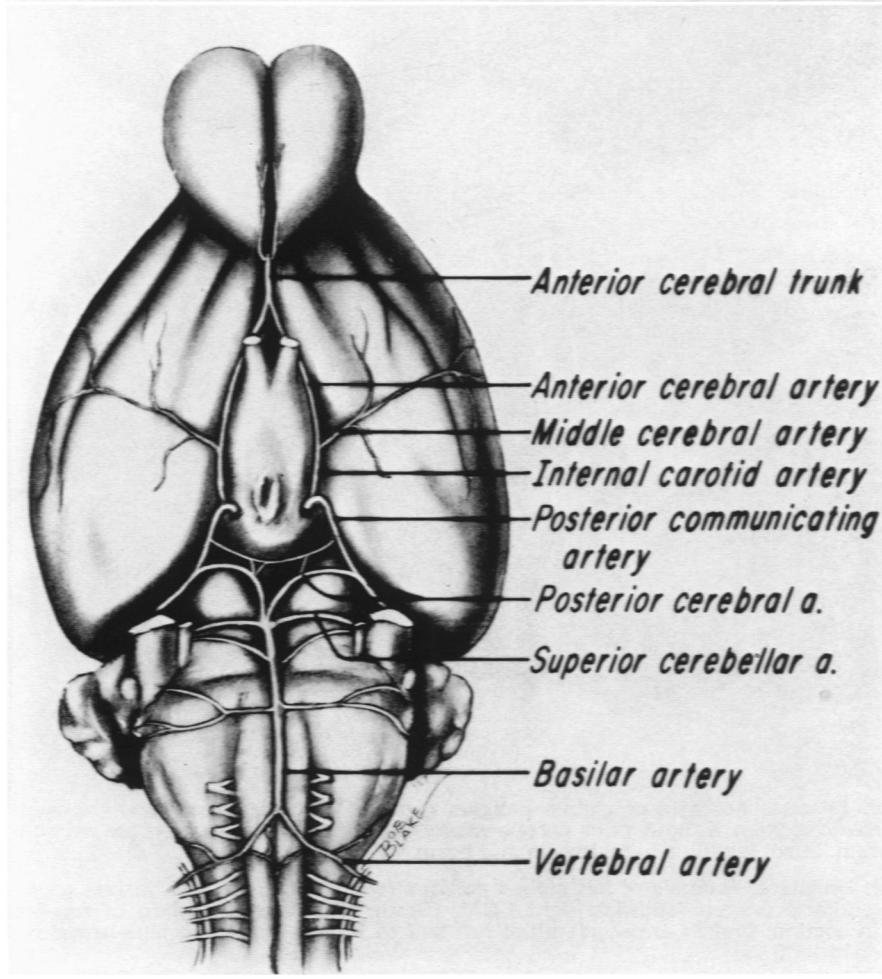
Anterior cerebral trunk
Anterior cerebral artery
Middle cerebral artery
Internal carotid artery
Posterior communicating artery
Posterior cerebral a.
Superior cerebellar a.
Basilar artery
Vertebral artery

2

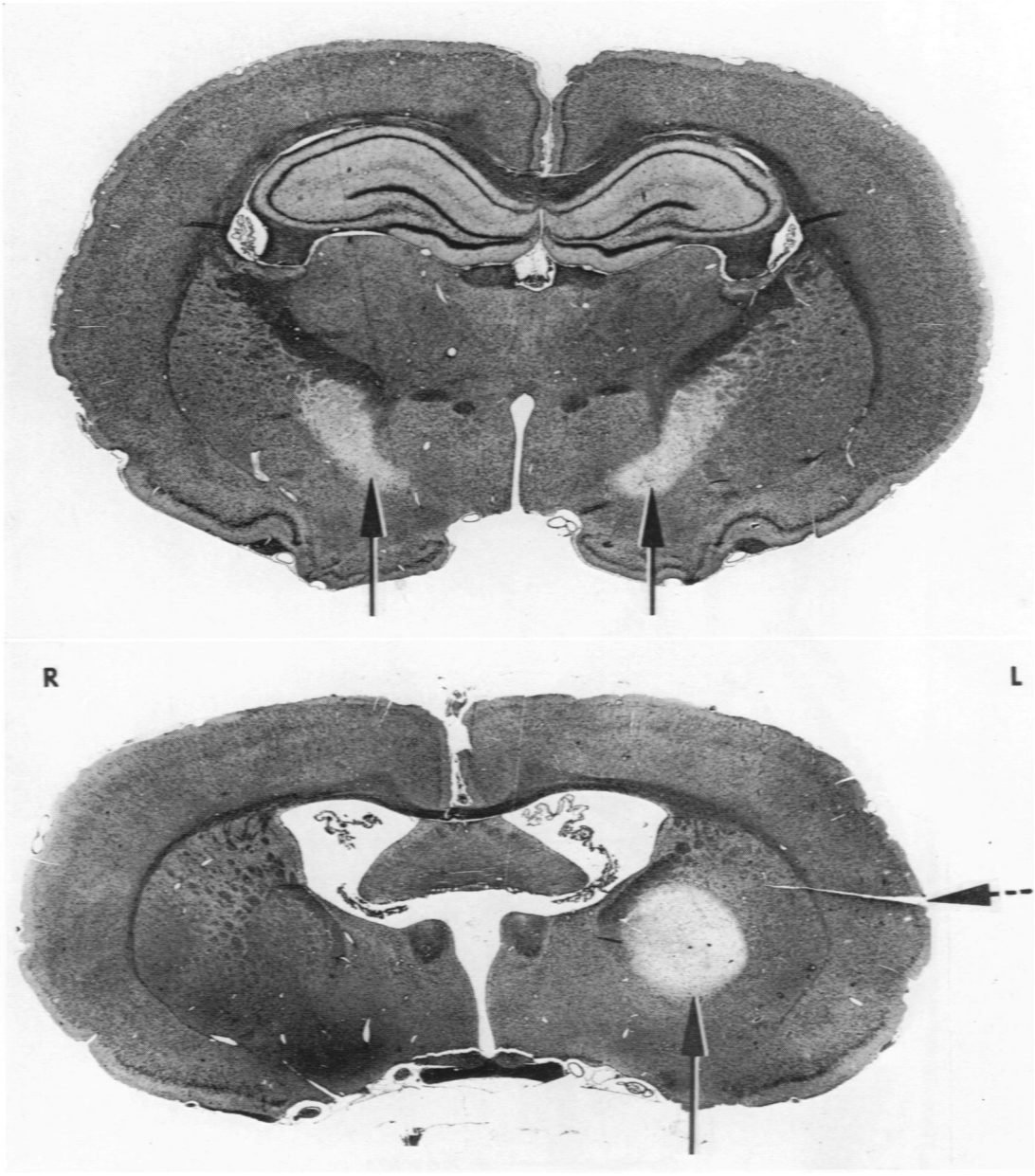

Fig. 3. Bilateral necrosis of globus pallidus *(arrows)* as seen in animals exposed to hyperbaric oxygen without prior carotid artery ligations (Group I). Coronal section of forebrain. Luxol fast blue-hematoxylin and eosin. × 9.

Fig. 4. Unilateral necrosis of left globus pallidus *(solid arrow)* as seen in rats exposed to hyperbaric oxygen following right UCAL (Group II). Coronal section of forebrain. (Cut in section, *broken arrow,* identifies left half of brain.) Luxol fast blue-hematoxylin and eosin. × 9.

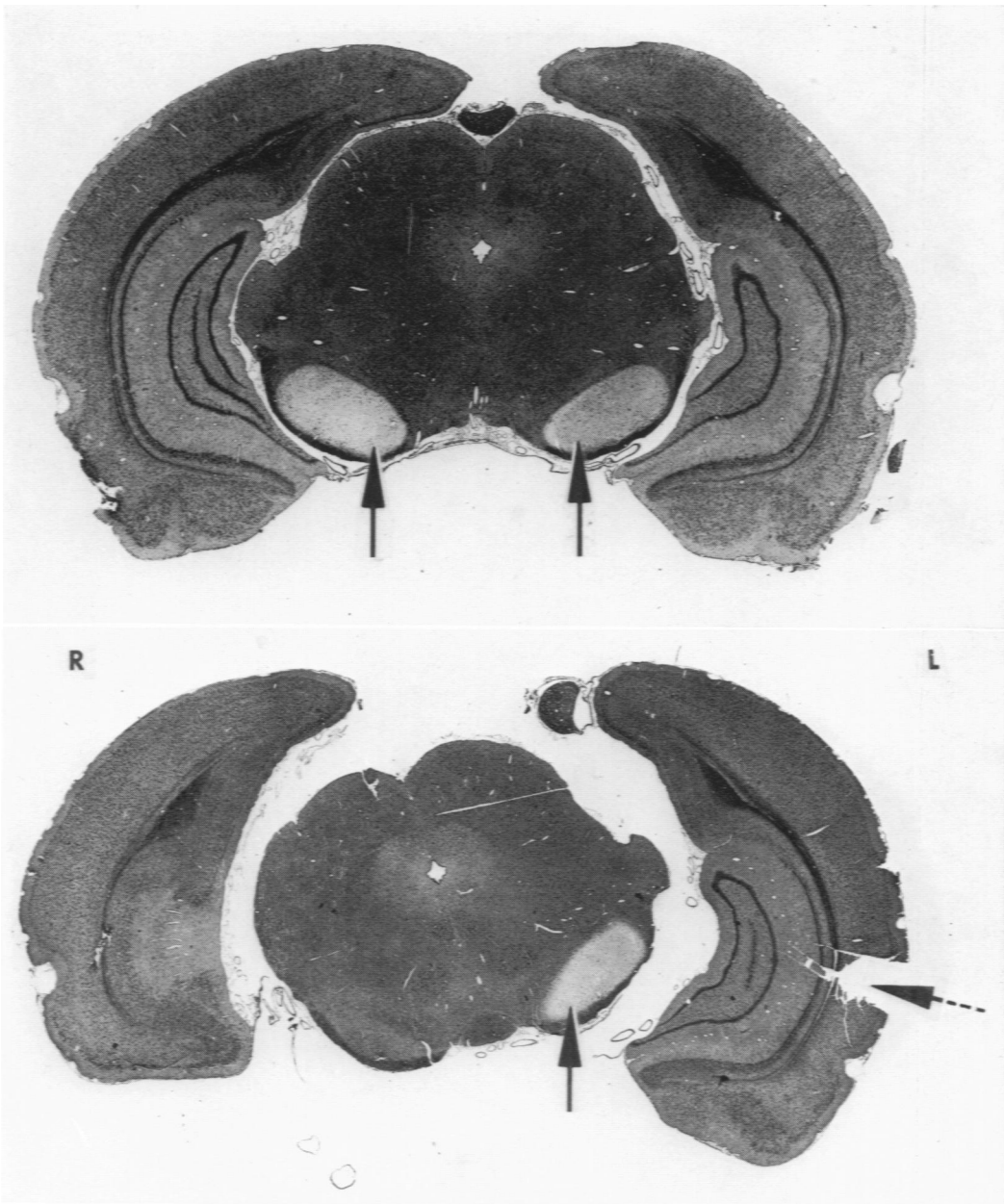

Fig. 5. Bilateral necrosis of substantia nigra *(arrows)* as seen in Group I rats. Coronal section through the forebrain and rostral midbrain. Luxol fast blue-hematoxylin and eosin. × 9. (From Balentine and Gutsche,[1] *Amer J Path.*)

Fig. 6. Unilateral necrosis of left substantia nigra *(solid arrow)* as seen in Group II rats. Coronal section through forebrain and rostral midbrain. (Cut in section, *broken arrow,* identifies left half of brain.) × 9.