obtaining both kinetic and approximate thermodynamic data from sorption experiments when there is a need to evaluate or screen a large number of compounds as to their potential interaction with a polymeric material. Research in this field is continuing, and the results will be reported in subsequent publications.

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Action of Lysergide, Ephedrine, and Nimergoline on Brain Metabolizing Activity

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Abstract □ The effects of lysergide, ephedrine, and nimergoline on the metabolizing ability (demethylation and acetylation of aminopyrine and glucuronoconjugation of oxazepam) of the dog brain isolated *in situ* were tested. The intracarotid perfusion with lysergide did not induce significant variations in aminopyrine and oxazepam metabolism, while the perfusion with ephedrine or nimergoline significantly increased the disappearance of the tested substances from the extracorporeal circuit, with an increase of some metabolic products. Activation of the tested biotransformation was accomplished by a decrease in cerebral vascular resistance and an increase in oxygen consumption. The action of lysergide, ephedrine, and nimergoline on the depression of the cerebral activity by hypoxia in dogs also was studied. Only ephedrine and nimergoline significantly improved the partial spontaneous reversion of the electroencephalogram depression, accomplished by a recovery of some enzymes (glutamic oxalacetic transaminase, lactate dehydrogenase, and alkaline phosphatase) in the cerebrospinal fluid, which was altered by the condition of hypoxia.

Keyphrases □ Metabolism, cerebral—lysergide, ephedrine, nimergoline effects, *in situ*, dogs □ Lysergide—cerebral metabolism effect, dogs □ Ephedrine—cerebral metabolism effect, dogs □ Nimergoline—cerebral metabolism effect, dogs

Many researchers have related changes in cerebral metabolism to changes in oxygen metabolism, cerebral blood flow, the electroencephalogram, and enzymes of the cerebrospinal fluid, for example. The present study was undertaken to investigate the action of lysergide, ephedrine, and nimergoline on these same parameters. To eliminate any interference from systemic response, the action of the drugs was investigated during a direct perfusion into the circle of Willis.

METHOD

The experiments were performed in the dog using two techniques: (*a*) the isolated perfused brain *in situ*, and (*b*) depression of cerebral activity by progressive hypoxia.

Isolated Perfused Brain *In Situ* Technique—*Animals and Anesthesia*—The experiments were carried out on 40 beagle dogs (10.7–14.6 kg. body weight), which were preanesthetized with urethan (0.4 g./kg. i.p.). Anesthesia was induced and maintained by nitrous oxide, cyclopropane, or ethyl ether in closed circuit. The animals were given artificial ventilation after tracheal intubation with a Warne tube, following succinylcholine chloride (1 mg./kg. i.v.) administration.

Operative Procedure—Benzi *et al.* (1) stated that the operative procedure consists mainly of the isolation of the external jugular veins and the common carotid arteries, with ligature of all their branches except the internal carotid arteries and the right thyroidea superior artery. In addition, the vertebral vessels are ligated before their entrance into the transverse foramen of C2 or C3. The numerous muscular branches arising from the vertebral vessels, the anastomosis between vertebral and carotid arteries, the anastomosis between vertebral and jugular veins, the internal jugular veins, the vascular branches of the neck, the vessels running under the carotid arteries and vagus nerves, and the zygomatic, maxillary, auricular, and supraorbital vessels are all occluded by ligature or compression. The occlusions of the sinus columnae vertebralis and the anterior spinal artery are made by opening the rachis in C2, ligating the spinal artery according to the method of Greeley and Greeley (2), and compressing the venous vessels around the spinal cord.

Both of the isolated jugular veins were ligated, cannulated, and connected to the venous reservoir of the pump-oxygenator system by gravitational flow. Both of the isolated carotid arteries also were cannulated and connected to the pump-oxygenator system. The isolated right thyroidea superior artery was cannulated by a polystan tube and connected to a perfusor apparatus (Palmer). Arterial blood pressure was measured from a cannula inserted into a femoral artery. Extradural electrodes were set in place some days before the experiments; during the experiments the electroencephalogram was

recorded continuously with both systemic and cerebral pressure on a 12-channel polygraph.

Brain Pump-Oxygenator System—The brain perfusion apparatus employed consists of a venous reservoir, an oxygenator with gasmeter, a roller-type pump with a flowmeter, two blood filters, an apparatus to eliminate blood foam, a perfusion pressure regulator with manometer, and a blood exchanger with telethermometer. Before the extracorporeal perfusion, the pump-oxygenator system was filled with 500 ml. of heparinized blood diluted with Tyrode solution (3:1) added to glucose (10%). The blood was obtained 20 min. prior to use to prevent the accumulation of lactic acid, which would subsequently require a considerable degree of neutralization. Before the perfusion, the diluted blood was filtered through glass wool and polyester staple and adjusted to pH 7.35 using 1 *M* sodium bicarbonate.

The priming blood, which was fully oxygenated and warmed, was circulated through the pump-oxygenator system. A flow of O_2 + CO_2 mixture (95:5), maintained at the rate of 5–8 l./min., was passed into the oxygenator during the extracorporeal brain perfusion; the blood flow rate was kept between 8 and 12 ml./min./kg., a pressure equal to the initial systemic pressure of the animal. The time of brain perfusion was limited to 60 min. and was related to the presence of a considerable cerebral electric activity. The eventual leakage of perfusate into the systemic circulation was evaluated: (*a*) during the metabolic research by taking samples from the general circulation of the blood and of the lymph to verify the absence of the tested substance or its metabolites, and (*b*) at the end of the metabolic research by adding either a dye or a radiopaque substance to the blood of the extracorporeal circuit.

Cerebral Metabolizing Activity—The metabolizing activity of the brain was investigated *in situ* by: (*a*) demethylation, by evaluating the transformation of aminopyrine to 4-aminoantipyrine using the method of Brodie and Axelrod (3) after addition of aminopyrine to the extracorporeal circuit; (*b*) acetylation, by evaluating the transformation of 4-aminoantipyrine to *N*-acetyl-4-aminoantipyrine under the above-mentioned conditions; and (*c*) glucuronoconjugation, by evaluating the transformation of oxazepam to glucuronide, using the method of Walkenstein *et al.* (4) after addition of oxazepam to the extracorporeal circuit.

Blood samples of the extracorporeal circuit were collected every 10 min. for determination of tested substances and their metabolites. Hematocrit, clotting time, artereovenous blood oxygen, pH, and lactic acid values were also measured. The brain concentrations of both oxazepam or aminopyrine and their metabolites were evaluated at the end of the perfusion.

Evaluation of Drug Action—Thirty minutes after the addition of aminopyrine or oxazepam to the extracorporeal circuit, the perfusion was started through the thyroidea superior artery (at the rate of 0.5 ml./min. for 30 min.) with: (*a*) saline solution; (*b*) *N,N*-diethyl-D-lysergamide (lysergide), 5×10^{-7} *M;* (*c*) 1-ephedrine chloride, 1×10^{-4} *M;* and (*d*) 1,6-dimethyl-8β-(5-bromonicotinoyloxymethyl)-10α-methoxyergoline tartrate (nimergoline), 5×10^{-6} *M.*

Depression of Cerebral Activity by Hypoxia—*Animals and Anesthesia*—The experiments were carried out on 36 mongrel dogs (8.5–14.4 kg. body weight) which were preanesthetized with urethan (0.4 g./kg. i.p.). Anesthesia was induced and maintained by nitrous oxide, cyclopropane, or ethyl ether in closed circuit. The animals were given artificial ventilation after tracheal intubation with a Warne tube, following succinylcholine chloride (1 mg./kg. i.v.) administration. The general condition of the animals was investigated by evaluation of the systemic arterial blood pressure (from a cannula inserted into a femoral artery) and the tone and motility of both the duodenum and small intestine (by a rubber balloon).

Operative Procedure—By drilling, monopolar electrodes were set in place in the left and right frontal, parietal, and occipital areas. Heated-type thermocouples were set in place on the ventral and dorsal surfaces of the brain, and electrical activity and cerebral blood flow were recorded continuously using a 12-channel polygraph.

Benzi *et al.* (5) stated that the operative procedure for arterial injection into the brain consists primarily of isolation of the common carotid arteries and of ligature of all their branches, except the internal carotid arteries and the right thyroidea superior artery. The right external jugular vein was isolated, and the vertebral vessels were ligated before their entrance into the transverse foramen of C2 or C3. The numerous muscular branches arising from the vertebral vessels, the anastomosis between vertebral and carotid arteries, the muscular vessels of the neck, the vessels running under the carotid arteries and vagus nerves, and the zygomatic, maxillary, auricolar,

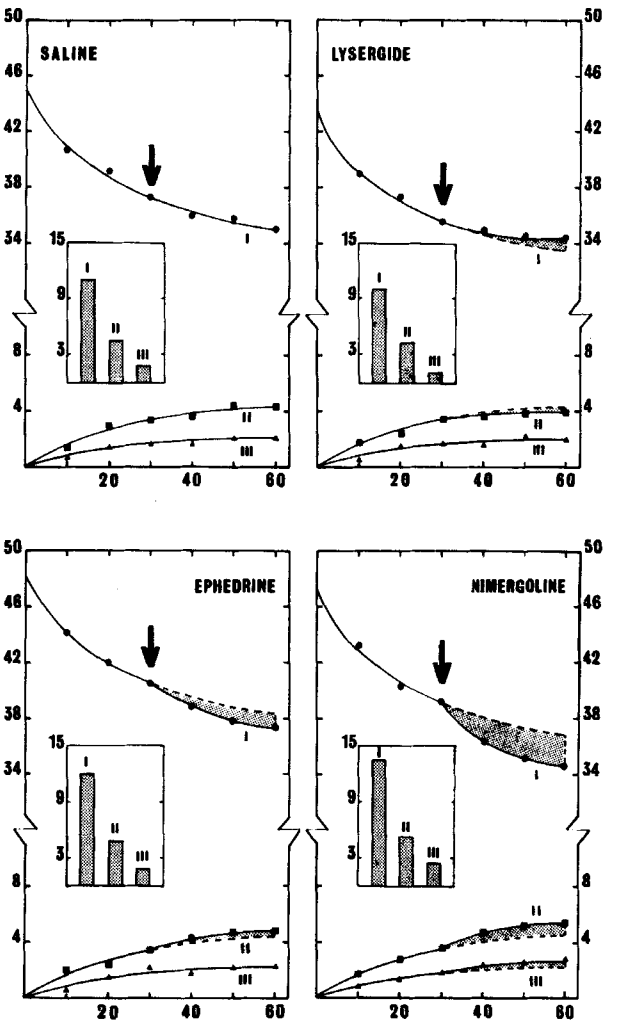

Figure 1—*Action of lysergide* (5×10^{-7} M), *ephedrine* (1×10^{-4} M), *and nimergoline* (5×10^{-6} M) *on aminopyrine metabolism investigated by the isolated perfused brain* in situ *of the beagle dog. Blood in the extracorporeal circuit = 500 ml.; blood flow rate of extracorporeal circuit = 10–12 ml./min./kg. On the ordinates are the plasma concentrations (micrograms per milliliter) of aminopyrine (I), 4-aminoantipyrine (II), and N-acetyl-4-aminoantipyrine (III), assayed for 60 min. at intervals of 10 min. (abscissae) after aminopyrine addition to the extracorporeal cerebral circuit; 30 min. after aminopyrine addition (at the arrow) cerebral perfusion with saline or drug solution (0.5 ml./min. for 30 min.) started. The unbroken line represents the experimental results, the broken line represents the expected results, and the hatched area depicts the difference between the expected and experimental results. There are no broken lines for III in the graphs for lysergide and ephedrine, indicating that the experimental and "expected" lines cross. Inserts show the brain concentrations (micrograms per gram ordinate) of aminopyrine (I) and its two metabolites (II and III), assayed after 60 min. of the extracorporeal brain perfusion with addition of aminopyrine.*

and supraorbital vessels were occluded by ligature or compression. The isolated thyroidea superior artery was cannulated by a polystan tube and connected to a perfusor (Palmer). The isolated right external jugular vein was cannulated by a polystan catheter. The basal electroencephalographic pattern of anesthesia, after the surgical procedure, was maintained at a fourth of Faulconer's level (6).

Depression of Cerebral Activity—Benzi *et al.* (5) showed that depression of the cerebral activity is produced during treatment with tubocurarine (0.1 mg./kg. i.v. every 30 min.) by repeated suppression of artificial ventilation. This condition induced a series of burst suppression patterns in the electroencephalogram. Myocardial depression began, and the animals were given artificial ventilation.

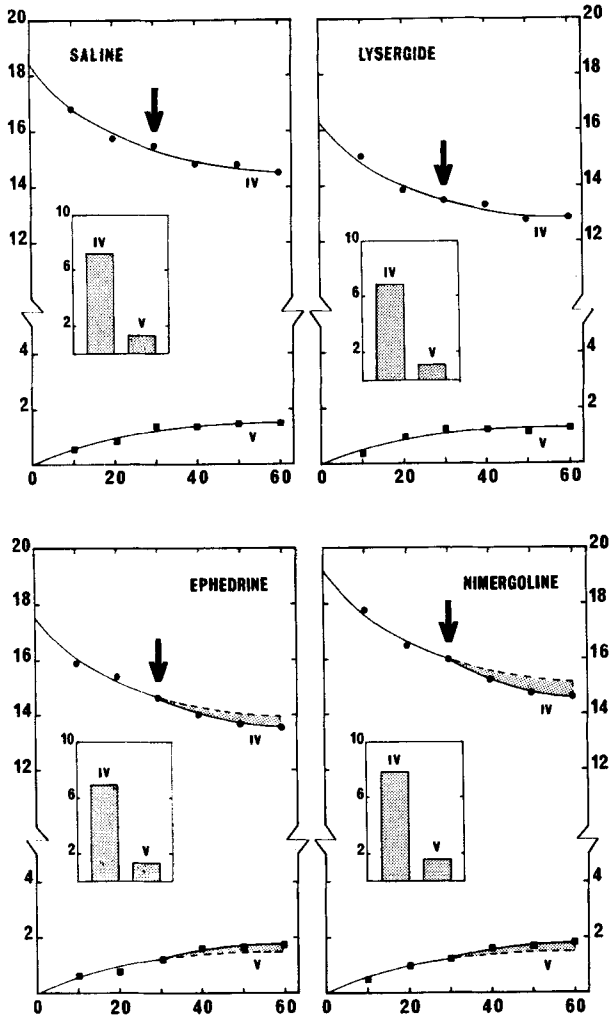

Figure 2—*Action of lysergide (5×10^{-7} M), ephedrine (1×10^{-4} M), and nimergoline (5×10^{-5} M) on oxazepam metabolism studied by the isolated perfused brain* in situ *of the beagle dog. Blood in the extracorporeal circuit = 500 ml.; blood flow rate of extracorporeal circuit = 10–12 ml./min./kg. On the ordinates are the plasma concentrations (micrograms per milliliter) of oxazepam (IV) and its glucuronide (V), assayed for 60 min., at intervals of 10 min. (abscissae) after oxazepam addition to the extracorporeal cerebral circuit; 30 min. after oxazepam addition (at the arrow) cerebral perfusion with saline or drug solution (0.5 ml./min. for 30 min.) started. The unbroken line represents the experimental results, the broken line represents the "expected" results, and the hatched area depicts the difference between the expected and experimental results. There are no broken lines for oxazepam and its glucuronide in the lysergide graph because the experimental and "expected" lines cross. Inserts show the brain concentrations (micrograms per gram, ordinate) of oxazepam (IV) and its glucuronide (V) assayed after 60 min. of the extracorporeal brain perfusion with addition of oxazepam.*

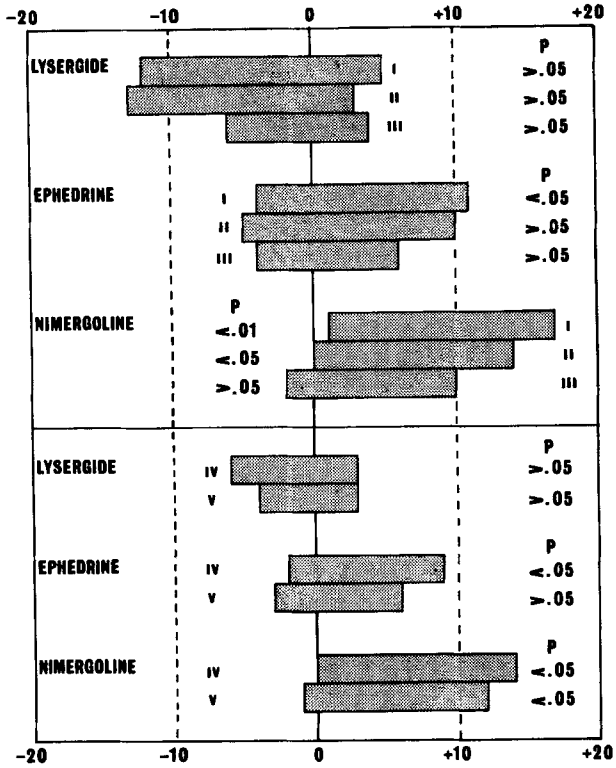

Figure 3—*Cerebral metabolizing activity investigated by the isolated perfused brain* in situ *of the beagle dog. Range of the percent variations (with probability levels; n = 5) between the expected and experimental values induced by the cerebral perfusion (0.5 ml./min. for 30 min.) of lysergide (5×10^{-7} M), ephedrine (1×10^{-4} M), and nimergoline (5×10^{-5} M) upon the decrease of aminopyrine (I) and oxazepam (IV) concentrations in the extracorporeal circuit, and the appearance of their metabolites 4-aminoantipyrine (II), N-acetyl-4-aminoantipyrine (III), and oxazepam glucuronide (V).*

Spontaneous partial recovery occurred in both electroencephalographic and myocardial activity; after 5–15 min., artificial ventilation was suppressed. This procedure was repeated many times (4–8). After the last myocardial depression, without cardiac arrest, artificial ventilation was given for 1 hr.

Cerebrospinal Fluid Enzyme Activity—Before and after induction of hypoxia and after perfusion with the tested drugs, the cerebrospinal fluid was evaluated for glutamic oxalacetic transaminase, lactate dehydrogenase, and alkaline phosphatase enzyme activity. These particular enzymes were chosen because of the well-known changes they undergo during experimental cerebral injury in laboratory animals and in neurological diseases in man.

Drug Tested—After the first 0.5 hr. of natural recovery, the perfusion through the thyroidea superior artery was started (at the rate of 0.5 ml./min. for 30 min.) with: (*a*) saline solution; (*b*) *N,N*-diethyl-D-lysergamide (lysergide), 5×10^{-7} *M*; (*c*) 1-ephedrine chloride, 1×10^{-4} *M*; and (*d*) 1,6-dimethyl-8β-(5-bromonicotinoyloxymethyl)-10α-methoxyergoline tartrate (nimergoline), 5×10^{-5} *M*.

RESULTS AND DISCUSSION

Isolated Perfused Brain *In Situ*—As observed previously (1), the *in situ* isolated brain shows a metabolizing activity; Figs. 1 and 2 show typical examples of the transformation rate of aminopyrine to the demethylated and acetylated products and of oxazepam to the glucuronoconjugated metabolite. In Figs. 1 and 2, it is possible to observe the effects, on this metabolizing ability, of the perfusion with drugs (lysergide, ephedrine, and nimergoline) compared with control conditions (perfusion with saline solution). The range of the percent variations between the expected values and the experimental values, induced by the perfusion with the drugs, is summarized in Fig. 3.

The perfusion (0.5 ml./min. for 30 min.) with lysergide (5×10^{-7} *M*) tends to decrease the cerebral metabolizing activity but without significant statistical differences; on the other hand, perfusion with ephedrine (1×10^{-4} *M*) significantly increases the disappearance of aminopyrine and oxazepam from the extracorporeal cerebral circuit without significant increase in the metabolites appearance. When nimergoline is perfused (5×10^{-5} *M*), there is a significant increase both in the disappearance of aminopyrine and oxazepam from the extracorporeal cerebral circuit and in the appearance of the demethylated and glucuronoconjugated metabolites.

As indicated in Fig. 4, during the cerebral perfusion with saline solution (control condition), at the constant rate of the blood flow into the extracorporeal circuit, the cerebral blood pressure increases significantly, without significant change in oxygen consumption. The cerebral perfusion (0.5 ml./min. for 30 min.) with lysergide (5×10^{-7}

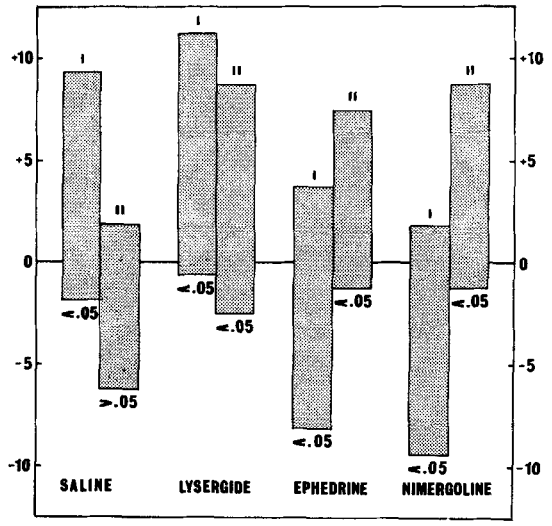

Figure 4—*Isolated perfused brain* in situ *of the beagle dog. Range of the percent variations from the initial values (with probability levels; n = 10) of the cerebral blood pressure (I) and oxygen utilization (II), induced by the cerebral perfusion (0.5 ml./min. for 30 min.) of saline solution, lysergide (5 × 10⁻⁷ M), ephedrine (1 × 10⁻⁴ M), and nimergoline (5 × 10⁻⁵ M).*

M) significantly increases this last parameter, and both ephedrine (1×10^{-4} *M*) and nimergoline (5×10^{-5} *M*) increase oxygen utilization and decrease cerebral blood pressure.

The increase in oxygen consumption by ephedrine and nimergoline can probably be related to the activation of specific biotransformations, such as the tested demethylation and acetylation of aminopyrine and the glucuronate conjugation of oxazepam. The increase in oxygen utilization by lysergide may be dependent, at least in part, on activation of energetic and kinetic reactions involved in processes inducing a pyretogenic effect. In fact, according to Horita and Dille (7), small doses of lysergide produce a hyperpyretic response in the trained rabbit; larger doses produce a fatal hyperpyrexia.

The activation of the tested biotransformation by ephedrine and, particularly, by nimergoline is accomplished by the decrease in cerebral vascular resistance, which can be dependent on: (*a*) direct action of the drugs on the blood circulation (8), and (*b*) indirect activation of the ganglion cell metabolism with detumescence of the glial process, because of the importance of astrocite pressure on the capillaries for vascular resistance (9–12).

Depression of Cerebral Activity by Hypoxia—The repeated suppression of artificial ventilation in curarized mongrel dogs induces a depression of the spontaneous electrical activity of the cortex, with only a partial natural reversion after 30 min. of recovery of artificial respiratory conditions; the "before" column of Fig. 5 shows the residual depression of the electroencephalogram pattern at this time. The subsequent infusion (0.5 ml./min. for 30 min.) with saline solution or lysergide (5×10^{-7} *M*), directly into the circle of Willis, does not induce any effects on electroencephalographic activity; ephedrine (1×10^{-4} *M*) and nimergoline (5×10^{-5} *M*) significantly improve the partial spontaneous reversion of the electroencephalogram depression, as summarized in the "after" column of Fig. 5.

As indicated in Fig. 6, during the induction of the severe hypoxia it is possible to observe that the depression of the spontaneous electrical activity is accomplished by an increase of: (*a*) some enzymes in the cerebrospinal fluid, at first glutamic oxalacetic transaminase and lactate dehydrogenase and subsequently alkaline phosphatase, and (*b*) the cerebral blood flow during the first phases, with a subsequent decrease at the terminal tested phase of hypoxia itself.

The increase of cerebrospinal fluid enzymes during experimental cerebral injury in laboratory animals and in neurological diseases in man has been demonstrated (13–30). In experimental conditions of this study, this increase can be explained at least in five different ways: (*a*) damage to the cerebral tissue with liberation of intracellular enzymes which, in the CNS, primarily pass into the cerebrospinal fluid space, as occurs in some neurological conditions (31); (*b*) vascular disturbance, with an increase of enzyme levels in damaged vascular endothelium and of their transmitting function across the barrier (32); (*c*) an increase of the permeability of the blood–cerebrospinal fluid barrier so that the enzymes, normally confined in serum in higher levels than in cerebrospinal fluid (33–35), pass into this fluid from the blood (36, 37); (*d*) a change in cerebral metabolic pathways with a decrease of metabolic activity of the ganglion cells accomplished both by an inverse proportional rise in enzyme concentration of astroglia and by accumulation of polysaccharides, mainly in the glial processes of astrocytes (38–41); and (*e*) a change in sodium and potassium brain homeostasis, dependent on oxidative metabolism and related to the electric activity (11, 12, 42, 43).

The change in cerebral blood flow can also be related to these five conditions, in particular to the metabolic change, with a reduction of cerebral metabolic rate for oxygen. The increase in cerebral blood flow is related to the increased cerebral production of CO_2 and acid metabolites, such as lactate (44). The subsequent moderate reduction of the cerebral blood flow is probably dependent on the initial condition of a general brain edema or on the local tumescence of particular cerebral cells (*e.g.*, astrocytes) related to the remarkable fluctuation in the degree of the activity of some enzymes (38, 45, 48).

The perfusion into the circle of Willis with ephedrine (1×10^{-4} *M*) and, particularly, with nimergoline (5×10^{-5} *M*) induces a partial

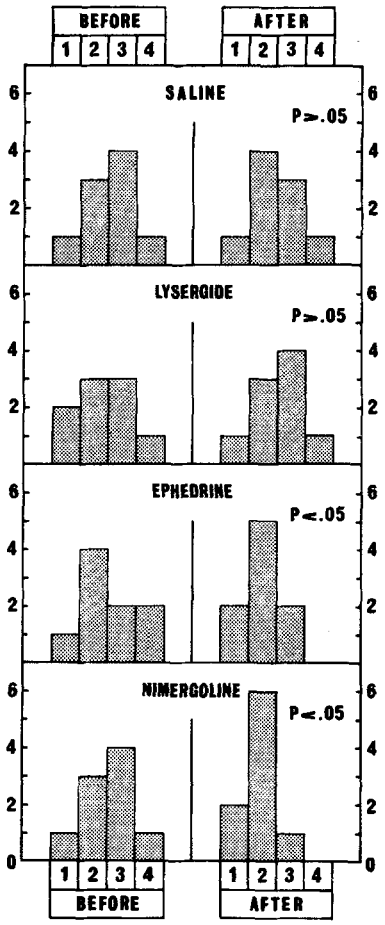

Figure 5—*Degree of the depressed spontaneous electrical activity of the cerebral cortex in the curarized mongrel dog obtained by severe hypoxia, before and after the direct perfusion into the circle of Willis (0.5 ml./min. for 30 min.) with saline solution, lysergide (5 × 10⁻⁷ M), ephedrine (1 × 10⁻⁴ M), and nimergoline (5 × 10⁻⁵ M). On the abscissae is the electroencephalogram pattern scale (related to control values = 100, corresponding to the fourth of Faulconer's levels): 1 = reduction <15%; 2 = reduction from 15 to 30%; 3 = reduction from 30 to 45%; and 4 = reduction from 45 to 60%. On the ordinate is the number of dogs. The figure indicates also the probability level (n = 9) of statistical difference between the distributions before and after the perfusion with drug. Note that the perfusion started 30 min. after the recovery of artificial respiratory ventilation.*

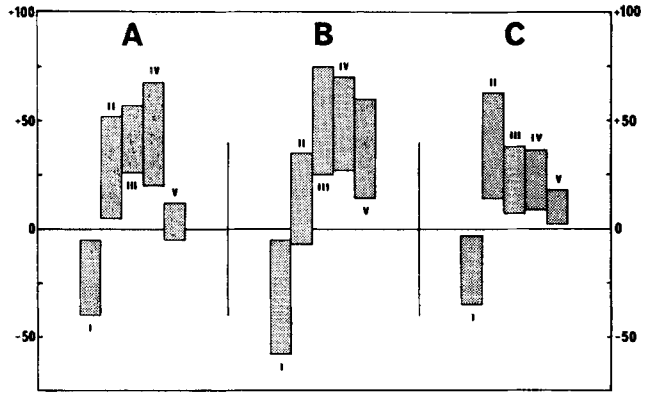

Figure 6—*Range of percent variations of electroencephalogram pattern (I), cerebral blood flow (II), and cerebrospinal fluid glutamic oxalacetic transaminase (III), lactate dehydrogenase (IV), and alkaline phosphatase (V) activity, from the control conditions before the induction of the severe hypoxia in mongrel dogs* (n = 9). *A = 40 ± 4 min. after hypoxia induction; B = at the end of the hypoxia condition; C = at the end of the perfusion (0.5 ml./min. for 30 min.), directly into the circle of Willis with nimergoline* (5×10^{-5} M). *Note that the perfusion started 30 min. after the recovery of artificial respiratory ventilation.*

recovery of the altered parameters (Fig. 6) by means of an activation of the cerebral metabolic activity and/or lowering the vascular resistance related to the change in cerebral blood flow (49).

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