as blood urea nitrogen concentrations in the hydrocortisone treated group rose in a linear fashion, those in the testosterone treated group showed a marked tendency to flatten out in the last day or 2 of the animal's life.

Summary. Hydrocortisone, given to anuric dogs, prolonged survival time with lower rates of accumulation for urea, creatinine and phosphorus in the serum. Testosterone in a second group of animals failed to influence survival time or urea accumulation.

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Elevation of γ-Aminobutyric Acid in Rat Brain with Hydroxylamine.* (25105)

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γ-Aminobutyric acid (γABA)† has been found in all areas of mammalian central nervous system which have been subjected to analysis. A number of hypotheses have been advanced to account for a variety of physiological effects attributed to γABA(1-11), but none has received unequivocal experimental proof. The levels of γABA in brains of adult animals are relatively constant and show little variation from one animal to another in inbred strains of mice(12). Cerebral levels of γABA have been lowered in rats and mice by administration of Vit. B_6 antimetabolites and carbonyl trapping agents(10,13-15), but a sustained elevation of γABA in brains of normal animals has not been produced. Injection of massive doses of γABA into mice, cats, and rats produced no significant changes in brain levels of γABA(12,16) or electroencephalographic recordings(4). More than ½ of any trace dose of isotopically labeled γABA injected intracerebrally into mice disappeared within 2 minutes from the brain(11) and rapid labeling of other amino acids and tricarboxylic acid cycle intermediates occurred indicating that there is a rapid rate of metabolism of exogenously supplied γABA. Selection of NH_2OH for the study reported here was based upon observations which indicated that *in vitro* NH_2OH was a more effective inhibitor of the γABA-α-ketoglutaric acid transaminase than of glutamic acid decarboxylase (17-19) and might, therefore, cause an accumulation of γABA.

Methods. All experiments were performed with male rats of either Sprague-Dawley or Wistar strain. Treated animals were injected intraperitoneally with a solution containing 50 mg/ml NH_2OH (pH 7.0). The toxic effects observed after administration of NH_2OH are believed to result from methemoglobin formation(20) and involvement of central nervous system(21). To minimize the hematological effect some rats received an intraperitoneal injection containing 10 mg of MB in 0.5 ml(22). The dye was administered 10 to 20 minutes prior to treatment with NH_2OH. Control animals received no treatment. Intraperitoneal injections of physiological saline previously had no effect on levels of γABA in the brains of rats(10). One to 1½ hours after injection of NH_2OH the rats were killed by decapitation. Brain areas were removed

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† The following abbreviations have been used: γ-aminobutyric acid - γABA, hydroxylamine - NH_2OH, methylene blue - MB, triphosphorpyridine nucleotide - TPN.

immediately, weighed and dropped into separate vials containing 4 to 6 ml of ice cold 80% ethanol. Samples were homogenized in Tembroeck homogenizer and transferred quantitatively to 12 ml centrifuge tubes. Two to 4 ml of 75% ethanol were used to rinse homogenizer and pestle. Rinsings were added to homogenate. The homogenate was spun at 2000 g for 15 minutes and supernatant decanted. The precipitate was resuspended twice in 3 ml aliquots of 75% ethanol and spun. Extracts of each sample were pooled in 20 ml beaker and evaporated to dryness. One ml of distilled water was added to dried extract for every 100 mg of original fresh wt of tissue. The extract was resuspended and the suspension was spun in Spinco ultracentrifuge at 20.000 g for 30 minutes and aliquots of supernatant (0.2 to 0.25 ml) pipetted into numbered optical tubes. The supernatant was evaporated to dryness and stored in vacuum desiccator. The extract in optical tubes was resuspended in 0.1 ml of water and 0.3 ml of pyrophosphate buffer and analyzed by the enzymatic method described below. Quantitative recoveries of γABA added to original homogenate were achieved routinely. γABA was measured enzymatically by a new method. The bacterium *Pseudomonas fluorescens* EC, when grown on pyrrolidine, contains an enzyme system which converts γABA to succinate *via* transamination and oxidation coupled to TPN reduction(25). A quantitative assay for γABA has been developed by use of this enzyme system(24). The method was adapted to measurement of γABA in extracts of rat brain. Fraction 3, the first dialyzed acetone fraction of the enzyme system (25), was used for all determinations of γABA. The assay system consisted of the following: 0.1 ml of tissue extract equivalent to 20 or 25 mg of fresh brain tissue; 0.3 ml of 0.1 M pyrophosphate buffer, pH 8.3; 0.1 ml mercaptoethanol, 2 mg/ml in pyrophosphate buffer; 0.1 ml TPN, 8 mg/ml; 0.1 ml enzyme; 0.1 ml α-ketoglutaric acid neutralized with sodium hydroxide, 14.6 mg/ml. Final volume of assay system was 0.8 ml. The mercaptoethanol, TPN, enzyme and α-ketoglutarate were premixed and stored on ice. The reaction was started by addition of 0.4 ml of the mixture to 0.4 ml of buffer and sample contained in optical tube at room temperature. Optical density at 340 mμ was read immediately after stirring the reaction mixture. All spectrophotometric readings were made with Beckman Spectrophotometer model DU, equipped with tube adapter(26). The reaction proceeded at room temperature. Maximal readings at 340 mμ were reached 15 to 20 minutes after starting the reaction.‡ Maximal change in OD_{340} from zero time was proportional to amount of γABA in a sample. One μg of γABA in the system corresponded to ΔOD_{340} of 0.055. This represents close to an isomolar relationship between γABA in a sample and formation of TPNH. Samples containing no γABA showed no changes in OD_{340}.

Results. Male Sprague-Dawley rats, pretreated with MB and receiving NH_2OH at 100 mg/kg body wt., convulsed within 5 minutes after intraperitoneal injection of the drug. Convulsions, which in no instance lasted longer than 10 minutes, were accompanied by visible blanching of extremities. Fifteen minutes after injection, breathing still was rapid and labored, but returned to normal within 30 to 60 minutes. At time of decapitation all treated animals appeared slightly sedated but otherwise normal. Blood showed some discoloration because of presence of MB and methemoglobin. There were marked elevations of γABA in all 8 brain areas of treated rats by comparison with controls (Table I). The largest relative increase in content of γABA was noted in the cortex. Chromatographic verification of elevation of γABA in the cortex of treated animals is shown in Fig. 1. Increase in freely extractable γABA was by far the most notable alteration produced in the content of detectable ninhydrin-reactive constituents.

In view of possible effect of MB on γABA levels, an experiment was performed with 4 weight-matched groups of 3 male Wistar rats each. Rats in group 1 received no treatment; group 2 received an injection of MB only;

‡ Enzyme concentration was so adjusted that 0.1 ml of enzyme in incubation mixture of 0.8 ml produced maximal reading at OD_{340}, 15 to 20 minutes after initiating the reaction.

TABLE I. Effect of Hydroxylamine upon Brain Levels of γABA in Rats Pretreated with Methylene Blue.

Area	Control γABA (mg %)		Treated γABA (mg %)		Increase (%)
Olfactory lobe	40*	37–43†	61	52– 69	53
Diencephalon	56	51–61	79	64– 98	41
Superior colliculi	57	56–58	91	81–109	60
Inferior "	45	40–50	80	68–103	78
Cortex	26	24–27	48	43– 59	85
Cerebellum	32	31–34	44	41– 52	38
Pons	25	23–28	36	32– 42	44
Medulla	26	22–29	33	29– 39	27

* Avg † Range

Experiment was performed with Sprague Dawley rats 200 to 250 g in wt. All treated animals received one intraper. inj. of methylene blue (10 mg in 0.5 ml) and 20 min. later an intraper. inj. of NH_2OH (25 mg in 0.5 ml pH 7). Animals were decapitated between 60 and 75 min. after inj. of NH_2OH.

group 3 received relatively low dose of NH_2OH (10 mg); and group 4 received both MB and higher dose of NH_2OH (15 mg). The results are shown in Table II. MB alone tended to decrease γABA levels. Despite lower dosage of NH_2OH employed, the brains of rats in group 3 (Table II) showed marked elevation of γABA levels. The blood of these animals showed no visible discoloration.

Changes of cerebral γABA with time in cortex of rats treated with NH_2OH are shown in Fig. 2. The results suggest that at the time of convulsions (5 minutes after injection) γABA levels in brains of treated rats were not elevated. Raised levels of γABA in the cortex occurred concomitantly with lethargic behavior of these animals 1 to 2 hours after injection. The following report will discuss electrophysiological findings(27).

Discussion. It has been difficult to show that a causal relationship exists between decrease in cerebral levels of γABA, antivit. B_6 activity, and seizures. Convulsions and seizures induced by administration of Vit. B_6 antimetabolites and by convulsant hydrazides are accompanied by lowering of γABA levels (13,10,15). Furthermore, it has been reported that such convulsions can be prevented or stopped by administration of Vit. B_6 itself, by pretreatment with parenterally-adminis-

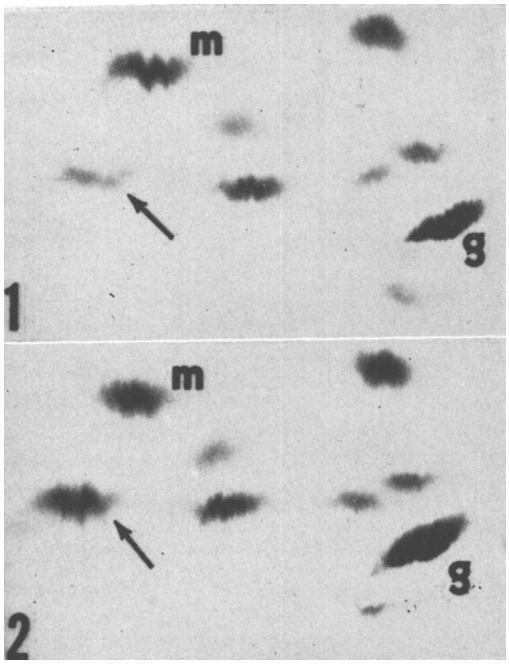

FIG. 1. Two-dimensional chromatograms of tissue extracts corresponding to 25 mg (wet wt) of cortex in untreated rats (1) and those treated with NH_2OH (2). Descending 2-dimensional chromatography was performed in water-saturated phenol and water-saturated lutidine. Amino acids were visualized by spraying with solution of 0.1% ninhydrin in butanol. The following symbols have been used to identify amino acids on chromatograms. Arrow points to γABA, g = glutamic acid, m = marker of α-aminobutyric acid.

TABLE II. Separate Effects of Methylene Blue and Hydroxylamine upon Brain Levels of γABA in Rats.

Group	Treatment		γABA levels in different areas of brain (mg %) Diencephalon	Colliculi	Olfactory lobes	Cortex	Cerebellum
1	Control		58	57	42	27	28
2	Methylene blue	10 mg	38	45	35	22	22
3	Hydroxylamine	10 "	71	71	55	47	40
4	Methylene blue + hydroxylamine	10 " 15 "	82	89	62	51	39

Each group consisted of 3 Wistar male rats 160 to 190 g in wt. Animals were decapitated 70 to 90 min. after last inj. and brain areas removed and extracted as described in text. Hydroxylamine inj. corresponded to dose of 50 to 65 mg/kg in Group 3, and 75 to 93 mg/kg in Group 4.

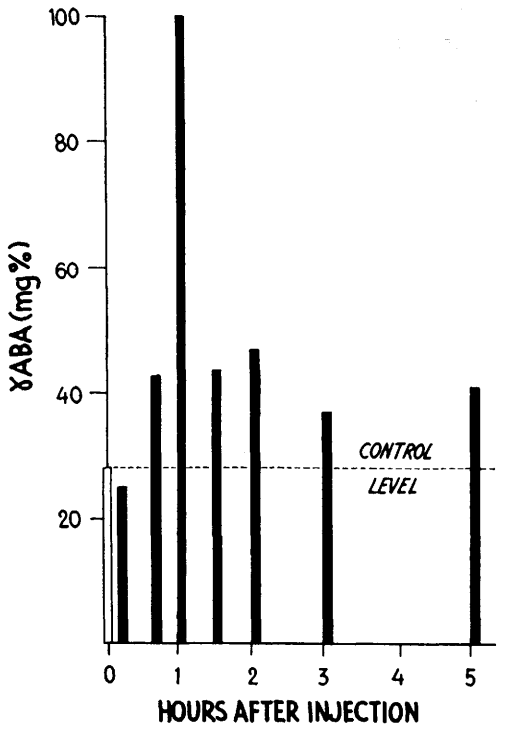

FIG. 2. Changes with time in levels of γABA after inj. of hydroxylamine. Each bar represents level of γABA in cortex of one Wistar male rat inj. with hydroxylamine and decapitated at different times after inj. The inj. dose corresponded to 10 mg/150 g body wt. Animals ranged in wt from 150 to 200 g.

tered γABA(14,28,29), or by topical application of γABA to specific areas of brain(30). Probably no appreciable amount of parenterally-administered γABA enters brain tissue unless the blood-brain barrier is destroyed(4) and the effects produced by topical application of γABA to exposed cortex are difficult to interpret(31).

The case for a causal relationship between increases or decreases in seizure susceptibility in specific brain areas with corresponding increases or decreases in γABA level could be strengthened if endogenously raised γABA levels would result in electrophysiologically observable effects which were opposite to those found with convulsant hydrazides or B_6 antimetabolites. A large number of drugs we tested failed to elevate γABA levels in whole brains of mice, rats, and rabbits(12). Experiments with NH_2OH are the first to show that increase in endogenous levels of γABA can be produced in normal animals by chemical means. If elevation of endogenous levels of γABA should decrease seizure susceptibility, then NH_2OH or one of its derivatives may find practical use in control of clinical seizures.

Although NH_2OH was tested because of its potent inhibitory action on γABA-α-ketoglutarate transaminase, it is by no means certain that this is the mechanism by which elevation is achieved *in vivo*. Besides combining with pyridoxal and keto acids, NH_2OH may also exert its effect through interaction with the DPN(32) of succinic semialdehyde dehydrogenase(33). Indirect effects of NH_2OH through action of its postulated metabolic products such as nitrite(34), nitrogen or ammonia(35), or through cerebral anoxia resulting from methemoglobinemia seem less probable, but cannot be completely excluded as contributing toward changes in levels of cerebral γABA. While evaluation of these possibilities is underway, the use of NH_2OH will provide a convenient tool for investigating the physiological effects which accompany elevated γABA levels in the central nervous system.

Summary. 1. Intraperitoneal injection of hydroxylamine resulted in elevation of levels of γ-aminobutyric acid in 8 brain areas of the rat. 2. These elevated levels of γ-aminobutyric acid were attained even after rats were pretreated with methylene blue to reduce methemoglobin formation. 3. The effect of a single injection of hydroxylamine persisted for at least 5 hours. 4. The potential value of this procedure for physiological study has been discussed.

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Anticonvulsant Properties of Hydroxylamine and Elevation of Cerebral γ-Aminobutyric Acid in Cats.* (25106)

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In the preceding paper(1) it was shown that elevation of brain levels of γ-aminobutyric acid (γABA) could be achieved in the rat by injection of hydroxylamine (NH_2OH). Since a correlation exists between decreases in brain γABA and increased seizure susceptibility(2,3 also see 1 for other pertinent references), it was of interest to determine whether a decrease in induced seizure activity would be found when brain levels of this substance are elevated. The present report is concerned with the effect of NH_2OH on electrically-induced seizure afterdischarges in acute experiments in cats.

Methods. The cats used were immobilized with gallamine triethiodide [Flaxedil®] and anesthetized locally with procaine at painful points. Preliminary surgical procedures, including a wide bilateral craniectomy, were carried out under ether anesthesia. The ether was blown off during 1½ hours of artificial ventilation. Two samples of cortex, one on

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