14. Armstrong, M. D., Chao, F. C., Parker, V. J., Wall, P. E., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 675.

15. Jacobson, E., Chodos, R., Falcon, W., *Clin. Res.*, 1959, v7, 33.

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Demonstration of Thiosemicarbazide-Induced Convulsions in Rats with Elevated Brain Levels of γ-Aminobutyric Acid.* (25861)

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The brain and spinal cord of vertebrate organisms contain high levels of GABA† (1). Physiological experiments have shown GABA to have inhibitory properties in invertebrate and vertebrate nervous systems (2,3). When TSC was injected into rats, it lowered cerebral levels of GABA probably by preferential inhibition of glutamic acid decarboxylase and increased susceptibility of these animals to convulsions (4). When NH_2OH was injected into rats, cats or monkeys, it elevated cerebral levels of GABA in all 3 species (5) presumably through inhibition of γ-amino-butyric acid-α-ketoglutaric acid transaminase (6). At the same time it was noted that rats were protected against Metrazol-induced convulsions after NH_2OH administration and that in the cat and monkey sensitivity of the cortex to electrical stimulation was decreased (7,8). These and similar observations suggested that certain types of convulsions and resistance to them may be related to levels of GABA in the brain of mammals (9,10). The experiments presented here demonstrate that the physiological effects of TSC and NH_2OH cannot be attributed solely to their effect upon the mechanisms which regulate synthesis and degradation of GABA and that a greatly elevated level of GABA can exist in most brain areas of rats undergoing thiosemicarbazide-induced convulsions.

Method. Twenty-eight male Sprague-Dawley rats weighing 190 to 210 g each, were selected for this experiment and divided into 6 groups. Rats in 5 groups were injected intraperitoneally either with $NH_2OH \cdot HCl$ (75 mg/kg neutralized) or with TSC (20 mg/kg) or with both at varying time intervals as indicated in Table I. Animals injected with $NH_2OH \cdot HCl$ only, were decapitated at 90 to 120 minutes after injection. *All* animals receiving TSC had convulsions within 90 to 135 minutes after injection. Pretreatment with NH_2OH tended to shorten the time period between injection of TSC and onset of convulsions. All TSC-treated animals were sacrificed after showing one maximal tonic extensor convulsion. Nine areas were removed from each brain and analyzed for content of GABA by an enzymatic procedure described previously (5).

Results. TSC alone lowered levels of GABA while NH_2OH alone elevated them in all areas (Table I), in agreement with previous observations (11,5).

In the 3 groups of animals receiving both TSC and NH_2OH, levels of GABA were significantly elevated in most of the areas examined. Several areas showed normal levels, but none were depressed at time of maximal convulsion. In comparing the results of the 3 groups of rats receiving both TSC and NH_2OH, it was found that level of GABA was elevated to a greater extent in the group receiving NH_2OH prior to TSC. This effect was especially noticeable in the colliculi, diencephalon and midbrain tegmentum, areas of brain with the highest levels of glutamic acid decarboxylase (Baxter and Roberts, unpublished).

Discussion. The above results do not ex-

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† Abbreviations: GABA: γ-aminobutyric acid; TSC: thiosemicarbazide; NH_2OH: hydroxylamine.

TABLE I. Influence of NH_2OH and TSC, Alone and in Combination, on GABA Levels in Rat Brain.

Brain areas	GABA levels (mg/100 g of tissue, wet wt) Control* (5)†	NH_2OH only* (3)	TSC only (4)	TSC ½ hr after NH_2OH (4)	TSC + NH_2OH together (5)	TSC ½ hr before NH_2OH (5)
Cortex	25‡ (24-27)	49 (45-54)	18 (16-20)	48 (42-57)	41 (36-45)	37 (28-47)
Medulla	25 (23-26)	33 (28-38)	17 (16-18)	34 (28-44)	29 (27-32)	26 (22-29)
Pons	27 (25-28)	42 (38-46)	18 (17-19)	42 (34-53)		31 (27-37)
Cerebellum	27 (25-30)	37 (35-38)	20 (19-23)	44 (38-51)	37 (30-49)	38 (29-43)
Hippocampus	31 (28-35)	41 (38-44)	20 (18-22)	42 (39-44)	39 (36-43)	39 (27-50)
Caudate nucleus	31 (29-33)	46 (42-52)	22 (20-24)	45 (31-54)	41 (33-55)	43 (29-51)
Olfactory lobes	48 (38-55)	61 (44-78)	43 (37-51)	65 (57-78)	62 (52-75)	61 (50-77)
Diencephalon	53 (46-60)	71 (62-75)	32 (27-36)	66 (54-88)	57 (52-63)	56 (42-71)
Colliculi	56 (52-60)	85 (77-95)	33 (31-35)	73 (60-94)	58 (54-65)	53 (38-66)

Animals receiving NH_2OH only, were sacrificed 90 to 120 min. after inj. All animals receiving TSC, alone or in combination with NH_2OH, were sacrificed at time of first maximal tonic convulsion.

* For more extensive series showing similar results see Baxter and Roberts(5).

† Figures in parentheses indicate No. of rats.

‡ Avg for group; figures in parentheses indicate range.

clude the possibility that a decrease occurred in level of GABA in specific small areas of brain. However, they indicate clearly that an overall depression of GABA levels is not a necessary requirement for TSC-induced convulsions. This conclusion is in keeping with recent physiological findings by Adey *et al.* (12) and by Terzuolo *et al.*(13).

Although in the past attention has been focused upon the function of TSC and NH_2OH as carbonyl trapping agents which affect Vit. B_6 dependent enzyme systems, these agents may well have additional effects upon various cellular constituents including enzymes which do not require pyridoxal phosphate as cofactor. TSC may exert an effect upon neuronal membranes or prevent formation of some physiologically active derivative of GABA. These and other possibilities are under investigation.

Summary. In confirmation of biochemical data for whole brain, injection of thiosemicarbazide produced convulsive seizures in rats concomitantly with decreases in levels of γ-aminobutyric acid in 9 areas of the brain. However, rats injected with both hydroxylamine and thiosemicarbazide had convulsive seizures when levels of γ-aminobutyric acid were either elevated or normal in different areas of brain. Seizures produced by administration of thiosemicarbazide are not related to a generally decreased level of γ-aminobutyric acid in the brain.

1. Roberts, E., Lowe, I. P., Guth, L., Jelinek, B., *J. Exp. Zool.,* 1958, v138, 313.

2. Kuffler, S. N., Edwards, C., *J. Neurophysiol.,* 1958, v21, 589.

3. Dasgupta, S. R., Killam, E. K., Killam, K. F., *J. Pharmacol. Exp. Ther.,* 1958, v122, 16A.

4. Killam, K. F., Bain, J. A., *ibid.,* 1957, v119, 255.

5. Baxter, C. F., Roberts, E., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 811.

6. ———, *Fed. Proc.,* 1960, v19, 1.

7. Eidelberg, E., Baxter, C. F., Roberts, E., Saldias, C. A., French, J. D., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 815.

8. Roberts, E., Baxter, C. F., Eidelberg, E., *Function and Structure of the Cerebral Cortex,* Elsevier Publishing Co., Amsterdam, 1960, in press.

9. Killam, K. F., Dasgupta, S. R., Killam, E. K., *Inhibition in the Nervous System and γ-Aminobutyric Acid,* Pergamon Press, London, 1960, p302.

10. Elliott, K. A. C., Jasper, H. H., *Physiol. Rev.,* 1959, v39, 383.

11. Killam, K. F., *J. Pharmacol. Exp. Ther.,* 1957, v119, 263.

12. Adey, W. R., Dunlop, C. W., Killam, K. F., Brazier, M. A., *Inhibition in the Nervous System and γ-Aminobutyric Acid,* Pergamon Press, London, 1960, p317.

13. Terzuolo, C. A., Sigg, B., Killam, K. F., *ibid.,* p336.

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