

Canine Epilepsy Caused by Flour Bleached with Nitrogen Trichloride (Agene). II. Role of Amino Acids.*

M. L. SILVER, E. P. MONAHAN, AND J. R. KLEIN.

From the U. S. Army Medical Nutrition Laboratory, and the Departments of Psychiatry and Biological Chemistry, University of Illinois College of Medicine, Illinois Neuropsychiatric Institute, Chicago, Ill.

Epilepsy in dogs fed bleached flour is attributable to the toxic effect of wheat proteins modified by nitrogen trichloride.^{1,2} The heat stability of the toxic material, its presence in agenized gliadin and casein, and the rapid onset of symptoms² suggest that the toxicity resides in a relatively small molecule, possibly an altered amino acid. Thus it seemed advisable to test amino acids treated with nitrogen trichloride for convulsant properties.

The results of such tests in control and epileptic dogs are reported in the present work, changes in electroencephalographic pattern being used as a measure of convulsant activity. The electroencephalograms of the epileptic dogs prior to administration of amino acids also provide further insight into the syndrome.

Method. A mixture of synthetic amino acids was prepared to resemble the composition of gliadin. One-half of this amino acid mixture was kept as a control, and the other half was "bleached," *i.e.*, treated with agene (NCl_3) in the same manner as the flour.² The mixed amino acids were exposed for 10 minutes to 1% NCl_3 in air saturated with water vapor. No analysis for lost excess gas was performed. 3.3% solutions of the amino

acid mixtures were prepared by adjusting aqueous suspension to pH 10 with 10N NaOH, and then adjusting the pH to 8.5 with concentrated HCl. They were then used for intravenous injection.

Dogs were prepared under vinyl ether for artificial respiration, and for intravenous infusion *via* the femoral vein. The electrical activity of the brain and the electrocardiogram were recorded by means of Grass amplifiers and inkwriters. Paralysis was initiated by the intravenous injection of 25 mg dihydro- β -erythroidine hydrobromide and maintained by infusion of the erythroidine (1 mg per ml in 0.156 M sodium chloride). Under artificial respiration, the vinyl ether was rapidly eliminated and within 15 minutes the electroencephalogram resembled that of the intact animal. The dog was now available for testing the convulsant properties of the amino acid mixtures.

Results. Eight dogs on the experimental diet containing agenized flour (*i.e.*, bleached flour) for periods up to 2 weeks were used. These showed abnormal electroencephalograms but no seizures during a pre-injection period of 1 to 2 hours. The pattern illustrated in Fig. 1b is characteristic of all dogs on the experimental diet, and resembles that reported by others.³

Seizures followed the intravenous administration of the agene-treated amino acid mixture in less than 5 minutes (Fig. 1d, e, f). The absolute amount of amino acids varied

* The bleaching equipment was supplied through the kindness of the Wallace and Tiernan Company, Chicago. The synthetic amino acids and β -erythroidine were supplied through the kindness of Merck and Co., courtesy of Dr. J. M. Carlisle.

¹ Mellanby, E., *Brit. Med. J.*, 1946, Dec. 14, No. 4484, 885.

² Silver, M. L., Zevin, S. S., and Johnson, R. E., *Proc. Soc. Exp. Biol. and Med.*, 1947, **66**, 408.

³ Newell, G. W., Erickson, T. C., Gilson, W. E., and Elvehjem, C. A., *Proc. Soc. Exp. Biol. and Med.*, 1947, **65**, 115.

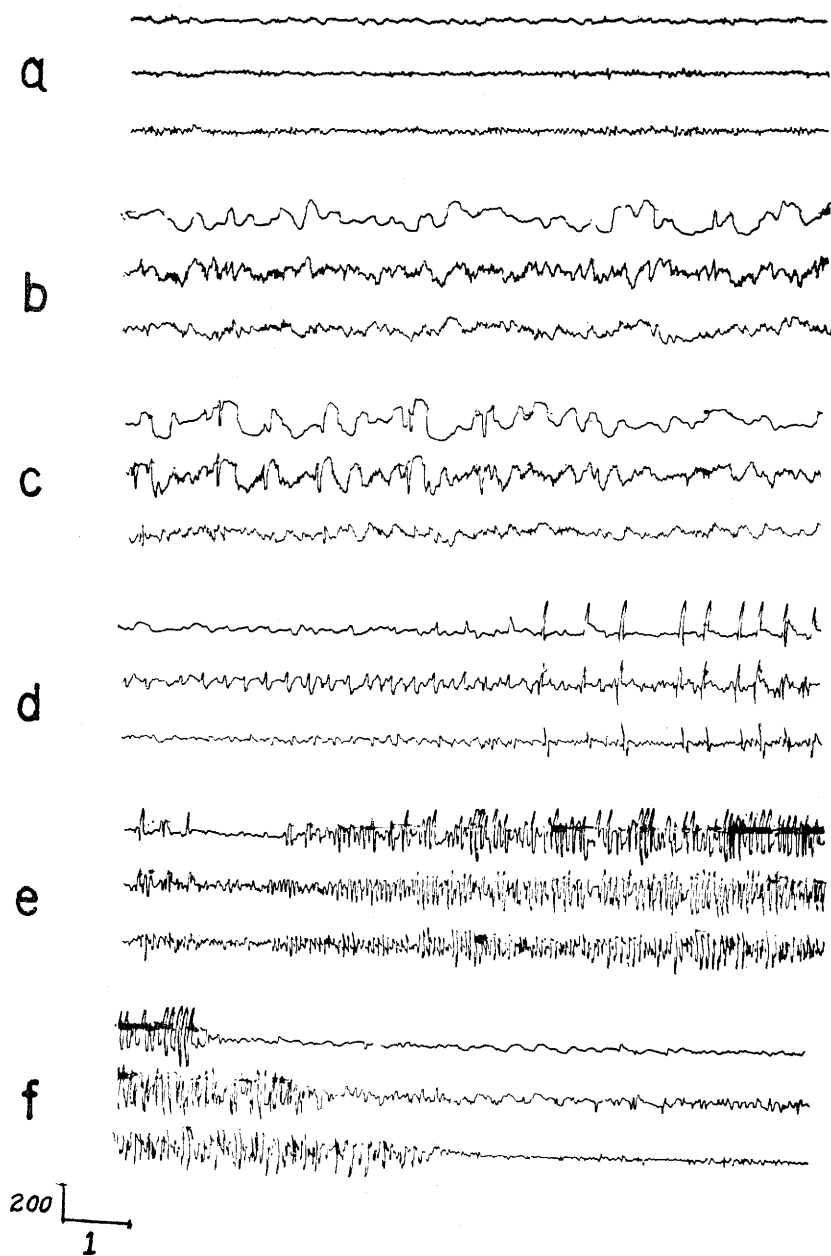


FIG. 1.

a. Normal electroencephalogram of dog on control (unbleached) diet. The upper lead is left parietal to ground; the middle lead is right parietal to ground; the lower lead is right parietal to left parietal (bipolar); this is constant in all tracings.

b, c. Abnormal E.E.G. of dog fed experimental (bleached) diet for three days. Note resemblance to petit mal activity.

d, e, f. In same dog as above, continuous record of a grand mal seizure occurring less than 5 minutes after the intravenous injection of "bleached" amino acid mixture. In small figure at lower left, voltage calibration is 200 μ V, time calibration is 1 second. Each frame is ten seconds.

from 0.2 to 1.5 g. The injection of control untreated amino acids was ineffective in 4 dogs even after the injection of 20 g in 120 minutes.

In 12 dogs which were on the control diet containing untreated flour (*i.e.*, unbleached flour), the intravenous administration of the agene-treated amino acid mixture was followed by seizure patterns in the electroencephalogram of 4 dogs, marked abnormalities in 3 dogs, and no significant change in 5 dogs. In the animals with abnormal E.E.G.'s produced by intravenously administered agenzized amino acids, the intracisternal injection of 1 to 2 cc of the NCl_3 -treated amino acid mixture was followed by seizures within 5 minutes after injection.

Experiments have been conducted with individual amino acids treated in their crystalline states and the results have been negative

for arginine, glutamic acid, glycine, histidine, lysine, methionine, tryptophane and tyrosine; equivocal for proline, serine, and aspartic acid; and positive for cysteine hydrochloride. A possibility that should not be overlooked is that the toxic compound in foodstuffs may be an altered polypeptide.

Summary. When an amino acid mixture approximating the composition of gliadin is treated with nitrogen trichloride gas (NCl_3) this mixture is so altered as to make it convulsant for dogs when given intravenously. The convulsant activity is most readily demonstrated in dogs which have been on a bleached (agenized) flour diet for 5 to 7 days, presumably because these animals have accumulated sufficient quantity of the toxic material *via* the alimentary tract, to make them highly susceptible to an additional increment received *via* the blood stream.

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Adrenal Cortical Activity in Urine of Horses.

EDWIN A. RISLEY, ANN B. SCHULTZ, WILLIAM B. RAYMOND, AND RICHARD H. BARNES.

From the Department of Biochemistry, Medical Research Division, Sharp and Dohme, Inc., Glenolden, Pa.

The presence of a substance in urine that protects adrenalectomized rats from the stress of cold was first noted by Anderson, Haymaker and Joseph¹ and Weil and Browne.² More extensive investigations have shown that extracts of urine from normal human subjects when tested in adrenalectomized rats

cause an elevation of liver glycogen,^{3,4} increased life maintenance,^{3,5} prolongation of muscle work performance,⁶ and prevention of water intoxication.⁷ Although the above studies have employed extracts of human urine, Selye and Dosne⁸ have reported that the urine of large domestic animals contains cortin-like activity. The purpose of this in-

¹ Anderson, E., Haymaker, W., and Joseph, M., *Endocrinology*, 1938, **23**, 398.

² Weil, P., and Browne, J. S. L., *Science*, 1939, **90**, 445.

³ Venning, E. H., Hoffman, M. M., and Browne, J. S. L., *J. Biol. Chem.*, 1943, **148**, 455.

⁴ Horwitt, B. N., and Dorfman, R. I., *Science*, 1943, **97**, 337.

⁵ Dorfman, R. I., and Horwitt, B. N., *Fed. Proc.*, 1943, **2**, 60.

⁶ Shipley, R. A., Dorfman, R. I., and Horwitt, B. N., *Am. J. Physiol.*, 1943, **139**, 742.

⁷ Schiller, S., and Dorfman, R. I., *Endocrinology*, 1943, **33**, 402.

⁸ Selye, H., and Dosne, C., *Lancet*, 1940, **239**, 70.