

Canine Epilepsy Caused by Flour Bleached with Nitrogen Trichloride (Agene). I. Experimental Method.*

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(Introduced by A. Earl Walker.)

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Many reports on canine hysteria have stressed the remarkable resemblance of this syndrome to human idiopathic epilepsy. The nervous disorder in dogs is characterized clinically by irregular "grand mal" seizures, "running fits," ataxia, and "hysterical" states, with apparent recovery between attacks. The electro-encephalograms of these dogs show a cerebral dysrhythmia similar to that of human epilepsy. Because of these common features, it seems proper to term the condition *canine epilepsy*.

A dietary etiology of this disease has been proposed by various investigators, *e.g.*, gliadin toxicity,¹ lysine deficiency from wheat flour diet² and wheat gluten flour toxicity.^{3,4} This etiology is supported and explained by the crucial experiment of Mellanby.⁵ He showed that the treatment of flour with agene gas, *i.e.*, nitrogen trichloride (NCl_3), a process used on approximately 90% of the white wheat flour milled in both England and the

United States, rendered that flour convulsant to dogs. The toxicity of the wheat fractions used in the investigations cited above was presumably due to agene treatment (NCl_3 treatment)[†] of the flour source, inasmuch as untreated wheat fractions showed no toxicity. The purposes of this communication are to describe a method of producing consistently and rapidly a convulsive disorder in dogs, and to delimit the toxic principle.

Method. A diet having a high flour component was prepared. The control ration, including supplementation with accessory food substances, and the actual analysis of this diet, is given below.

The diet, with water added, was baked into biscuits at 395°F for 30 minutes. The biscuits were canned and pasteurized after sealing. The analysis shown in Table I was made of these biscuits 2 weeks after baking.

The experimental ration was identical except that the flour component was heavily treated with agene. This was done in a bleaching unit in which the gas is generated by the interaction between chlorine gas and 1% ammonium chloride solution at a pH of 3.0. The NCl_3 gas thus formed was blown over the flour by means of an air stream, and was applied as a 1% solution of NCl_3 in air saturated with water vapor. Agene gas was used as the bleaching agent for all experimental flour samples, in a concentration of 30 g per 100 lb of flour, this value being determined by controlling the amount of chlorine gas available for reaction. No attempt was made to determine how much of the NCl_3 gas evolved had reacted with the flour.

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¹ Melnick, D., and Cowgill, G. R., *J. Nutrition*, 1937, **14**, 401.

² Arnold, A., and Elvehjem, C. A., *J. Am. Vet. Med. Assn.*, 1939, **95**, 303.

³ Wagner, J. R., and Elvehjem, C. A., *J. Nutrition*, 1944, **28**, 431.

⁴ Newell, G. W., Erickson, T. C., Gilson, W. E., and Elvehjem, C. A., *Proc. Soc. Exp. Biol. and Med.*, 1947, **65**, 115.

⁵ Mellanby, E., *Brit. Med. J.*, 1946, Dec. 14, No. 4484, 885.

† The word "agene" is used throughout interchangeably with nitrogen trichloride gas, NCl_3 .

TABLE I.
Basic Diet Used in Producing Canine Epilepsy.

Composition			Analysis
	%		%
Casein	5	Protein	17.5
Liver powder	5	Carbohydrate	65
Salts IV	4	Fat	5
Sucrose	6	Ash	4
Corn oil	5	Moisture	8.5
Flour	75		
	100		100
Supplementation			
	mg/kilo		mg/kilo
Thiamine	5.6	Thiamine	4.3
Riboflavin	5.6	Riboflavin	10
Pyridoxine	4		
Niacin	60	Niacin	94
Pantothenic acid	15		
Para amino benzoic acid	15		
Inositol	15		
Ascorbic acid	80	Ascorbic acid	120
Halliver oil	500		
α -tocopherol	100		

Results. When the control ration was fed to dogs of 5 to 10 kg weight, in quantities of 250 to 300 g per day, they gained weight, had a good appetite, and showed no symptoms of neurologic disorder or deficiency disease in the periods up to 45 days during which they were observed.

When the experimental diet (bleached flour) was fed to dogs, regardless of age, sex or the breed used, all the animals developed convulsions within 5 days, together with marked behavior disturbances and an ataxic gait. The electroencephalogram showed marked abnormalities, appearing between the 2nd and 3rd day of the experimental diet. No changes appeared after 45 days on the control diet. In all, 24 dogs were fed the experimental diet, and all 24 dogs developed canine epilepsy.

The wheat flour was fractionated after trials with numerous fat solvents showed that Bloor's mixture (75% anhydrous ethyl alcohol, 25% di-ethyl ether) was most satisfactory. By repeated extractions, it was possible to remove 1.4% fat from a straight grade of hard red winter wheat, the flour thus obtained containing less than 0.15% of fat by analysis. The solvent was removed from the fat at 100°C and a pressure of 20 mm Hg with a counter current of nitrogen,

and both fat free flour and fat, were treated with agene. Feeding experiments showed that the toxicity remained unimpaired in the flour fraction, while the fat (although it became dark and rancid) did not prove toxic to test animals. Separation of gluten from the fat free flour showed that convulsive activity was retained in the protein fraction. Further confirmation of the relation between toxicity and the protein fraction is offered by the fact that casein treated with agene proved convulsant to 4 dogs.

The extraction of fat is emphasized, because commercially produced gluten has the lipid so firmly bound that it is not fully extractable at this stage. Hard red winter, hard red spring, and soft red spring wheat of clear, straight and patent grades all produced convulsions after treatment with agene although straight grade (so-called "family-grade") of hard red winter wheat was used in most of these experiments.

Monkeys were also used. The control diet seemed adequate to maintain nutrition in 6 monkeys for 3 weeks. The experimental diet did not produce convulsions, but the monkeys developed a tremor of the extremities and weakness of the hind limbs, within 5 days after changing to the experimental diet. This syndrome persisted 6 weeks later.