Development of Seizure Patterns in Newborn Animals. Significance of Brain Carbonic Anhydrase.* (23411)

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The pattern and threshold of electroshock convulsions have been investigated by Gellhorn and Ballin(1) in rats 4 weeks of age and older. With increasing age, susceptibility to seizures was diminished and, in older animals, a stronger electroshock stimulus was required in order to induce a tonic seizure pattern.

The susceptibility of young rats to experimental seizures is in accordance with the peak incidence of seizures in childhood. However, since seizures of the cryptogenic type are unusual during early infancy, and the major tonic pattern is observed rarely at this age, it seemed important to investigate further the convulsive pattern and threshold in young animals and to study susceptibility to induced seizures from the time of birth. Animal species of different degrees of maturity at birth were selected so that changes in seizure threshold which might occur with increasing age and concomitant alterations in brain chemistry could be correlated.

Carbonic anhydrase is one of a number of enzymes in the brain of the young rat which shows a sharp increase in activity during the first month of life(2). Acetazolamide (Diamox), an inhibitor of this enzyme, has an anticonvulsant effect(3), and previous observations of the mechanism of action of this drug suggest that brain carbonic anhydrase is of possible significance in the seizure process (4). In the present investigation, the relation of the enzyme to the seizure threshold has been studied further. Levels of carbonic anhydrase activity in the brain of the young rat and of the guinea pig, an animal more mature at birth, have been correlated with development of susceptibility to seizures induced by electroshock stimuli.

Methods. The young rats used in this experiment were bred from the Holtzman albino strain. Seizure patterns were studied first in rats of 3 weeks of age and were graded from 1 to 5, according to the type of response with successive increments in strength of an electroshock alternating current. The current was applied by corneal electrodes for 0.2 sec. The threshold stimulus required for maximal tonic seizures (50 m.amps.) in these animals was used subsequently for the examination of seizure patterns in rats between the ages of 1 and 35 days.

Of 10 litters of rats included in the study, 3 litters were tested only once, 3 litters twice, and 4 litters on 3 occasions. The interval between repeated electroshock stimuli was 3 to 16 days with an average of 7 days and, in agreement with the findings of Woodbury and Davenport(5) in older rats, was sufficient to permit complete recovery from the previous seizure response. The response to stronger electroshock stimuli of 100 and 200 m. amps. was observed in additional litters of rats between 4 and 24 days old. Sixteen guinea pigs in 5 age groups between 1 and 31 days were tested principally with current stimuli of 100 to 400 m.amps.

In parallel with the investigation of seizure patterns, the carbonic anhydrase activity in saline extracts of brain of rats and guinea pigs was estimated by the manometric method of Meldrum and Roughton(6). Animals were decapitated and, after removal of macroscopic blood, the cerebral cortex and subcortical white matter were weighed and homogenized with 0.9% sodium chloride solution, in a dilution of 1:20. The entire cerebral hemisphere of the young rat and a portion of the hemisphere (temporal pole) of the guinea pig brain were employed. The carbonic anhydrase activity of the supernatant fluid was determined after centrifugation. In 4 rats of different

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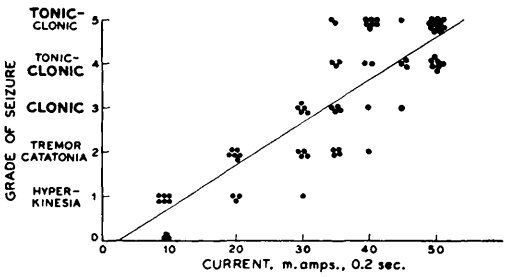

FIG. 1. Grade of electroshock seizure in 21-day-old rats in relation to strength of current stimulus. Each point represents response of one test animal.

ages, the enzyme activity in contaminating red cells was estimated and was found to be less than one per cent of that in the brain tissue specimen. The wet and dry weights of the brain were recorded for rats and guinea pigs in the age range studied.

Results. Seizure patterns. In rat 21 days old, grade of electroshock seizure was related to strength of the current stimulus as follows (Fig. 1): *grade 1,* hyperkinetic behavior sometimes associated with transient opisthotonus and breast-stroke swimming movements (10 m.amps); *grade 2,* stunning, tremor of the head, abduction of fore-limbs, and catatonic posture (20 m.amps.); *grade 3,* generalized symmetrical clonus with loss of posture (30 m.amps.); *grade 4,* transient tonic flexion and extension of the forelimbs followed by prolonged generalized clonus (40 m. amps.); *grade 5,* tonic flexion and prolonged tonic extension of both fore and hindlimbs followed by mild clonus and post-ictal depression (50 m.amps.). Similar seizure patterns in response to electroshock stimuli of different intensity have been reported in older rats(5).

Fig. 2 shows the seizure patterns of rats between 1 and 35 days of age in response to the threshold electroshock stimulus for maximal tonic seizures in 21-day-old rats (50 m. amps. applied for 0.2 sec.). In rats aged 20 to 35 days, the electroshock seizure was mainly *tonic* in pattern (grade 5); between 10 and 20 days, the *clonic* type of seizure predominated (grade 3); and from birth to 10 days, neither tonic nor symmetrical clonic seizures could be elicited with a current stimulus of 50 m.amps. The grade of response was more severe with the stronger stimuli (100 and 200 m.amps.) but, in rats aged 1 to 15 days, a maximal tonic seizure could not be elicited.

In the guinea pig, unlike the rat, there was little change in the electroshock seizure response from birth to 35 days of age (Fig. 3). The clonic response predominated and a maximal seizure with a hind-limb tonic exten-

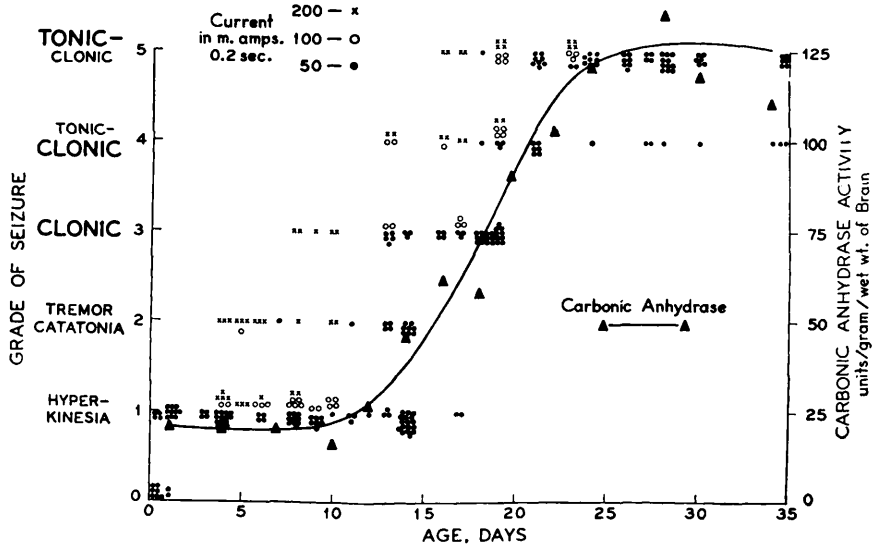

FIG. 2. Grade of electroshock seizure in young rats in relation to age and to level of brain carbonic anhydrase activity. Each point, open circle, and cross represents response of one test animal. Each triangle represents the mean of separate estimations on each of 2 to 4 animals.

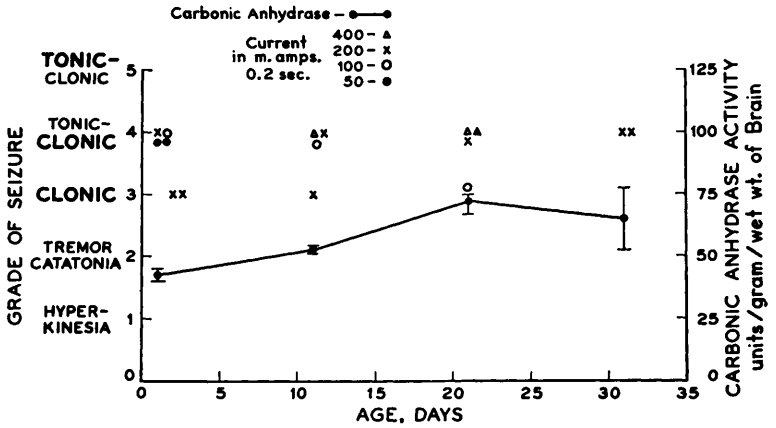

FIG. 3. Grade of electroshock seizure in young guinea pigs in relation to age and to level of brain carbonic anhydrase activity. Points and vertical lines represent respectively mean value and range of separate estimation on each of 2 or 3 specimens.

sor component could not be induced, despite the use of current stimuli of 100 to 400 m. amps.

Carbonic anhydrase determination. The carbonic anhydrase activity per unit weight of brain in relation to the age of the young rat is shown in Fig. 4. The manometric method for determination of carbonic anhydrase employed in this study yielded higher levels of enzyme activity than those obtained in a previous investigation(2); in addition, with this method carbonic anhydrase activity was demonstrated in the brain of rats at birth. A 6-fold increase in activity occurred between 10 and 25 days of age. During this interval, the water content of the brain fell from 86% to 80% (Fig. 5), and increase in enzyme activity was 4-fold when expressed as units per gram of dry brain. In contradistinction to the slow increase in body weight of the rat during this developmental period, the total brain weight rose sharply and the enzyme activity, calculated as units per whole brain, showed a 30-fold increase. In Fig. 2, the developmental pattern of brain carbonic anhydrase and the grades of electroshock seizure in the rat are superimposed.

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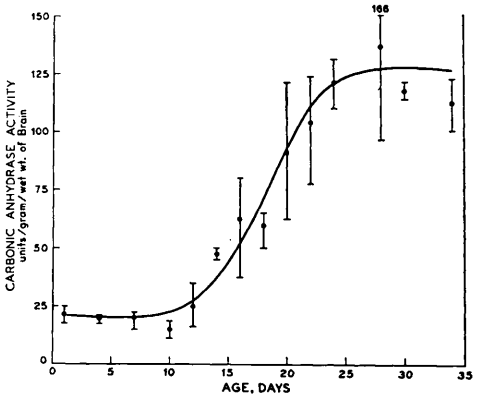

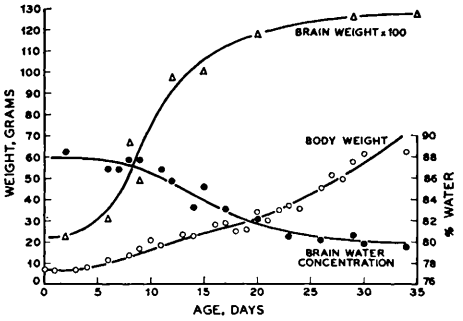

FIG. 4 (left). Development of carbonic anhydrase activity in the brain of the young rat. Points are mean values and vertical lines represent range of separate estimations on each of 2 to 4 animals.

FIG. 5 (right). Body wt, total brain wt, and brain water concentration in relation to age of young rats. ○, mean wt of 12 rats. △, mean wt of 5 brains. ●, mean water conc. of 3 brains.

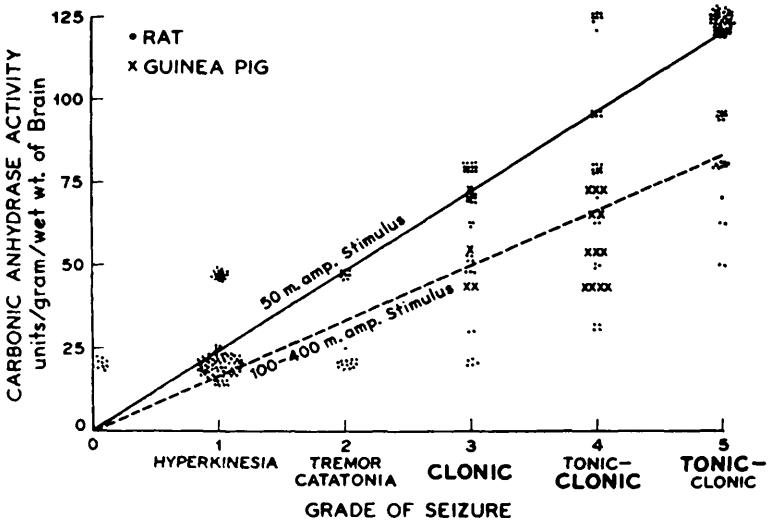

FIG. 6. Grade of electroshock seizure in rats and guinea pigs aged 1 to 35 days in relation to brain carbonic anhydrase activity. Continuous and broken diagonal lines indicate respectively the relationships obtained with currents of weak and strong intensity. Guinea pigs tested principally with stronger stimuli.

variation in electroshock seizure response remained correspondingly small during this age period (Fig. 3). In addition, there was no significant change in total weight and water content of the brain, but a 3-fold increase in body weight was observed. The degree of maturation of the guinea pig corresponded with that of the 15- to 20-day-old rat, both with regard to the grade of seizure response and to the level of brain carbonic anhydrase.

Discussion. A close correlation between the developmental pattern of brain carbonic anhydrase and the grade of seizure in response to electroshock has been demonstrated in 2 animal species of different degrees of maturity at birth. When the brain enzyme activity in rats and guinea pigs, in the age range tested, is plotted against the grade of seizure in response to stimuli of fixed intensity, a linear relationship is observed of the same order of magnitude in both animal species (Fig. 6). Biochemical maturation and susceptibility to seizures were directly related to total weight and solid constituents of the brain but not to body weight. In the young guinea pig, an increase in body weight occurred without significant alteration of the seizure threshold and, in the rat between 10 and 25 days of age, a slow increase in body weight was associated with a rapid fall in the seizure threshold. In older rats, however, Davenport(7) found that the electroshock threshold was directly related to the body weight.

The inability of the newborn rat to respond maximally with a tonic electroshock *seizure pattern* is in accordance with clinical observations and the infrequent occurrence of generalized tonic-clonic seizures in immature infants. The present findings agree also with the report by Grossman(8), of an absence of "tonic" or fast spike discharge in response to electrical stimulation of the cortex of immature kittens. The high *seizure threshold* in the newborn rat corresponds with the low susceptibility of the young infant to convulsions associated with fever and to seizures of the cryptogenic type.

In previous work(4), it has been shown that the anticonvulsant action of acetazolamide (Diamox)† in mice is directly related to inhibition of brain carbonic anhydrase activity. That this enzyme is of significance in the seizure process is suggested by this study and is supported further by the present demonstration of a relation between the susceptibility to induced seizures and the level of carbonic anhydrase activity in the brain. Pre-

† Diamox was kindly supplied by Dr. James D. Gallagher, Lederle Medical Research Department, American Cyanamid Co.

liminary studies of the effect of Diamox on electroshock seizures in young rats have shown a specific activity against the maximal seizure response. While the *tonic* type of seizure exhibited by the older rats was abolished by very small doses of the carbonic anhydrase inhibitor, the *clonic* pattern was controlled only by large doses, and the hyperkinetic behavior of 10-day-old rats was refractory to the drug.‡ Similar results were obtained with Diamox in infants and children with grand mal and generalized myoclonic seizures; the tonic seizure was controlled more effectively than the myoclonic pattern(9).

These observations suggest that brain carbonic anhydrase is of functional significance in development of susceptibility to maximal *tonic* convulsions, in which a generalized spread of the seizure discharge is probably involved. The significance of this enzyme in the mechanism of clonic seizure patterns is less well defined and, on the basis of present evidence, the following possibilities are suggested. Such seizures, which are minor in character and which probably involve focal neuronal discharge, may be unrelated to the activity of brain carbonic anhydrase. As the result of observations in the rabbit of the effects of carbonic anhydrase inhibition by thiophene-2-sulfonamide, Davenport(10) has concluded that, when stimulated locally, the electrical activity of the cerebral cortex is independent of carbonic anhydrase; and the resistance to Diamox of the minor convulsive activity observed in the newborn rat would lend support to this conclusion.

Alternatively, an impairment of diffusion of the drug might explain the relatively low anticonvulsant potency of Diamox in the minor hyperkinetic and clonic seizure patterns of the newborn rat, when compared with its activity against tonic seizures in the older rat. The potency of brain enzyme inhibition *in vivo* may be lower than that expected by *in vitro* tests(4). The increase in brain carbonic anhydrase activity with age corresponds temporally with the rapid increase in weight and in solid constituents of the brain. The greater anticonvulsant potency of the enzyme inhibitor observed in older animals might then be associated with changes in cerebral localization of the enzyme and possibly, with facilitation of diffusion of the drug. The possible role of brain carbonic anhydrase in development of susceptibility to minor or clonic seizures remains to be clarified.

Summary. Electroshock seizures have been studied in young rats and guinea pigs, and a correlation has been demonstrated between the developmental pattern and threshold of the induced seizures and the level of carbonic anhydrase activity in the brain. In young rats aged 20 to 35 days, the seizures were mainly tonic in pattern, whereas the clonic type of seizure predominated in rats aged 10 to 20 days. Failure to induce convulsions in the newborn rat was associated with a low level of carbonic anhydrase activity in the brain. The degree of maturation of the newborn guinea pig was equivalent to that of the 15-day-old rat, both with regard to the type of seizure response and the level of brain carbonic anhydrase activity. That brain carbonic anhydrase is of functional significance in the generalized spread of the seizure discharge and in the production of a maximal tonic convulsion is suggested by these data and by observations of the anticonvulsant activity of acetazolamide.

1. Gellhorn, E., and Ballin, H. M., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 540.

2. Ashby, W., and Schuster, E. M., *J. Biol. Chem.*, 1950, v184, 109.

3. Bergstrom, W. H., Carzoli, R. F., Lombroso, C., Davidson, D. T., and Wallace, W. M., *A.M.A. Am. J. Dis. Child.*, 1952, v84, 771.

4. Millichap, J. G., Woodbury, D. M., and Goodman, L. S., *J. Pharm. and Exp. Therap.*, 1955, v115, 251.

5. Woodbury, L. A., and Davenport, V. D., *Arch. int. Pharmacodyn.*, 1952, v92, 97.

6. Meldrum, N. U., and Roughton, F. J. W., *J. Physiol.*, 1934, v80, 113.

7. Davenport, V. D., and Davenport, H. W., *J. Nutrition*, 1948, v36, 139.

8. Grossman, C., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 43.

9. Millichap, J. G., *Neurology*, 1957, v6, 552.

10. Davenport, H. W., *J. Neurophysiol.*, 1946, v9, 41.

‡ Millichap, J. G: unpublished observations.

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