Development of Susceptibility to Seizures in Young Animals III. Brain Water, Electrolyte and Acid-Base Metabolism.* (24228)

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In a previous study concerning development of susceptibility to experimental seizures in young animals, newborn rats were found to be much less susceptible than older rats(1). From birth to 10 days of age, the response to an electroshock stimulus of 50 m. amps. consisted of hyperkinetic behavior and opisthotonic spasms, and neither clonic nor tonic convulsions could be induced. Between 10 and 20 days, a rapid increase in seizure susceptibility was observed and the severity of the seizure pattern progressed from minor tremors to generalized clonic convulsions. Susceptibility was greatest at 25 to 35 days of age, when a maximal seizure response was obtained with tonic extension of all limbs. In older and adult rats, stronger electroshock stimuli are required for induction of major seizures and reduction in susceptibility occurs with increasing age(2). In the guinea pig, unlike the rat, there was little change in the electroshock seizure response from birth to 35 days of age. The clonic response predominated and a maximal seizure with a hindlimb tonic extensor component could not be induced.

In order to elucidate possible mechanisms in the seizure process, the changes observed in the seizure threshold with age have been studied in relation to development of carbonic anhydrase activity in the brain. In the rat, a sharp increase in brain carbonic anhydrase occurs at 10 to 20 days of age whereas in the guinea pig, little change in enzyme activity is observed during the first month of life. The degree of maturation of the newborn guinea pig corresponds with that of the 15- to 20-day-old rat, both with regard to seizure susceptibility and level of brain carbonic anhydrase activity. By developmental studies and by the use of acetazolamide, an anticonvulsant which inhibits carbonic anhydrase, a direct relation has been demonstrated between activity of the enzyme in the brain and susceptibility to experimental seizures in young animals of different species(3). The specific catalytic activity of brain carbonic anhydrase appears to be essential for propagation of the seizure discharge.

Studies in adult rats have shown that the anticonvulsant effect of acetazolamide is associated with an increase in the cellular total carbon dioxide concentration in the brain and with changes in the distribution of brain electrolytes.‡ Woodbury *et al.* have drawn attention to the anticonvulsant properties of carbon dioxide and the effects of brain electrolyte alterations on the seizure threshold (4). In the present developmental study, susceptibility to experimental seizures and concentration of brain carbonic anhydrase have been correlated with brain water and electrolyte metabolism in rats and guinea pigs and with the carbon dioxide content of the brain in rats.

Methods. Newborn, one-month-old and adult rats of the Holtzman and Sprague-Dawley strains and newborn guinea pigs were used as experimental animals. The percentage of animals which showed a maximal tonic extensor seizure in response to an electroshock stimulus of 50 m. amps. applied for 0.2 sec. was used as a measure of seizure susceptibility. An average of 25 rats in each age group and 12 guinea pigs were employed.

Water and electrolyte analyses. Animals of the same age as those tested for seizure susceptibility were used for estimations of water and electrolyte concentrations of plasma and brain tissue. Animals were killed by de-

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capitation, blood was collected in tubes containing heparin, and samples of the cerebral cortex and subcortical white matter were transferred to tared beakers, after removal of macroscopic blood and surface vessels. In newborn rats, samples from one litter of 8 to 10 animals were pooled for each determination. In older and adult rats and in guinea pigs, samples from 2 to 4 animals were pooled. Plasma and brain tissue were dried at 105°C to a constant weight; water content was calculated from the difference between wet and dry weights. Nitric acid was added to the dried tissues which were digested by gentle heating; the resulting solutions were analyzed for sodium and potassium by means of a Process and Instruments flame-photometer with an internal lithium standard. Tissue chloride determinations were performed by the method of Van Slyke and Sendroy(5); plasma chloride determinations, by the method of Schales and Schales(6). In calculations of cellular concentrations of water, sodium and potassium, the chloride space was used as a measure of extracellular fluid volume in the brain(7). As a method for estimation of absolute values, the limitations of the chloride space are recognized but, as a measure of relative values and of directional changes in the volume of the fluid compartments of the brain with growth, objections to the method might be lessened. In order to make possible the calculations of standard errors of values for water and electrolyte concentrations for comparative purposes, an analysis of intracellular cations was made for each pooled sample rather than using the mean values calculated for each group of animals.

Tissue carbon dioxide determinations. The method of Danielson and Hastings(8) was used for estimation of the total carbon dioxide content of the cerebrum of rats. The brain was removed after decapitation and was transferred rapidly to the tube containing ferric fluoride and air-free sodium hydroxide. Estimations were performed on 0.5 to 1 g of tissue and the brains of 2 to 4 newborn rats were pooled. A total of 47 determinations were made in animals at 9 different ages between

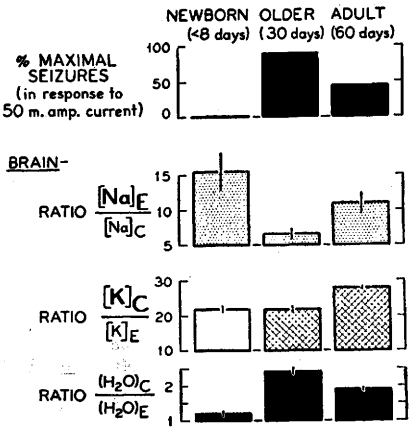

FIG. 1. Susceptibility to maximal electroshock seizures in newborn, older and adult rats in relation to distribution of brain water and electrolyte concentrations.

3 and 38 days. Values obtained in rats bred from the Holtzman strain were compared with those of the Sprague-Dawley strain. Since the consistency of the brain of older rats differed from that of the newborn, the values obtained with whole brain were compared with those for brain brei. A magnetic stirring rod was used to agitate the tissue and facilitate the evolution of the carbon dioxide. In addition to developmental studies, the brain carbon dioxide was estimated in 7 rats of the Sprague-Dawley strain aged 24 days, which were killed 2 hours after the subcutaneous injection of acetazolamide, in a dose of 1 g/kg/body weight. Control animals were examined after injection of an equal volume of isotonic saline. The collection of blood under anaerobic conditions was impractical in newborn rats and a comparison of intracellular pH and carbon dioxide concentration in the brain of newborn and older rats was not possible.

Results. Susceptibility to seizures. Fig. 1 shows degree of susceptibility to electroshock seizures in rats tested with a current of 50 m. amps. for 0.2 sec. Rats aged 30 days were most susceptible; a maximal response with tonic extension of the hind-lambs occurred in 90% of animals. Newborn rats between 4 and 7 days of age failed to respond maximally and seizure activity consisted of hyperkinetic behavior. In adult rats aged 60 days (55 to 65) maximal seizures were obtained

TABLE I. Concentrations of Water and Electrolytes in Brain and Plasma of Rats and Guinea Pigs.

		Cerebrum					Plasma			
Age of animal	No. of samples	H_2O, %	Na	K	Cl	No. of samples	H_2O, %	Na	K	Cl
			meq/km/wet tissue					meq/kg/plasma		
Newborn rat (4-7 days)	27[a]	87.61 ± .16*	61.45 ± .58	82.43 ± .56	43.02 ± .75	18	94.89 ± .32	135.9 ± 1.7	7.68 ± .21	98.33 ± 1.54
P_1 values		<.001	<.001	<.001	<.001		<.01	N.S.	N.S.	<.001
Older rat (28-32 days)	11[b]	81.30 ± .71	47.9 ± 1.03	99.2 ± 1.89	30.8 ± .68	11	93.4 ± .28	139 ± 1.33	7.4 ± .20	114.5 ± 2.22
P_2 values		<.01	N.S.	<.02	N.S.		<.01	<.05	N.S.	<.001
Adult rat (55-65 days)	5[c]	78.3 ± .40	48 ± .43	104.5 ± .50	31.6 ± .89	5	92.1 ± .18	143.7 ± 1.43	6.97 ± .06	101.5 ± .55
Newborn guinea pig (3-5 days)	6[d]	81.4 ± .20	52.7 ± .64	97.2 ± .72	37.5 ± .66	6	93.2 ± .37	136.1 ± 2.98	6.74 ± .08	109.4 ± 1.59
P_3 values		<.001	<.001	<.001	<.001		<.01	N.S.	<.01	<.05

[a,b,c,d]; each sample represents pooled specimens of brain or plasma obtained from 10, 4, 3, and 2 animals respectively.

P_1, P_2; tests of significance for differences between newborn and older, and older and adult rats, respectively.

P_3; tests of significance for difference between newborn rats and newborn guinea pigs.

* Mean ± stand. error.

only in 45% of the animals tested; a generalized clonic seizure was observed in the remainder. In the newborn guinea pig, seizures in response to a 50 m. amp. electroshock stimulus were unlike those in the newborn rat and corresponded more closely to responses obtained in the rat aged 15 to 20 days and the adult rat. Generalized clonic movements were preceded by tonic extension of the forelimbs but a maximal seizure with extension of the hind-limbs was observed rarely.

Water and electrolyte analyses. Table I shows concentrations of water and electrolytes in brain and plasma of newborn, 1-month-old and adult rats and of newborn guinea pigs. Table II shows the intracellular $()_C$ and extracellular $()_E$ concentrations of water, sodium and potassium in the brain. In Fig. 1, ratios of cellular to extracellular brain water and electrolyte concentrations are shown in relation to seizure susceptibility in rats. In newborn compared with older and adult rats, the potassium concentration in whole brain is 18% lower and the sodium and chloride, 27% and 40% higher, respectively. The water concentration of the newborn compared with adult brain is 10% higher but the ratio of cellular to extracellular brain water is approximately one-half that of the older animals. Ratios of cellular to extracellular potassium and of extracellular to cellular sodium in the brain of 30-day-old rats are lower than in adults. The highest ratio of extracellular to cellular sodium in the brain is found in newborn rats. In the newborn guinea pig, the concentration and distribution of water and electrolytes in the brain are significantly different from the values obtained in the newborn rat. The newborn guinea pig corresponds more closely to the older and adult rat, both with regard to seizure susceptibility and brain water and electrolyte metabolism.

Brain carbon dioxide content. Fig. 2 shows total carbon dioxide content of the brain in

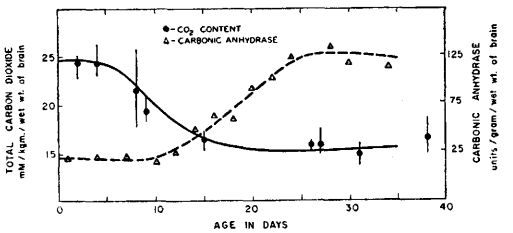

FIG. 2. Total carbon dioxide content of brain of rats 1 to 38 days of age in relation to brain carbonic anhydrase activity. Each point and vertical line represents respectively the mean and range of separate estimations on an avg of 5 specimens. Triangles are mean values of separate estimations on each of 2 to 4 animals.

TABLE II. Distributions of Water and Electrolytes in Brain of Rats and Guinea Pigs.

Age of animal	Water			Sodium			Potassium		
	Intracellular	Extracellular	Ratio†	Extracellular	Intracellular	Ratio†	Intracellular	Extracellular	Ratio†
	% whole tissue			meq/kg water			meq/kg water		
Newborn rat (4-7 days)	47.30 ± 1.36*	40.20 ±1.48	1.23 ± .09	137.5 ± 1.94	15.15 ± 3.28	15.45 ± 2.73	169.0 ± 3.53	7.76 ± .23	22.0 ± .62
P_1 values	=.001	<.001	<.001	<.05	N.S.	<.01	N.S.	N.S.	—
Older rat (23-32 days)	57.20 ± 1.44	24.20 ± .93	2.42 ± .13	143.0 ± 1.43	22.1 ± 2.27	6.46 ± .67	165.3 ± 6.37	7.5 ± .22	22.0 ±1.04
P_2 values	<.01	<.01	<.01	<.01	<.01	=.02	<.001	<.02	<.001
Adult rat (55-65 days)	50.80 ± .77	27.60 ± .65	1.85 ± .07	149.8 ± 1.67	13.2 ± 1.27	10.90 ± 1.56	202.1 ± 2.73	7.25 ± .06	27.9 ± .51
Newborn g. pig (3-5 days)	50.7 ± .39	30.7 ± .32	1.65 ± .03	142.1 ± 3.37	18.1 ± 2.20	8.92 ± 1.82	187.2 ± 1.27	6.91 ± .03	27.1 ± .24
P_3 values	<.05	<.001	<.001	N.S.	N.S.	=.05	<.001	<.001	<.001

* Mean ± stand. error. † Mean of ratios obtained from individual samples.
P_1, P_2; tests of significance for differences between newborn and older, and older and adult rats respectively.
P_3; tests of significance for difference between newborn rats and newborn guinea pigs.

rats 2 to 38 days of age, bred from the Holtzman strain. When compared with carbonic anhydrase, a relatively high level of carbon dioxide in the newborn brain is associated with a low enzyme concentration. In 26-day-old rats which have a high level of brain enzyme activity, brain carbon dioxide content is 38% lower than in newborn rats ($P<.001$). When compared with those observed in rats of the Holtzman strain, the values obtained in 6 Sprague-Dawley rats were equal in the 4-day-old but higher in 26-day-old animals. In this strain, a difference of 20% in the brain carbon dioxide content of newborn and older rats was observed ($P<.001$). In an initial study of 8 rats aged 38 days and 2 aged 2 days, the values obtained with brain brei were 1 mM/kg lower than those with whole brain. The difference was not significant ($P>0.1$), but all subsequent estimations were performed on whole brain.

Effect of acetazolamide on brain carbon dioxide. In 2 groups of 24-day-old rats treated with acetazolamide the brain carbon dioxide content was 20.3 and 23 mM/kg; values obtained in control animals were 18 and 19.2 mM/kg, respectively. Thus, inhibition of carbonic anhydrase by acetazolamide was associated with an increase in brain carbon dioxide of 12% ($P<.05$) in one group and 20% ($P<.001$) in a second group of rats.

Discussion. In the present series of investigations, susceptibility to maximal electroshock seizures in rats was greatest at one month of age. In newborn and adult rats, a low degree of susceptibility or high threshold to electroshock seizures was observed. The inability of the newborn rat to respond maximally with a tonic seizure pattern is in accordance with clinical observations and the infrequent occurrence of generalized tonic-clonic seizures in immature infants. We have observed in the newborn that the clinical pattern of a seizure consists principally of tremors and opisthotonic and myoclonic spasms. In infants and in adults, history of an inherited lowered threshold to seizures is relatively uncommon and seizures are associated usually with structural brain lesions. The age of greatest susceptibility is between 4 and

8 years, when incidence of those seizures unassociated with brain pathology is highest.

Changes in susceptibility to seizures with age have been correlated with the water, electrolyte and acid-base metabolism of the brain in animals of different species. Susceptibility to electroshock seizures of newborn, older and adult rats is related inversely to the ratio of extracellular to cellular sodium ($[Na]_E/[Na]_C$) and directly, to the ratio of cellular to extracellular water ($(H_2O)_C/(H_2O)_E$) in the brain. The large extracellular fluid space observed in the newborn may be explained by the relatively small nerve cells and the paucity of glia and cell processes at this age. However, the possible effect of an increased permeability of cell membranes to chloride ions cannot be excluded. In newborn and adult rats, susceptibility to electroshock seizures is related inversely to total carbon dioxide content of the brain. The reciprocal relation between carbon dioxide and carbonic anhydrase concentrations in the brain is seen in Fig. 2. In agreement with observations in adult rats,‡ inhibition of carbonic anhydrase by acetazolamide in 1-month-old animals is associated with an increase in carbon dioxide content of the brain. In the newborn guinea pig, an animal more mature than the rat at birth, degree of seizure susceptibility and concentrations of water and electrolytes in the brain resemble those of older and adult rats. In addition, brain carbonic anhydrase activity in the newborn guinea pig is greater than that in the newborn rat and corresponds more closely with the level of enzyme activity observed in older rats(1).

The relation between seizure susceptibility, carbonic anhydrase activity, and water, electrolyte and acid-base metabolism demonstrated in developmental studies is supported by observations in adult animals treated with acetazolamide. The anticonvulsant effect of acetazolamide is related directly to inhibition of brain carbonic anhydrase(9); it is associated with an accumulation of carbon dioxide in the brain which, in certain concentrations, is known to depress seizure activity. Acetazolamide decreases the permeability of brain cell membranes and the rate of uptake of radiosodium; anticonvulsant doses effect an increase in the ratio of extracellular to cellular brain sodium.‡ An increase in ratio of cellular to extracellular potassium ($[K]_C/[K]_E$) observed in adult rats treated with acetazolamide is not demonstrable in newborn rats which have a low level of brain carbonic anhydrase and a high threshold to seizures. However, when results obtained in 30-day-old and adult rats are compared, the correlation between seizure susceptibility and distribution of potassium is similar to that observed in acetazolamide-treated animals. The catalytic activity of carbonic anhydrase and the local acid-base changes produced are involved in transport and distribution of ions in brain tissue. Development of susceptibility to seizures in young animals is related to the maturation of carbonic anhydrase activity and to the effects of the enzyme on acid-base and water and electrolyte metabolism of the brain.

Summary. In newborn, 1-month-old and adult rats, susceptibility to maximal electroshock seizures has been correlated with distribution of water and electrolyte concentrations and total carbon dioxide content of the cerebrum. Seizure susceptibility is greatest at one month of age. It is related inversely to the ratio of extracellular to cellular sodium and directly to the ratio of cellular and extracellular brain water. The relation to distribution of potassium is inconstant. The newborn guinea pig, an animal more mature than the rat at birth, corresponds more closely to the older and adult rat, both with regard to seizure susceptibility and brain water and electrolyte metabolism. Failure to induce convulsions in the newborn rat is associated with a high level of total carbon dioxide in the brain. Development of seizure susceptibility in older rats is related to a rise in brain carbonic anhydrase activity and to a reciprocal fall in total carbon dioxide content of the brain.

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Hepatic Uptake and Biliary Excretion of Indocyanine Green in the Dog.* (24229)

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Indocyanine green is a water-soluble tricarbocyanine dye developed by Brooker and introduced by Fox *et al.*(1,2) for measurement of cardiac output by the indicator dilution technic. For this purpose its absorption spectrum and protein-binding characteristics (1) are ideally suited. The results of the present investigation indicate that the dye may also be of great value in study of hepatic function.

Methods. All studies were conducted on 2 trained unanesthetized female dogs weighing 18.9 and 22.0 kg. Several months previously each had undergone splenectomy, cholecystectomy, and preparation of a permanent duodenal fistula using the device described by Thomas(3), which permitted access to the common bile duct. The dogs remained in good health, and hepatic function, as judged by repeated measurements of sulfobromophthalein (BSP) transfer maximum(4), continued unimpaired. At the time of each study an olive-tipped ureteral catheter (5 or 6 Fr.) was inserted through the ampulla of Vater and advanced about 5 to 6 cm into the common bile duct. The dogs were then placed upright in a sling and bile was collected by gravity or, when it was particularly viscous, by gentle aspiration with a tuberculin syringe. Intravenous injections were given through a polyethylene catheter introduced through a large bore needle in a vein of the foreleg and advanced to the region of the right atrium.

Indocyanine green§ was made up to 250 mg% in distilled water for injections. In one study a continuous infusion of the dye was given. This was prepared by mixing 24 ml of 500 mg% indocyanine green with 51 ml of normal saline and 5 ml of dog plasma. (Indocyanine green is unstable on standing in aqueous solution, and the presence of plasma was found empirically to retard its breakdown.) Concentration of indocyanine green in plasma was measured in a Beckman DU spectrophotometer at 810 mμ after 11-fold dilution with normal saline. Concentration standards for this determination were also prepared in dog plasma. (Dilute aqueous standards are unsatisfactory because of marked instability.) Bile samples were diluted 25 to 100 times with water. One ml of diluted bile was then added to 1 ml of dog plasma and 9 ml of normal saline and the absorption measured at 810 mμ. Although the dye is quite stable in bile even when diluted, the addition of plasma, for reasons not apparent, was necessary for full color development. BSP concentrations

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