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Carbon Dioxide Seizures in Immature Rats.* (32073)

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It is well known that both exposure to and withdrawal from high concentrations of CO_2 can produce seizures in experimental animals and man(1,2). Although the exact mechanisms responsible for these seizures have not been elucidated, Woodbury and his associates have presented evidence that changes in central nervous system excitability caused by CO_2 are related to rapid changes in brain intracellular bicarbonate levels which, in turn, result in changes in brain cell pH (3,4).

In a recent paper(5), it was reported that immature rats, as contrasted with adult animals, have very high cellular bicarbonate levels in brain. Thus, in view of the intracellular acid-base data of Woodbury and coworkers cited above, very young rats might be expected to have different seizure responses to CO_2 challenges than do adult animals. This report is a summary of experiments in which this possibility was studied in detail by comparing the responses of immature and adult rats to various high CO_2 atmospheres.

Methods. General. Entire litters of Sprague-Dawley baby rats along with their mothers were obtained from a local supplier. All immature animals less than 3 weeks old remained with their mothers in their own cages until used; hence, variations in body temperature and fluid balance were minimized. Selection of young animals for tests was on the basis of age only; no attempt was made to group animals according to sex, weight, etc. Animals classified as adults in the results reported below were male Sprague-Dawley rats of 200-300 g body weight.

CO_2 Seizures. Animals of different ages were placed in a plastic chamber filled with various high concentrations of CO_2 and 20% oxygen. Usually, a total of 6 to 10 animals was observed at one time. Care was taken to have a wide representation of ages in each experimental group. Because of reported tolerance to CO_2(6) and adverse effects of the gas on growth(7), each animal was exposed to CO_2 only once. If an animal exhibited either jaw or forelimb clonus, or both, while being exposed to CO_2 in the plastic box, this animal was said to have had an in-chamber or exposure seizure.

After remaining in the chamber for approximately 8 minutes, all animals were taken from the plastic box and allowed to breathe room air. In susceptible animals, so-called withdrawal seizures produced by this maneuver were manifested as very rapid jaw and forelimb clonus, marked salivation, piloerection, and a rearing-up movement.

Additional details of the apparatus and

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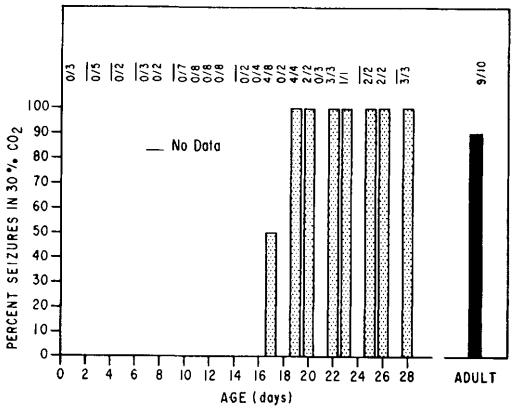

FIG. 1. Incidence of seizures in immature and adult rats during exposure to 30% CO_2 for 8 minutes. Numbers across top of figure represent number of animals that were tested and responded in each age group studied.

techniques used for studying CO_2-induced seizures can be found in the publications of Woodbury and coworkers(1,8).

Results. The responses of immature and adult rats during exposure to 30% CO_2 are given in Fig. 1. With 2 exceptions, namely, 18- and 21-day-old rats, most animals older than 17 days of age had jaw and forelimb clonus that could be distinguished from normal activity of rats of the various age groups. No clonic activity was observed in even a single animal younger than 17 days of age. These data thus suggest that the response to CO_2 in maturing animals is not gradual in age of onset but appears suddenly at age 17 days. Also, the percentage of infant animals responding to CO_2, after they reach the susceptible age of 17 days, is about the same as that observed in adult animals.

The incidence of seizures produced by withdrawal from 40% CO_2 in infant rats of various ages and in a single adult group is depicted in Fig. 2. Once again, no responses were observed in very young animals, but older maturing rats could manifest withdrawal seizures after reaching 16 days of age. Whether the incidence of seizures in rats less than one month old is the same as or geater than that observed in adults cannot be stated with certainty from these data, but additional results presented later clarify this point.

Experiments similar to those summarized in Fig. 1 and 2 were repeated at other concentrations of CO_2. Altogether, a total of 400 rats were exposed to and withdrawn from atmospheres containing from 5 to 50% CO_2. The distribution of animals according to age was as follows: 1 to 9 days old, 39; 10 to 16 days old, 151; 16 to 28 days old, 130; adults, 80. In these tests, CO_2 caused no observable effects in rats less than 16 days of age, that is, neither exposure to nor withdrawal from any of the CO_2 atmospheres used caused seizures in any animals in this age range. Because the results in rats between birth and 16 days of age were completely negative, a breakdown of these immature animals into age or CO_2 exposure groups is not given here.

A detailed analysis of the incidence of CO_2 seizures in animals more than 16 days old is given in Fig. 3 and 4. Because of the large number of animals necessary to obtain a CO_2 response curve, it was not feasible to study in detail the effects of CO_2 in homogeneous age groups of rats in the 2- to 4-week-old age ranges. Thus, the curves labeled "young" in these two Figures represent a heterogeneous age group of rats between 16 and 28 days old, and were constructed by pooling all the results obtained at a given CO_2 concentration when rats included in these age limits were tested. The curves labeled "adult" in these Figures represent a homogeneous group of older animals, age approximately 2½ months.

In Fig. 3, the curves representing the

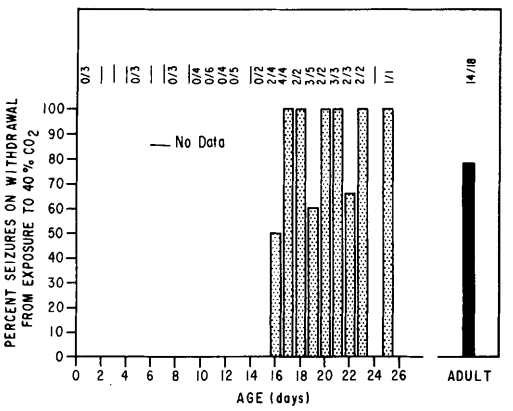

FIG. 2. Incidence of seizures in immature and adult rats breathing room air after 8 minutes of exposure to 40% CO_2. See legend to Fig. 1 for explanation of numbers at top of figure.

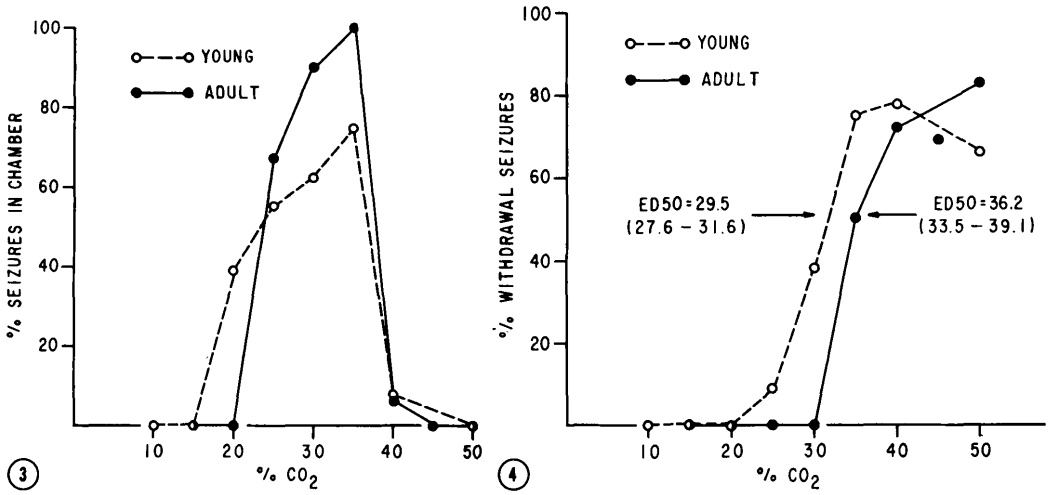

FIG. 3. Per cent seizures in infant and adult rats exposed to various concentrations of CO_2. The curve labeled "young" represents pooled data from a heterogeneous group of animals between 16 and 28 days of age. The adult group was a homogeneous group of rats approximately 2 1/2 months old.

FIG. 4. Per cent seizures in infant and adult rats after withdrawal to room air from various CO_2 concentrations. See legend to Fig. 3 for explanation of animals group labeling.

young and adult rats are qualitatively similar since the concentration of CO_2 which produces the highest incidence of seizures is the same (35%) in both groups of animals. However, the percentage of rats that had seizures was higher in adult animals at 25, 30, and 35% CO_2 concentrations.

The patterns of CO_2 withdrawal seizures for young and adult animals depicted in Fig. 4 show that the two age groups have responses that are, once again, qualitatively similar, that is, the maximum incidence of withdrawal seizures is about the same in both groups, and the CO_2 response curves obtained in young and adult rats have similar shapes. However, withdrawal of rats from a given CO_2 concentration is more likely to produce seizures in the younger animals as indicated by the displacement of the seizure incidence curve for young rats to the left of that for the adult rats. An analysis of these data by a modification of the Litchfield-Wilcoxon method(9) showed that the ED50 of CO_2 for withdrawal seizures was significantly lower in the younger rats.

Discussion. The experiments reported above were done to determine whether immature rats with high intracellular brain bicarbonate concentrations responded to CO_2 challenges in a manner different from that of adult rats with much lower bicarbonate levels. The results given in Fig. 1 and 2 show conclusively that rats less than 16 days of age do not have CO_2 exposure or withdrawal seizures. Further, the data depicted in Fig. 3 and 4 suggest that rats between the ages of 16 and 28 days of age respond differently to CO_2 than do adults. There are, however, some factors that complicate interpretation of these results.

In the first place, functional neural development of rat cerebral cortex is not complete until about 15 days after birth(10,11). Since excitation of the cerebral cortex is necessary for the exhibition of the clonic seizures induced by CO_2(1,2,4), the lack of maturity of the cortex of the rat could account for the absence of CO_2 seizures in rats less than 16 days old. In other words, no unique resistance of rats to CO_2 need be proposed to account for the results presented above in Fig. 1 and 2.

A second point that must be considered in discussing the absence of response to CO_2 reported here is the proposal that intracellular acid-base distortions produced by CO_2, and responsible for seizures, might be different in very young and adult rats. In a series of

related experiments, Woodbury and his associates have pointed out the importance of carbonic anhydrase for the rapid hydration of CO_2 and perhaps the rapid dehydration of carbonic acid in brain cells(3,4). Thus, the rate of uptake or loss of CO_2 by brain cells during exposure to or withdrawal from high concentrations of CO_2 could be expected to differ in normal animals as compared to animals with either enhanced or reduced brain carbonic anhydrase activity. Since rats less than 3 weeks old have lower brain carbonic anhydrase activity than older animals(12,13), it is not unreasonable to suggest that brain intracellular acid-base parameters do not change at the same rate in immature and adult rats. It is therefore possible that part of the apparent insensitivity of immature rats to CO_2 challenge is due to an inadequate stimulation of these animals by the CO_2 exposure.

Finally, it is still a moot point as to whether young rats between 16 and 28 days of age are more or less susceptible to CO_2 than are much older animals. The results presented in Fig. 4 indicate that the young rat is more susceptible to CO_2 since the ED50 for CO_2 withdrawal seizures is lower in this group than in adults. On the other hand, the data depicted in Fig. 3 show that the CO_2 concentration which causes the greatest incidence of exposure seizures is the same for young and adult rats, and that the incidence of seizures is greater in adult than in young animals. Neither of the findings depicted in Fig. 3 is consistent with the notion that young animals are more sensitive to CO_2. It is also of interest to note that the results summarized in Fig. 3 and 4 are not the same as those seen in adult animals treated with a carbonic anhydrase inhibitor, since acetazolamide treatment decreases the incidence of withdrawal seizures at any given CO_2 level and makes any given CO_2 tension more effective in producing exposure convulsions in older rats(4). At present, no explanation for these seemingly aberrant results is forthcoming. It should be remembered, however, that the data for young rats summarized in both Fig. 3 and 4 represent those obtained in a heterogeneous sample of rats and may not faithfully depict the responses of any single age group. Also, there are probably different underlying mechanisms responsible for exposure and withdrawal seizures(4). Thus, until more precise data are available, it is perhaps unwise to extrapolate the present results too far with regard to the question of the relative sensitivity of very young rats exposed to high concentrations of CO_2.

Summary. The incidence of seizures caused by exposure to and withdrawal from CO_2 concentrations ranging between 5 and 50% was determined in adult rats and immature rats less than one month old. It was found that neither exposure nor withdrawal seizures could be elicited in animals less than 16 days of age, even though the same CO_2 atmospheres produced seizures in older animals. It was concluded that the lack of response of very young rats to CO_2 was primarily a manifestation of the general central nervous system immaturity of the infant rat, even though other factors, *e.g.*, lack of CO_2 equilibration, might also affect the CO_2 response.

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