THE ROLE OF CARBON DIOXIDE IN THE NERVOUS SYSTEM

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CHANGES in P_{CO_2} in an organism are capable of producing marked alterations in the functioning of the central nervous system. The gross actions of CO_2 upon central nervous system activity, however, are not simple because they include both depression and excitation, ranging from anesthesia to seizures. Carbon dioxide is not the only gas capable of affecting the activity of the central nervous system, but CO_2 is of special interest and importance because it is one of the principal end products of metabolism.

The mechanism of action of CO_2 on the nervous system is not understood; however, the gas participates in several reaction systems. The most recognized function of CO_2 is in the principal buffering system of the body; changes in P_{CO_2} result in changes in acid-base balance. Some of the significance of physiological variations in *p*H is probably derived from the dependence of enzyme activity on hydrogen ion concentration, and hence changes in *p*H can influence the rates of various metabolic reactions.

Carbon dioxide may also serve as a metabolic substrate in such known reactions as "CO_2-fixation" and carbamate formation. The rate of incorporation of CO_2 is at least partially dependent upon P_{CO_2}; therefore, any situation altering P_{CO_2} could conceivably affect the kinetics of such reactions. The significance of these reactions is not understood, except in the case of carbaminohemoglobin formation.

Carbon dioxide is not chemically or physiologically inert; consequently, the maintenance of a constant level of CO_2 is an important physiological function. The steady-state level of CO_2 in tissue depends upon two factors: (1) the rate of its metabolic production, and (2) the rate of its elimination. Any condition which influences either one of these factors can change the level of CO_2 and hence can produce profound functional alterations.

Numerous substances are capable of influencing either the rate of production or of elimination of CO_2. This review will present evidence that such substances exert a profound effect on the nervous system due to the fact that they alter the concentration of CO_2 in the brain. The brain is particularly sensitive to changes in CO_2 concentration and slight variations produce marked neurophysiological, neurochemical, respiratory, and vascular responses. Only neurophysiological and neurochemical responses will be discussed in this paper.

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THE EFFECTS OF EXOGENOUS CARBON DIOXIDE ON BRAIN FUNCTION

NEUROPHYSIOLOGICAL EFFECTS: *Effects of Various Concentrations of Carbon Dioxide on Brain Excitability.* The effects of various concentrations of CO_2 on brain excitability as measured by the threshold for electrically- and chemically-induced seizures have been tested. The effects on electroshock seizure threshold (EST) are shown in figure 1.[1] The EST of both mice and rats was elevated by increasing the concentrations of inhaled CO_2 until a peak was reached at 12.5 per cent CO_2 for mice and 15 per cent CO_2 for rats. Thereafter the EST declined until at 30 per cent CO_2 it reached the pretreatment level. In mice, a concentration of 40 per cent CO_2 lowered the EST. In rats approximately 30 per cent CO_2 induced seizures and hence the EST could not be determined. The seizures produced by moderate concentrations of CO_2 first appeared at a concentration of 25 to 30 per cent, reached a peak incidence at 35 per cent, and thereafter rapidly declined concomitant with the marked central depression which occurred at higher CO_2 concentrations. The animals exposed to concentrations of 40 per cent CO_2 appeared to be anesthetized.

The experiments on the effects of various concentrations of CO_2 on EST and overt activity indicate that with regard to excitability of the nervous system CO_2 exerts a triphasic effect which can be explained as follows: The

first phase, that of decreased cortical excitability, appears to be due to direct cortical depression and occurs with low concentrations of the gas; cortical excitability progressively decreases with increasing concentrations of CO_2. The *second phase*, that of increased cortical excitability, appears to be due to the activation of subcortical centers by CO_2. Gellhorn and co-workers [2, 3] have demonstrated that cortical excitability can be enhanced by stimuli which activate the hypothalamic-cortical system, a part of the reticular activating system [4] involved in arousal mechanisms and activation of the EEG. It thus appears likely that CO_2 stimulation of the hypothalamic-cortical system enhances cortical excitability to the extent that localized clonic seizures occur. Although the concentrations of CO_2 required for activation of subcortical centers are higher than those necessary for cortical depression,[5] the stimulation seems to be sufficiently intense to overcome mild cortical depression and to produce seizures. The hyperexcitability in this phase is apparently enhanced by the release of epinephrine and of adrenal cortical hormones of the cortisol type as a result of CO_2-induced stimulation of the hypothalamus. Previous work has shown that both of these classes of hormones enhance excitability of the cortex.[6, 7] The *third* or anesthetic phase in response to CO_2 inhalation is attributed to the marked cortical and subcortical depression caused by high concentrations of the gas. The inhibition of subcortical centers by high CO_2 concentrations is probably due to inhibition of the central reticular activating system in a manner similar to the blockade produced by barbiturates and certain other anesthetic agents.[8, 9]

Other evidence for an effect of CO_2 on excitability is provided by the fact that low concentrations of this gas abolish the tonic extensor phase of the maximal electroshock seizure (MES) pattern in experimental animals (mice, rats, and rabbits) [1, 10-14] and in man.[15] This can be adduced as evidence for a decrease in excitability (first phase of CO_2 action). In addition, low concentrations of CO_2 protect against chemically-induced seizures produced by such agents as pentylenetetrazol,[10, 14, 16] strychnine,[16, 17] insulin,[18, 19] picrotoxin,[16] camphor,[16] coriamyrtin,[16] and absinthe.[16]

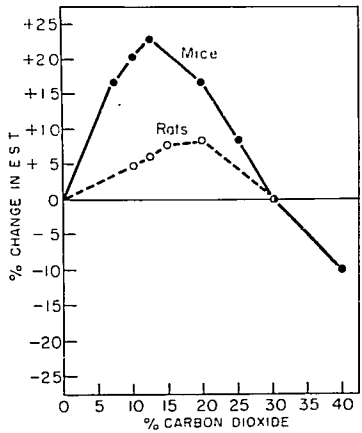

FIG. 1. Effect of inhalation of various concentrations of CO_2 on electroshock seizure threshold (EST) of mice and rats.[1] *Ordinate* is percentage change in EST referred to control values. *Abscissa* is inhaled CO_2 concentration in per cent. See text for discussion.

Effects on the EEG. Inhalation of CO_2 in low concentrations usually results in an increased frequency of cortical potentials and can interrupt petit mal seizure discharges as well as the clinical seizure.[20, 21] On the other hand, decreased P_{CO_2} (induced by hyperventilation) results in slowing of frequency with the appearance of high voltage, slow-wave discharges particularly in the frontal leads. Exposure to high (anesthetic) concentrations of CO_2 usually causes flattening of the EEG in man and in experimental animals.[1, 17] For example, in rats, 50 per cent CO_2 sharply depressed the EEG activity, and produced flattening of the normal waves. In contrast, when 30 per cent CO_2 was inhaled the EEG record showed intermittent bursts of high-voltage discharges. These bursts were of short duration and coincided with the episodes of overt clonic seizure activity which characterizes the hyperexcitable phase induced by CO_2.[1] Thus the triphasic effect of CO_2 on the nervous system is also evident from the EEG measurements.

Effects on the Spinal Cord. The effects of CO_2 inhalation on spinal cord activity and on polysynaptic and monosynaptic pathways have been studied by a number of investigators. Inhalation of 10 per cent CO_2 abolished the knee-jerk reflex of intact but not of spinal or decerebrate animals, and hence the inhibitory effect was thought to be of cerebral origin.[22] However, a direct effect of CO_2 on the cord itself has been demonstrated by other workers. Carbon dioxide in a concentration of 8.9 per cent induces a greater depression of monosynaptic than of polysynaptic spinal reflexes.[23] This observation was confirmed and extended by Esplin *et al.*[24] who found that low concentrations of CO_2 or acetazolamide, in striking contrast to other known depressant drugs, selectively depress monosynaptic (2N) pathways in the spinal cord of cats. With 10 per cent CO_2, 2N spike was reduced about 60 per cent, whereas polysynaptic response area was reduced only 5 per cent. In spite of their marked depressant effect, these agents are without influence on synaptic recovery, and they depress post-tetanic potentiation (PTP) only slightly. These data have been interpreted[25] to mean that CO_2 (and acetazolamide) affects some neuronal process which is more critically concerned in the spinal cord monosynaptic pathway than in polysynaptic circuits. The most outstanding difference between the component synapses of these two systems is that the safety factor with which transmission occurs is lower for monosynaptic than for polysynaptic pathways.[26] It was tentatively suggested, therefore, that CO_2 (and acetazolamide) decreases the safety factor of transmission, and that depression would be produced by this drug at all synapses in which the safety factor is low, as it is in the spinal monosynaptic pathways.

Carbon Dioxide-Withdrawal Seizures.[1] Rats exhibit clonic seizures usually within one minute after removal from anesthetic concentrations of CO_2 (35 per cent or higher). These seizures involve the face, jaw, and foreleg muscles and are accompanied by salivation, lacrimation, and often piloerection. The seizures appear to result from the fact that on withdrawal from anesthetic doses of CO_2 the rats pass through the hyperexcitable (middle) phase of CO_2 effect, and exhibit seizures. Such seizures are prevented by anticonvulsant agents like phenobarbital, trimethadione, and acetazolamide, and are enhanced by diphenylhydantoin in low doses.[1]

Metabolic Effects of Exogenous Carbon Dioxide: *Effects on Electrolytes and Acid-Base Balance.* Inhalation of a concentration of CO_2 (12.5 per cent) which elevates EST (first phase) results in the following changes in brain electrolyte and acid-base composition.[27] There is a decrease in intracellular Na concentration (increase in ratio of extracellular to intracellular Na, Na_e/Na_c) and an increase in intracellular K concentration. Cellular HCO_3^- concentration increases; however, H_2CO_3 concentration increases to a greater extent and hence brain cell *p*H decreases. Such changes are consistent with the decrease in excitability which is observed in the animal exposed to this concentration of CO_2, and add additional support to the observations previously described (see Woodbury and Esplin[25] for summary) of a direct correlation between brain excitability and the Na_e/Na_c.

When rats were exposed to 50 per cent CO_2, there were changes in electrolyte and acid-base balance in the brain.[1, 28] There was a decrease in brain intracellular Na and K (increase in Na_e/Na_c and decrease in K_e/K_c), and a cellular acidosis (*p*H 6.41) resulted which was characterized by a large increase in both HCO_3^- and H_2CO_3. The total cation (assumed to be Na plus K) content in the brain cells was decreased, probably as a consequence of the reduction in organic anions resulting from the intracellular acidosis produced by CO_2. Nonbicarbonate, organic polyvalent anions (assumed to be total cation minus bicarbonate) decreased markedly in the brain of rats exposed to 50 per cent CO_2. The decrease in organic anions is probably due to a shift in the ionization of the cellular proteins toward the acid side of their isoelectric point as a result of the acidosis. Furthermore, additional observations from our laboratory have demonstrated that 50 per cent CO_2 decreases the brain concentrations of glutamic and aspartic acids (see below). Thus less total anion is present, necessitating a decrease in cation in order to maintain electrical neutrality.

Thirty seconds after abrupt withdrawal of

rats from an atmosphere of 50 per cent CO_2, the lowered intracellular brain Na concentration increases rapidly, but K concentration remains decreased; seizures appear at this time. Thus there is a markedly increased Na influx into brain cells associated with the rapid loss of CO_2 and the occurrence of seizures. The rapid loss of CO_2 is associated with an increase in *p*H of the brain cells to the level of that in the cells of rats exposed to only 30 per cent CO_2 (*p*H 6.76). In addition to the Na change which occurred on withdrawal from 50 per cent CO_2, there were changes in H_2CO_3 and HCO_3^- concentrations. The values for HCO_3^- and H_2CO_3 in plasma decreased below those observed in rats exposed to 30 per cent CO_2, and the brain intracellular H_2CO_3 and HCO_3^- concentrations fell into the same range of values as observed in the animals exposed to 30 per cent CO_2. The intracellular HCO_3^- concentration in the withdrawal animals was increased over that seen in the rats exposed to 50 per cent CO_2. Since rats exposed to 30 per cent CO_2 and those abruptly withdrawn from 50 per cent CO_2 exhibit seizures, and since the acid-base and presumably the electrolyte changes are similar in the two groups of rats, it is tempting to ascribe the seizures to either the electrolyte changes or the acid-base changes, or both. The seizures in the CO_2-withdrawal animals are thus associated with a rapid influx of Na and probably HCO_3^- into brain cells and consequently a decrease in brain Na ratio. This influx of Na into the brain appears to be of sufficient magnitude to precipitate the seizures and may be presumed to be the same process that renders the peripheral nerve axon unstable; the passage across the membrane of sufficient charge explosively increases permeability to Na and an action potential is generated. In the brain, where millions of neurons are involved, seizure activity might be the consequence.

The cause of the influx of Na into the brain during CO_2-withdrawal is unknown, but the following hypothesis can explain the results. The sudden and marked loss of CO_2 from the cell is unaccompanied by an equal loss of HCO_3^- because the cell membrane is less permeable to HCO_3^- than CO_2; as a result, cellular *p*H increases. The increase in cellular *p*H probably decreases the ionizable calcium of the cell; such a decrease is known to enhance the permeability of the cell to Na. Therefore Na pours into the cell as the *p*H increases in order to satisfy the increase in anions resulting from the excess HCO_3^- in the cell and the increase in the anion charges on the protein. Evidence for a role of calcium in precipitating the seizures is provided by the fact that administration of calcium chloride to rats protects against both withdrawal seizures and 30 per cent CO_2-induced seizures (unpublished observations). Also, Carey and Schaefer[29] have shown that the plasma Ca level markedly decreases immediately after withdrawal of rats from an atmosphere of 50 per cent CO_2.

TABLE 1

EFFECT OF INHALATION OF VARIOUS CONCENTRATIONS OF CARBON DIOXIDE ON FREE AMINO ACID LEVELS IN CEREBRAL CORTEX OF RATS *

Treatment	Glutamic Acid (mg. %)	Aspartic Acid (mg. %)	Glutamine (mg. %)	GABA (mg. %)
Controls	103±4†	54±7	47±4	21.5
12.5% CO_2	110±6	70±9 (0.05)	39±4	36
50% CO_2	50±13 (0.02)	48±5	39±4	32
Withdrawal from 50% CO_2	58±1 (0.01)	42±4	38±6	19

* From unpublished observations of Woodbury, D. M., Vernadakis, A. and Timiras, P. S.

† Values are mean ± standard error. Numbers in parentheses are *P* values for significance of treated animals from the controls.

Effects of Carbon Dioxide on Carbohydrate and Amino Acid Metabolism. The effects of inhalation of various concentrations of CO_2 on free amino acid concentrations in brain of rats are shown in table 1. Inhalation of 12.5 per cent CO_2 resulted in a slight increase in glutamic acid, a marked increase in aspartic acid, and a slight decrease in glutamine concentrations. In addition, the concentration of γ-aminobutyric acid (GABA) was increased considerably but the data are insufficient to establish the level of significance of this change. The increase in glutamic acid plus aspartic acid is consistent with an increase in

total cation found in the brain of rats treated with 12.5 per cent CO_2. However, this accounts for only part of the increase in cation; the remainder is due to the marked increase in HCO_3^- concentration.[27] Thus quantitatively the increase in cation content of the cell is accounted for mainly by (or is a consequence of) an increase in the labile anions (glutamic acid, aspartic acid, bicarbonate).

In contrast to the animals receiving 12.5 per cent CO_2, the rats exposed to 50 per cent CO_2 showed a marked decrease in glutamic acid, a small decrease in aspartic acid, a slight decrease in glutamine, and an increase in GABA concentrations. The decrease in glutamic acid plus aspartic acid concentrations counterbalances the marked increase in HCO_3^- concentration [28] induced by this concentration of gas. However, the decrease in total cation was accompanied by a marked cellular acidosis (pH 7.04 to 6.41) which would result in a decrease in polyvalent anion, consequently maintaining electrical neutrality.

On abrupt withdrawal from 50 per cent CO_2 there was a slight increase in glutamic acid, a slight decrease in aspartic acid and glutamine, and a striking decrease in GABA concentrations. Since the withdrawal animals were killed and the values measured just prior to the onset of seizures, the values for GABA would be expected to decrease even more as the seizures develop.

The values for GABA in the different experimental situations correlate fairly well with the level of excitability of the animals in the same situation. Both 12.5 per cent and 50 per cent CO_2 decrease excitability and increase GABA concentration, but the increase in GABA is greater in the 12.5 per cent CO_2 group which is the more excitable of the two groups. On withdrawal from 50 per cent CO_2, excitability increases markedly and the animals exhibit seizures, at which time the GABA concentration decreases markedly. Therefore, in all three of these experiments there is a rough inverse correlation between the level of excitability and the GABA concentration in the brain. These data are consistent with additional observations by us [29] and others (see Tower [31] for summary) which suggest the same correlation.

It is evident from these data that CO_2 has a profound influence on the amino acid metabolism of the brain, and that such effects are related to the electrolyte and acid-base metabolism of the brain and ultimately to the level of excitability of the nervous system.

Since selective electrolyte accumulation in cells has been shown to be an active process which requires energy, and since CO_2 affects electrolyte metabolism, it is likely that CO_2 exerts an influence on reactions yielding or transferring energy. It is of interest in this regard that Bain and co-workers [32, 33] have shown that inhalation of CO_2 (10 to 30 per cent) in normal cats resulted in lowered brain lactate and pyruvate levels with little or no effect on glucose concentration and on levels of high-energy phosphate. However, in cats undergoing electroshock seizures during the inhalation of CO_2, the expected rise in brain lactate, pyruvate, and inorganic phosphate concentrations was limited; the expected fall in high-energy phosphate level was prevented, and a rise in brain glucose concentration occurred. The brain/plasma glucose ratio, nevertheless, remained the same. These results might be interpreted to mean that CO_2 inhibits the utilization of glucose by the brain, with a consequent decrease in brain pyruvate and lactate concentrations. Inhibition of a major energy-yielding process would delay recovery from an energy-consuming process, such as occurs during a maximal electroshock seizure. It is of interest that CO_2 does indeed delay recovery from MES in mice.[10] The fact that the glutamic acid decreases markedly in rats exposed to 50 per cent CO_2 is also consistent with the hypothesis that CO_2 inhibits glucose utilization because glutamic acid can be used as a substrate for oxidative metabolism in the brain. It is likely that, under conditions which inhibit glucose oxidation, glutamic acid substitutes for glucose and thereby maintains cellular respiration and brain function.

Drugs Which Influence Endogenous Carbon Dioxide Metabolism: Their Effect on Brain Function

Drugs Which Increase Carbon Dioxide Production: Further evidence for a role of carbon dioxide in regulating the excitability of the nervous system is provided by the use of

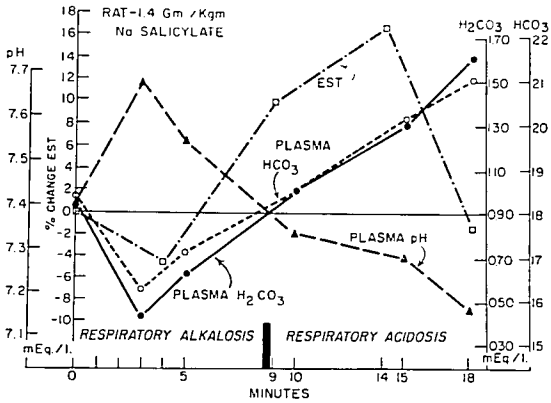

FIG. 2. Effect of acute intraperitoneal administration of Na salicylate on electroshock seizure threshold (EST) and acid-base balance of rats at various times after injection. *Ordinates* on left are blood *p*H values and percentage change in EST referred to pretreatment control values; on right, plasma H_2CO_3 and HCO_3^- concentrations in mEq./l. *Abscissa* is time in minutes. See text for discussion.

agents which influence the endogenous production of CO_2. For example, salicylates enhance both the oxygen uptake and the carbon dioxide production of the body as a whole and of various tissues (see review by Smith [31]). The increase in CO_2 output is generally accompanied by a stimulation of respiration which more than compensates for the increased production and lowers P_{CO_2}. As a result, a respiratory alkalosis develops. However, if the dose of salicylate is large, respiratory depression soon follows and P_{CO_2} increases since CO_2 production exceeds the capacity of the respiratory system to eliminate the excess CO_2. As a result, a respiratory acidosis develops. It was of interest, therefore, to study the acute effects of sodium salicylate on the central nervous system during the state of respiratory alkalosis when the P_{CO_2} was low and during the state of respiratory acidosis when the P_{CO_2} was high. If the CNS effects were related to the P_{CO_2}, then such effects should follow changes in endogenous P_{CO_2}. The experiments were carried out in rats given an LD_{50} of Na salicylate (1.4g./kg.).[35] The EST was measured and electrolyte and acid-base values were determined at various times after intraperitoneal administration of the salicylate. The results are shown in figure 2. An initial respiratory alkalosis developed within 2 minutes and lasted for 9 minutes; this was followed by a respiratory acidosis which was severe at 15 minutes when seizures followed by death occurred. Consequently, the seizures appeared during the stage of respiratory acidosis, not during respiratory alkalosis. The EST decreased in the alkalotic phase, increased in the acidotic phase and decreased suddenly just prior to the onset of seizures. The changes in EST paralleled changes in plasma (and brain cell) carbonic acid concentration. The results from the salicylate experiments indicate that a lower than normal P_{CO_2} increases brain excitability, whereas a higher than normal P_{CO_2} decreases brain excitability up to a certain point and thereafter increases it to the extent that seizures occur. Thus the curve for the EST changes produced by salicylate, after the initial excitatory phase due to the low P_{CO_2}, resembles exactly the first two stages of the effect of exogenous CO_2 on EST as shown in figure 1. These data, therefore, suggest that the EST changes associated with high doses of salicylate are secondary to the changes in carbonic acid concentration in plasma and brain cells. In

addition, with the doses of salicylate used, there is also some inhibition of brain carbonic anhydrase (unpublished observations), and this effect tends to enhance the accumulation of CO_2 in the brain.

Other drugs which increase CO_2 production also have effects on the nervous system. 2,4-Dinitrophenol (DNP) produces central nervous system depression in rats but does not produce spontaneous seizures even in lethal doses. DNP also lengthens the tonic flexor phase and shortens the tonic extensor phase of the MES, facts indicative of anticonvulsant activity. Apparently DNP possesses the first phase of the CO_2 triphasic response (unpublished observations); however, in combination with other drugs which alter CO_2 metabolism, DNP produces more profound CNS effects (see next section).

Drugs Affecting Carbon Dioxide Release From Brain (Carbonic Anhydrase Inhibitors): *Neurophysiological Effects.* Although carbonic anhydrase is found in the central nervous system,[36] its precise role in this tissue has not been elucidated. However, the fact that carbonic anhydrase inhibitors, such as acetazolamide, have marked effects on central nervous system function suggests that this enzyme is involved in the regulation of brain excitability.

Thus, acetazolamide has been shown to be a potent anticonvulsant agent, both in laboratory animals and in epileptic patients. In experimental animals, acetazolamide abolishes the tonic extensor component of the MES in mice and rats,[13, 37-40] and potentiates the effects of CO_2 on MES.[37] The anticonvulsant action of acetazolamide is not abolished by nephrectomy and hence this action is independent of the systemic acidosis which results from the inhibition of carbonic anhydrase activity in the kidney.

In addition, acetazolamide, in higher doses than are necessary to modify MES, elevates EST about 16 per cent. But still higher doses diminish the elevation in EST, thus indicating, as is the case with moderately high concentrations of CO_2, an excitatory component of action.[12] However, acetazolamide even in extremely large doses does not produce seizures as do moderate concentrations of CO_2, but the drug does produce seizures when combined with small doses of CO_2 (see below). Fulbriard and Gangloff [41] studied the effects of acetazolamide on the threshold voltage for stimulation of the cortex, diencephalon, and rhinencephalon in rabbits. They observed that there was a slight decrease in the excitability of the cortex and a marked decrease in the excitability of the diencephalon. Thus acetazolamide, like CO_2, exhibits effects on cortical and subcortical centers. The drug has also been reported to obtund audiogenic seizures.[42]

A number of reports attest to the efficacy of acetazolamide against various types of epilepsy. The drug was first introduced for the therapy of epilepsy by Bergstrom *et al.*[43] and subsequent reports [44-47] confirmed its effectiveness in all types of epilepsy (grand mal, petit mal, and psychomotor seizures).

In addition, acetazolamide, like CO_2, protects against seizures evoked by various pharmacological agents. It reduces the intensity of spinal discharges induced by convulsive doses of strychnine (Esplin, personal communication), and it antagonizes pentylenetetrazol-induced seizures, although only to a limited extent (unpublished observations). Finally, acetazolamide protects against seizures produced by inhalation of 30 per cent CO_2 or by withdrawal from 50 per cent CO_2.[1] It is evident from the comparative observations that acetazolamide and CO_2 possess a wide and similar spectrum of anticonvulsant action on the CNS.

Further evidence that the action of acetazolamide on the CNS is identical to that of CO_2 is derived from the influence of the drug on spinal cord activity. As discussed previously, acetazolamide produces exactly the same effect on the spinal cord as does CO_2, and potentiation occurs when it is given in combination with CO_2.[21] Thus acetazolamide, like CO_2, selectively depresses monosynaptic (2N) pathways in the spinal cord without affecting synaptic recovery or post-tetanic potentiation.

There is much experimental [12, 37, 48] and clinical [45, 46] evidence that tolerance develops to the anticonvulsant effects of acetazolamide. If the anticonvulsant activity of acetazolamide is due to a localized increase in P_{CO_2} in neuronal cells, it follows logically that the tolerance which is known to develop to the anticonvu-

s.at effect of acetazolamide should be accompanied by tolerance to the anticonvulsant effect of CO_2. That this assumption is correct was demonstrated by Koch and Woodbury who found that tolerance developed to the repeated administration of acetazolamide and that cross tolerance concurrently developed to CO_2.[13] These data provide further strong evidence for a common mode of action of CO_2 and acetazolamide.

Since nitrate ion (NO_3^-) in rather high concentrations (24 mEq./l.) has been shown by Roughton and Booth [10] to inhibit carbonic anhydrase *in vitro*, it was of interest to test the anticonvulsant effect of this ion which is chemically unrelated to acetazolamide. If carbonic anhydrase inhibitors are acting by causing accumulation of CO_2 in brain cells, then it follows that such agents should be effective anticonvulsants and tolerance should develop to the anticonvulsant effect on repeated administration. Such is the case for NO_3^-; it protects against MES in mice and rats in the same ratio as does CO_2 (5.7) and tolerance develops to its anticonvulsant effect.[13]

Millichap [50, 51] and Millichap and co-workers [52] have presented convincing evidence that carbonic anhydrase is of functional significance in the development of the electroshock seizure pattern in developing rats and guinea pigs. They suggested that this enzyme is important in the development of the ability to exhibit maximal tonic convulsions, in which a generalized spread of the seizures discharge is probably involved, but that the enzyme probably is not involved in the capacity to exhibit clonic seizures, which involve localized neuronal discharges. This suggestion is supported by their observations that the hyperkinetic behavior induced by electroshock in rats 10 days old or less is refractory to acetazolamide, that the clonic seizures induced by electroshock in 10 to 20-day old rats were abolished only by very large doses of this drug, and that the tonic type of seizures seen in 21-day or older rats was abolished by low doses of acetazolamide.

Further evidence that carbonic anhydrase is involved in the spread of discharges was presented by Davenport [53] who observed in rabbits treated with a carbonic anhydrase inhibitor, thiophene-2-sulfonamide, that local stimulation of the cerebral cortex did not affect the general electrical activity of the stimulated area.

Carbonic anhydrase inhibitors other than acetazolamide have also been shown to have effects on the nervous system. Sulfanilamide abolishes the tonic extensor component of the MES in experimental animals [28] and possesses a weak antiepileptic effect in man.[54, 55] Methazolamide and ethoxzolamide, congeners of acetazolamide, exhibit anticonvulsant activity in experimental animals [39, 40] and in man.[56] Various other substances which are carbonic anhydrase inhibitors *in vitro* or *in vivo* have also been demonstrated to possess anticonvulsant activity. These include salicylate, NO_2^-, I^-, and Br^-,[57] but it is yet to be established conclusively that they exert their CNS activity through inhibition of this enzyme.

Metabolic Effects of Carbonic Anhydrase Inhibitors. The observations of Millichap *et al.*[58] have established that there is a direct relation between anticonvulsant effect and inhibition of brain carbonic anhydrase in mice. They compared the activity of acetazolamide with that of sulfanilamide, a much less potent inhibitor, and found that acetazolamide was approximately twice as potent as sulfanilamide, both as an anticonvulsant and as an inhibitor of brain carbonic anhydrase. In addition, the time of maximum degree of enzyme inhibition produced by these two drugs corresponded with their time of peak anticonvulsant effect.

These data support the hypothesis that carbonic anhydrase is integrally involved in regulation of CNS excitability. If this is the case, then inhibition of the enzyme should cause metabolic changes which are related to CNS activity. The following data show that this assumption is correct and lead to a theory of the mechanism of action of acetazolamide.

The evidence presented above indicates that the CNS effects of acetazolamide are mediated through CO_2 accumulation. Hence treatment with acetazolamide should increase the concentration of total CO_2 in the brain. The data presented in table 2 show that this drug does indeed elevate the CO_2 content of brain. In high doses, however, the CO_2 level is lowered and this is consistent with the lower EST of rats given high doses of acetazolamide.[13] Therefore, it appears from these data, and from those of the effect of salicylate on EST

TABLE 2

EFFECT OF ACETAZOLAMIDE ON TOTAL CO_2 CONCENTRATION IN CEREBRAL CORTEX OF RATS

Treatment	Total Intracellular CO_2 in mM/kg. Cell H_2O	Reference
Adults		
control	14.7	27
acetazolamide (20 mg./kg.)	17.3	
12.5% CO_2	22.7	
	Total CO_2 in mM/kg. wet brain	
24 day old		
control	18.6	53
acetazolamide	21.7	
Adults		
control	11.5	Heninger and
acetazolamide (10 mg./kg.)	13.2	Woodbury, unpublished
acetazolamide (200 mg./kg.)	10.7	observations

presented previously, that the excitability of the brain as measured by EST is inversely proportional to the CO_2 level in the brain.

Inasmuch as exogenous CO_2 profoundly influences electrolyte metabolism of the brain and the resulting changes in electrolytes are correlated with brain excitability, it was of interest to determine the effects of acetazolamide on brain electrolytes. The results [27] will be only briefly summarized here. The effects of acetazolamide on brain electrolytes were studied in nephrectomized animals in order to eliminate all renal effects of the drug. There was a significant decrease in the concentration of intracellular brain Na and an increase in brain cell K; hence both the Na_c/Na_e and K_c/K_e ratios were increased. These effects are the same as those produced by inhalation of 12.5 per cent CO_2 (see above). Animals rendered tolerant to acetazolamide showed an increase in both cellular Na and K concentrations and were hyperexcitable. These data lend support to previous observations that there is a correlation between Na_c/Na_e and brain excitability (see references 6, 25, and 27 for summary).

In order further to analyze the effect of acetazolamide on cellular Na, the influence of the drug on the rate of uptake of isotopic Na was studied in nephrectomized rats.[27] Acetazolamide decreased the uptake of Na^{22} by brain tissue as compared with the controls. The finding of an increased Na_c/Na_e coupled with a decreased rate of Na turnover has been interpreted to mean that acetazolamide decreases the rate of Na influx into brain cell, *i.e.*, decreases the permeability of the cell membrane to Na (see Koch and Woodbury [27] for evidence and review of literature). The relation of this effect of acetazolamide on brain cell Na metabolism to its mechanism of action is discussed below, after the role of carbonic anhydrase in the nervous system has been considered.

An impressive body of evidence has been collected which indicates that the neurophysiological and neurochemical effects of acetazolamide are mediated through CO_2 accumulation. This evidence will now be summarized. (1) Acetazolamide and CO_2 have the same spectrum of activity on experimental seizures and on the spinal cord. In addition, the effects of acetazolamide on the MES and on spinal cord synaptic transmission are potentiated by CO_2. (2) The changes in brain excitability induced by various concentrations of CO_2 are enhanced by acetazolamide (fig. 2). (3) Seizures induced by agents which enhance CO_2 production by brain cells (salicylate, DNP) are enhanced by acetazolamide. (4) Acetazolamide increases the total CO_2 concentration in brain cells. (5) Acetazolamide produces the same effects on brain electrolyte concentrations as does CO_2. (6) The effects of acetazolamide and CO_2 on brain amino acid concentrations are similar. (7) Rats which develop tolerance to the anticonvulsant effect of acetazolamide on MES are also tolerant to the anticonvulsant effect of CO_2.

COMBINATIONS OF DRUGS WHICH INCREASE CARBON DIOXIDE PRODUCTION OR INHIBIT ITS RELEASE: THEIR EFFECTS ON BRAIN FUNCTION: Further support for the concept that salicylate, DNP, and acetazolamide alter brain function through their influence on CO_2 metabolism is provided by experiments in which various combinations of these drugs were tested for their effect on CNS activity. The results of these experiments are summarized in table 3.

The effects of the drugs, alone or in combination, were tested on the MES pattern and on the development of clonic seizures similar to those produced by 30 per cent CO_2 as described above. When either salicylate, DNP, or acetazolamide was combined with CO_2 there was a marked potentiation of the anticonvulsant effect of CO_2 on MES. The drugs were combined with CO_2 in doses which by themselves had very little effect on the MES (table 3). In addition, the drugs when combined in subconvulsive concentrations with a subconvulsive concentration of CO_2 (15 to 20 per cent) produced severe clonic seizures (table 3). Thus both the anticonvulsant effects of low CO_2 concentrations and the seizure-producing effects of moderate doses of CO_2 are enhanced by salicylate and DNP, both of which increase CO_2 production, and by acetazolamide which inhibits CO_2 elimination from brain cells.

The combination of salicylate and DNP also enhanced the anticonvulsant effect of either one of the drugs given alone, and DNP also enhanced the seizures produced by salicylate. Acetazolamide and DNP are similar in the respect that either agent by itself is only capable of producing depression and not seizures. The combination of acetazolamide and DNP in low doses results in an enhancement of their respective anticonvulsant effects; in contrast, the combination in high doses results in the production of mild clonic seizures. The rats receiving this combination were identical in all their responses with those of animals receiving salicylate alone (salivation, diuresis, piloerection, seizures). Since the combination of DNP and acetazolamide produces effects identical with those of salicylate, and salicylate enhances CO_2 production and also inhibits brain carbonic anhydrase, it appears that the CNS effects of these drugs are mediated through CO_2 accumulation. The reason salicylate produces powerful effects on the CNS is that it possesses both the ability to increase CO_2 production and to inhibit CO_2 release.

It might be argued that the enhanced effect on the CNS produced by DNP, salicylate, or acetazolamide in combination with CO_2 is due to the acidosis produced by the gas and not the P_{CO_2}. The resultant acidosis would reduce the charge on the drugs which are weak acids; consequently, a higher percentage of the molecules would be in the noncharged form. It has been demonstrated (see Brodie and Hogben [58] for summary) that uncharged molecules penetrate the brain more readily than do charged ones. Therefore, the acidosis would increase the concentration of DNP, salicylate, or acetazolamide in the brain and thereby enhance the CNS effect of CO_2. Although this may occur to a certain extent, it cannot be the explanation of the findings shown in table 2. When a degree of acidosis comparable to that caused by CO_2 was produced by ammonium chloride (NH_4Cl), only equivocal effects were produced on MES, and no seizures appeared. When NH_4Cl was combined with salicylate in a dose which produced the same degree of acidosis as does the combination of CO_2 and salicylate, neither the anticonvulsant nor the convulsant effect of salicylate was enhanced. It seems evident, therefore, that it is the CO_2 and not the H^+ concentration which produces the CNS effects of salicylate, DNP, and acetazolamide. In addition, the enhancement of effects produced by the combination of DNP with salicylate and

TABLE 3

CENTRAL NERVOUS SYSTEM EFFECTS OF DRUGS WHICH ENHANCE CARBON DIOXIDE PRODUCTION OR INHIBIT ITS RELEASE FROM BRAIN CELLS

Drug Treatment	Anticonvulsant Effect on MES (Low Doses)	Effect on CNS activity (High Doses)	
		Clonic Seizures	Depression
15–20% CO_2	+++	−	+
30–40% CO_2	−	+	−
Salicylate	−	+	−
2,4-Dinitrophenol (DNP)	+	−	+
Acetazolamide	+++	−	+ (very high doses produce some excitation)
CO_2 (15–20%) + Salicylate	++++	+++	−
CO_2 + DNP	++++	+++	−
CO_2 + Acetazolamide	++++	+++	−
Salicylate + DNP	++	+++	−
Salicylate + Acetazolamide	++++	+++	−
DNP + Acetazolamide	++++	+ (resembles salicylate)	−
NH_4Cl*	±	+ (only in extremely large doses)	−
NH_4Cl + Salicylate	+	+	−

* Dose necessary to produce an acidosis equivalent to that produced by CO_2.

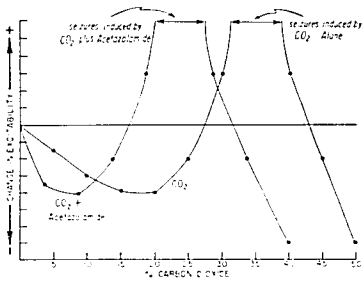

FIG. 3. Effect of CO_2 alone and in combination with acetazolamide, on brain excitability. *Ordinate* is change in excitability on an arbitrary scale. *Abscissa* is inhaled CO_2 concentration in per cent. See text for discussion.

by the combination of acetazolamide with salicylate or DNP cannot be due to acidosis because there was no appreciable change in plasma *p*H when these experiments were performed.

Finally, the observation that a combination of DNP and acetazolamide causes clonic seizures indistinguishable from these produced by salicylate or CO_2 cannot be explained by increased penetration of DNP or acetazolamide into the brain as a result of an acidosis produced by one of these agents. DNP by itself, in doses as high as an LD_{50}, causes only mild depression, as is indicated by its anticonvulsant effect on MES as well as by the overt signs and symptoms (unpublished observations). Also acetazolamide causes only depression, except in very large doses which produce some excitatory phenomena. In the doses used in the combination experiments, only depression would have been expected even if three times as much DNP or acetazolamide had entered the cell as a result of an acidosis caused by either of these agents. However, since seizures rather than depression occurred, it appears that these two drugs increased the CO_2 level of the brain sufficiently to put the animals into the second or hyperexcitable phase of CO_2 effect.

Further evidence for a role of endogenous CO_2 in regulating CNS function is provided by experiments in which acetazolamide is combined with various concentrations of CO_2. These data are summarized in figure 3. The ordinate is brain excitability in arbitrary units and the abscissa is per cent CO_2. It can be seen from this figure that acetazolamide potentiated the anticonvulsant effect of a low concentration of CO_2 [27]; but, when given to rats which were exposed to 15 per cent CO_2, the drug caused seizures. However, when acetazolamide was administered to rats exposed to 30 per cent CO_2, the seizures were prevented, an indication that the animals were elevated to the third or anesthetic phase of CO_2 effect. Thus the addition of acetazolamide appears to be the equivalent of exposing animals to a CO_2 concentration about 10 per cent higher than the actual concentration. These data provide convincing evidence that acetazolamide and CO_2 have the same mode of action.

It appears from the available data that agents which enhance CO_2 production can affect CNS excitability if the rate at which the CO_2 is produced exceeds the rate of elimination of the gas from the brain cells, thus resulting in a net accumulation of CO_2. It has been shown that accumulation may occur by at least three mechanisms: (1) decreased rate of elimination of the CO_2 from the lungs secondary to respiratory depression; (2) decreased rate of release of CO_2 from the brain cells; and (3) rate of CO_2 production exceeding the normal excretory capacity of the respiratory system for CO_2. Examples of the first mechanism are effects produced by barbiturates, trimethadione, and morphine which depress respiration and enhance the toxicity of salicylate and DNP. Salicylate (unpublished observations) and DNP[59] have also been reported to enhance barbiturate-induced sleep time. This is probably due to CO_2 accumulation as a result of increased CO_2 production coupled with respiratory depression. Examples of the second mechanism are the effects produced by carbonic anhydrase inhibitors, such as acetazolamide, sulfanilamide, etc. Examples of the third mechanism are effects produced by combinations of two drugs, such as salicylate and DNP, each of which enhances CO_2 production.

ROLE OF CARBONIC ANHYDRASE IN THE CENTRAL NERVOUS SYSTEM

In the previous sections of this discussion some of the effects of CO_2 on the central nervous system were reviewed. It is clear that

any experimental condition which changes the CO_2 tension in the brain is also capable of affecting brain function. The nature of the mechanism by which a change in CO_2 tension can produce changes in function remains obscure. The effects of carbonic anhydrase inhibitors upon the central nervous system demonstrate that carbonic anhydrase is important in regulating CO_2 tension in the brain. An understanding of the role of carbonic anhydrase in brain may yield some information on the biochemical role of CO_2 in the central nervous system.

The following discussion of the role of carbonic anhydrase in the central nervous system involves a fundamental assumption that the enzyme catalyzes only one set of reactions which include the hydration of CO_2 and the dehydration of carbonic acid. In a physiological medium this equation is generally represented as follows:

$$CO_2 + H_2O \xrightleftharpoons{\text{Carbonic Anhydrase}} H_2CO_3$$

It must be remembered that the assumption of specificity of action of carbonic anhydrase may not be a valid one; however, at present the hydration and dehydration of CO_2 are the only reactions known to be catalyzed by carbonic anhydrase, and therefore they are the only ones which can be considered.

In discussing a reversibly catalyzed set of reactions one must consider which direction the steady-state condition will favor; the direction depends solely upon the fate of the reaction products and the concentration of the substrate. For example, in the lung, CO_2 escapes the pulmonary vascular system, and hence this favors the generation of CO_2 and H_2O. However, the direction is reversed in blood at the tissue level because CO_2 cannot escape and the resulting high CO_2 tension in red blood cells drives the reaction in the direction of H_2CO_3 and HCO_3^-. Similarly, in organs which secrete H^+ or HCO_3^-, such as kidney or pancreas, carbonic acid formation can predominate.

The reactions, reversibly catalyzed by carbonic anhydrase, are somewhat unique among the enzyme-catalyzed systems in that they proceed at an appreciable rate in an uncatalyzed medium.[50] The fact that the uncatalyzed rate is significant is important because it implies that the inhibition of carbonic anhydrase in a sequence of reactions cannot completely block the sequence, but the resulting reduction in the rate may render the carbonic anhydrase catalyzed step a rate-limiting one. This is an important point to consider in evaluating the role of carbonic anhydrase in a physiological system.

It is relatively easy to appreciate the essentiality of carbonic anhydrase in activities associated with blood and other organs such as kidney and pancreas, but in brain a role for the enzyme has not been completely defined. In general, two functions for carbonic anhydrase are possible: one is the dehydration of carbonic acid to CO_2 and the other is the hydration of CO_2 to carbonic acid. The immediate question with respect to the brain is which of these reactions is utilized? In tissue as complex as brain, it is also possible that both functions are of equal importance, as in blood. In any event, the role of carbonic anhydrase in the brain revolves around the relation of the enzyme to endogenous CO_2 and H_2CO_3, the substrates of the enzyme.

Characteristics of Carbonic Anhydrase Activity in Brain: *Influence of Aging.* The influence of aging upon carbonic anhydrase activity in 2 to 38 day old rats was studied by Millichap *et al.*[50, 51, 52] who reported an increase in activity in developing animals. They observed an inverse relation between enzymatic activity in brain and excitability as measured by an electroshock method. An additional study of the relation of age to carbonic anhydrase activity has indicated that the activity in cerebral cortex continuously increases with increasing age from newborn rats to animals over two years old.[61] The relation between brain excitability and the progressive increase in enzymatic activity remains to be determined.

Intracellular Distribution. A study of the intracellular distribution of carbonic anhydrase has added another consideration to the problem of the role of the enzyme. In cerebral cortex [62] and other parts of the brain (unpublished observations) the enzyme is present in two subcellular fractions, mitochondrial and supernatant. As the total enzymatic activity of the tissue increases with age so does the activ-

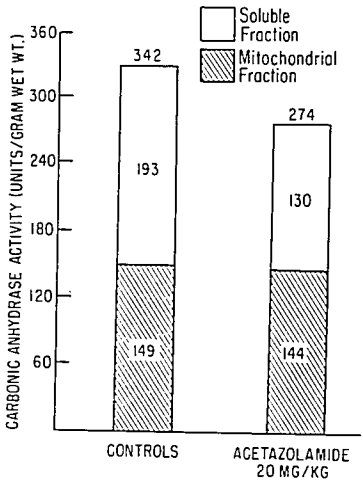

FIG. 4. Effect of acetazolamide on the intracellular distribution of carbonic anhydrase in rat cerebral cortex. Perfused cortex homogenized in 0.25 *M* sucrose solution (1:10) and the mitochondrial fraction separated from the supernatant by centrifuging 10,000 × *g* for 25 minutes a homogenate which had previously been centrifuged for removal of the nuclear fraction. Carbonic anhydrase activity was measured by a modification of the manometric method of Meldrum and Roughton. Acetazolamide dose was 20 mg./kg. intraperitoneally, and animals were sacrificed 4 hours after drug administration.

ity in the two subcellular fractions.[61] The presence of the enzyme in different subcellular components introduces the problems of the role of the enzyme in each locus and of the functional relation between the two enzyme loci.

Effect of Acetazolamide. A study of the effect of acetazolamide upon the intracellular distribution of carbonic anhydrase activity demonstrated that low doses of the drug *in vivo* preferentially inhibited the supernatant over the mitochondrial fraction of the enzyme. An example of the results of such an experiment is given in figure 4.

In-vitro experiments have indicated that acetazolamide is capable of inhibiting both cellular fractions containing carbonic anhydrase. As yet, there is no explanation for the greater vulnerability *in vivo* of the enzyme in the supernatant of the cytoplasm compared to that in the mitochondrial fraction. It is possible that the mitochondrial membrane *in vivo* is relatively impermeable to the drug; no information, however, is available on this point. Another possibility may lie in the biochemical nature of the two cellular enzymes. For example, it is not known if the enzymes in the two cell fractions are identical. The only biochemical characterization which has been made on these enzymes was a comparison of their activity-concentration curves. These curves have identical characteristics which provide some evidence that the enzymes are identical (unpublished observations). With respect to the problem of selectivity of drug action, one important biochemical characterization which is needed is the dissociation constants for the two enzyme-drug complexes. A difference in these constants could account for the preferential inhibition of one enzyme fraction over the other.

SOURCE AND NATURE OF CARBON DIOXIDE PRODUCED IN TISSUES: The principal source of CO_2 in tissues is probably the decarboxylation reactions associated with the tricarboxylic acid cycle. The enzymes involved in the tricarboxylic acid cycle are generally considered to exist intracellularly within mitochondria; therefore, the mitochondria are probably the major site of CO_2 production in cells. The presence of carbonic anhydrase in the mitochondrial fraction may indicate that there is an intimate relation between the enzyme and metabolically produced CO_2.

Another known source of CO_2 in cells arises from the decarboxylation of amino acids. In the central nervous system, the decarboxylation of glutamic acid to γ-aminobutyric acid is an example of such a reaction. Information on the intracellular localization of amino acid decarboxylases is meager; however, at least some of them are associated with the soluble fraction of cells. A qualitative study of glutamic decarboxylase in brain showed that it is also present in the soluble fraction (unpublished observations). It is again interesting to note that the intracellular distribution of carbonic anhydrase corresponds to a major site of CO_2 production.

Nature of Metabolically Produced Carbon Dioxide. The customary biochemical repre-

sentation of CO_2 produced during metabolism conveys the impression that CO_2 is split off directly from the decarboxylated product. It is true that, due to its volatility, CO_2 is an ultimate product of respiration, whether it is measured in the whole organism or in biochemical preparations; however, because of the kinetics of the hydration-dehydration reactions it is not known whether CO_2 or a hydrated product (carbonic acid or bicarbonate) is initially produced in metabolic reactions.

Attempts have been made to determine the original nature of the CO_2 liberated by decarboxylating enzyme systems by measuring the rate of CO_2 production in the presence and absence of carbonic anhydrase. On the basis of such experiments, Krebs and Roughton [63] concluded that reaction systems utilizing yeast carboxylase or urease produce CO_2 as the initial product. Conway and O'Malley [64] confirmed these results with respect to the urease system; however, with respect to the carboxylase system they maintained that the original nature of the CO_2 varied, being either the gas itself or H_2CO_3, depending upon experimental conditions.

Another approach to the study of the formation of CO_2 in enzymatic reactions was presented by Rothberg and Steinberg [65] who employed O^{18}-labelled water in reaction systems containing various bacterial amino acid decarboxylases. They observed that the CO_2 produced was essentially free of O^{18} and concluded that these pyridoxal-catalyzed decarboxylations were nonhydrolytic; consequently CO_2 was the initial product. The results support a mechanistic model for pyridoxal-dependent decarboxylase reactions proposed earlier by Metzler *et al.*[66]

The above-cited studies on the nature of CO_2 produced by decarboxylation reactions were necessarily restricted to isolated enzyme systems as opposed to tissue slices or homogenates because it was not technically possible to measure directly rates of CO_2 production in a respiring system. Ultimately, it is necessary to study the problem in respiring systems because they are closer to the situation *in vivo*. Some indirect studies of the effect of carbonic anhydrase inhibitors on the rate of CO_2 production in intact tissues have been reported. Shanes [67] suggested (on the basis of a hydrogen-potassium exchange hypothesis) that the increase in potential produced by sulfanilamide could be explained if H^+ and HCO_3^- were the first products of decarboxylation rather than CO_2.

Koch and Woodbury [27] observed that the total CO_2 content in the cerebral cortex of acetazolamide-treated animals was higher than normal values (table 2). They concluded that the inhibition of carbonic anhydrase reduced the rate of CO_2 escape from the tissue, an indication that CO_2 is derived from carbonic acid. It is of interest to note that the experimental conditions of these experiments were similar to those discussed earlier which demonstrated that the soluble fraction of brain carbonic anhydrase activity was selectively inhibited over the mitochondrial enzyme activity. The increase in total CO_2 content of cortex produced by acetazolamide, consequently, appears related to inhibition of carbonic anhydrase activity in the supernatant fraction of cells.

We have extended the study of the origin of CO_2 produced during respiration by employing a completely different system utilizing an infrared CO_2 analyzer. With a CO_2 analyzer it is now possible for the first time to record continuously the rate of CO_2 production

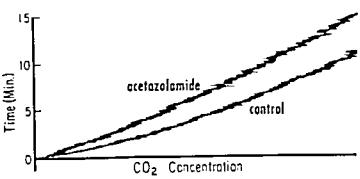

FIG. 5. Inhibitory influence of acetazolamide on the rate of CO_2 production in rat brain homogenate. Study conducted in a standard Warburg vessel at 38 C. Respiratory system contained 3.0 ml. of a 0.25 *M* sucrose homogenate (1:5) of brain, 0.6 ml. of 0.25 *M* glucose solution, and 0.4 ml. of either H_2O (control) or 10^{-4} *M* acetazolamide solution; final acetazolamide concentration equalled 10^{-5} *M*. This figure represents a photograph of the actual recording with the ordinate as time and the abscissa as CO_2 concentration. Note that rate of CO_2 production in the control system exceeds that of the system to which acetazolamide has been added. For example, the CO_2 concentration reached in the control system at 10 minutes is not attained in the acetazolamide inhibited system until approximately 15 minutes.

in tissue homogenates of brain during aerobic metabolism. Examples of such recordings are shown in figure 5. These recordings illustrate that the rate of CO_2 production decreased following the addition of acetazolamide. Furthermore, it was shown that the decreased rate of CO_2 production was not due to a decreased respiratory rate because oxygen consumption was unaffected by the concentrations of acetazolamide used. The fact that the *apparent* rate of CO_2 production decreased following the addition of acetazolamide supports the concept that respiratory CO_2 is derived from carbonic acid.

It is interesting to compare the relation of the depression of CO_2 production to the degree of carbonic anhydrase inhibition. The degree of depression in CO_2 production was approximately the same in the presence of either a 10^{-5} *M* or 10^{-7} *M* acetazolamide solution; however, a 10^{-5} *M* solution completely inhibited the carbonic anhydrase, whereas a 10^{-7} *M* solution inhibited only 10–15 per cent of the total tissue enzyme activity. Reactions containing a 10^{-7} *M* solution are comparable to the concentrations used in the *in vivo* experiments discussed earlier which demonstrated an increase in total CO_2 and selective inhibition of the supernatant carbonic anhydrase activity. The equal inhibition of CO_2 production obtained by widely different degrees of enzyme inhibition remains to be explained.

The studies on CO_2 production which have been made with the CO_2 analyzer were performed on whole tissue homogenates. However, since the major sites of CO_2 production in tissue are probably mitochondrial and supernatant fractions, it is important to study the nature of the CO_2 produced in these two isolated cellular fractions in order ultimately to understand the intracellular relationship of the carbonic anhydrase function in these two fractions. Such studies are now being carried out in our laboratory.

Mechanism of Anticonvulsant Action of Carbon Dioxide on the Nervous System

A hypothesis for the mechanism of anticonvulsant action of CO_2 on the CNS has been presented by Koch and Woodbury[57] and will only be summarized here. The effect of giving exogenous CO_2 or acetazolamide is to increase the steady-state concentration of H_2CO_3 in brain cells. In the case of acetazolamide, the inhibition of carbonic anhydrase reduces the rate at which metabolically derived CO_2, which is produced as H_2CO_3, is dehydrated. Because H_2CO_3 is much less diffusible than CO_2, the inhibition of carbonic anhydrase results in an increase in the steady-state concentration of H_2CO_3 in the brain. The increased H_2CO_3 concentration, produced by CO_2 and acetazolamide, reduces the permeability of the cell membrane to Na probably as a result of an increase in ionizable calcium which occurs from the local increase in acidity induced by H_2CO_3. Increased amounts of calcium ion are known to decrease cell permeability to Na. Inasmuch as Na-pump activity is not affected by H_2CO_3, the decreased Na influx results in a lower steady-state concentration of Na within the cells and a higher ratio of Na across the membrane. The decrease in permeability of the membrane to Na would be predicted to raise the threshold of firing of neurons and reduce, to some extent, the height of the propagated impulse. A very slight effect of this nature would be difficult to detect along the major portion of an axon, but could exert a marked effect on the postjunctional elements of a synapse.[68] Hence, carbon dioxide would be expected to block the abnormally high number of impulses impinging on the dendrites of central neurons and thereby to reduce considerably the probability of successful synaptic transmission. As a consequence, activity from an epileptogenic focus would tend to remain a localized phenomenon instead of progressively spreading to involve the whole cerebral cortex.

Summary

It has been shown that various concentrations of CO_2 exert inhibitory and excitatory effects on the central nervous system (CNS). In addition, several drugs which mimic the effects of CO_2 on the CNS possess this activity by virtue of their ability to alter the tissue level of CO_2. The mechanism of the CNS action of CO_2 appears dependent upon specific electrolyte and amino acid changes. These studies have also demonstrated that carbonic anhydrase is important in the regulation of the level of brain CO_2 and excitability and

that the role of carbonic anhydrase in brain is intimately concerned with the conversion of metabolically produced H_2CO_3 to CO_2. Furthermore, this functional role of the enzyme appears associated with the soluble fraction of the cell.

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