Inhibitory action of carbon dioxide on experimental convulsions

III) Experiments on cryoepilepsy in the frog.
Mechanism of the inhibitory action of carbon dioxide on convulsion

by

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In the two previous parts of this work (1) we studied the inhibitory action of carbon dioxide on strychnine convulsions in rabbits and on convulsions caused by direct chemical excitation of the cerebral cortex in dogs (strychnine and acetyl choline.) We also discussed in the introduction the findings of M. Ozorio de Almeida (2) who studied the action of carbon dioxide on convulsions caused by sudden chilling of the spinal cord of the frog (cryoepilepsy) and observed that when these animals breathed a gaseous mixture of atmospheric air containing carbon dioxide in a concentration of over 20% for 20 minutes, it was no longer possible to obtain the attack of cryoepilepsy.

This effect of carbon dioxide on cryoepilepsy was used by us to study the mechanism of the inhibitory action of this gas. Authors who have investigated the action of carbon dioxide on convulsions have directed their studies to find out whether this gas acted specifically or if its action was connected with an alteration in the pH of the intercellular medium. The same problem is met with when studying other physiological and pharmacological actions of carbon dioxide and knowledge on this point would bring light on these other actions. For instance in the regulation of respiratory movements by carbon dioxide where the question of the mechanism of action is still open. However we will keep exclusively to the study of the mechanism of carbon dioxide on convulsions.

In the introduction to the first part we summarized the results of the principle works on the influence of carbon dioxide on convulsions. The formation of carbonic acid by the solution of carbon dioxide in the blood, and the consequent modification of the pH, caused many
workers to consider the influence of the pH on convulsive attacks. In a series of studies, experimental and clinical, these authors studied the variations in the pH of the blood before, during and after the convulsive attacks, arriving at results which took them to a pathogenic interpretation of epilepsy. These studies took on a particular interest after the work of Foerster (2a) and Rosett (3), using the hyperpnoea test in the diagnosis of epilepsy. Collip and Backus (4) and Davis, Haldane and Kellaway (5) studied the blood changes during the neuromuscular hyperexcitability and tetanic contractions, caused by the hyperpnoea, finding a correlation between these phenomena and the alkalosis that follows hyperpnoea. As in the hyperpnoea test where there is an alkalosis following on the great elimination of carbon dioxide, the convulsive attack produced will be caused by the alkalosis and, in the spontaneous convulsions there would be an alkalosis related to either a diminution in the carbon dioxide tension of the blood, for some uncontrollable cause. It remains to be seen if there is always a preconvulsive alkalosis and there the findings are not in agreement.

Observations on the influence of starvation and ketogenic diets which increase the amount of acid formed by the body, with good results in human epilepsy, supports the viewpoint of an influence of the reaction of the blood on epilepsy. These results lead to the following general conclusions: factors which alter the pH of the blood to the acid side prevent the appearance of convulsions; factors which tend to raise the pH favour convulsions. One could at once make restrictions to such a general point of view: in diabetic coma and consequently with frank acidosis, convulsions have been observed.

The good effects of carbon dioxide seen in convulsive attacks could be explained by the acidosis that is produced. With the object of producing acidosis Lennox (6) made an epileptic breathe his own expired air, that is, an atmosphere with an increasing percentage of carbon dioxide and decreasing oxygen, noting a sedative action on the frequent convulsive attacks the patient suffered. He also observed that, adding carbon dioxide to the inspired air, the hyperpnoea test was negative (7); the patients did not have convulsions. There first results led Lennox and collaborators (8, 9, 10, 11,) to investigate the importance of oxygen and carbon dioxide in convulsions and, later, on the electrical activity of the cortex.

Experimentally the importance of acidosis in the inhibition of convulsive attacks was elaborated by various authors. Fröhlich and Solé (12) perfused frogs with strychnine in Ringer's solution and found that the convulsions were attenuated if the solution was acidified and increased if it was alkalinized. Also Lennox, Nelson and Beethan (13) by the injection of lactic acid, attenuated convulsions in rabbits produced by the injection of tujona or homocanfina. Swingle, Wennner and Stanley (14) studied the effect of carbon dioxide on tetany produced in dogs by the removal of the thyroid and parathyroids; they found carbon dioxide exerts an inhibitory action. They tried to explain the mechanism of this inhibitory and, based on satisfactory results obtained
by other authors with the administration of hydrochloric acid, calcium chloride and ammonium chloride, put forward the hypothesis that the effect of carbon dioxide was due to the acidosis it caused. In their experiments these authors titrated the total blood calcium, the carbon dioxide content and determined the pH. Under the action of carbon dioxide the pH of the blood showed a marked fall but the carbon dioxide content also fell which led them to admit that the acidosis was not caused by the carbon dioxide but by the increase in the blood of an organic acid, probably lactic acid. Later experiments with animals with parathyroid tetany, treatment with carbon dioxide showed them to have a marked increase of lactic acid in the blood. During the period that the animals were showing a high level of lactic acid in the blood, convulsions did not occur. The fall in the lactic acid was accompanied by a reappearance of tetany. As a final explanation Swingle and collaborators admitted that carbon dioxide increased the amount of lactic acid in the blood; this acidosis made the serum calcium more diffusible and probably stimulated the excessive secretion of phosphorus which inhibited parathyroid tetany. We showed in a previous work (15) that this mechanism would not apply to the inhibitory action of carbon dioxide on convulsions caused by strychninization of the cerebral cortex of dogs. In these experiments we found that the inhalation of 18% carbon dioxide, a concentration which inhibits convulsions caused by strychninization of the cerebral cortex, produced a fall in the lactic acid of the blood in normal dogs, the same result as that found by Anrep and Canna (16), and others. In dogs with convulsions, suspending the inhalation of carbon dioxide sometimes allowed the convulsions to reappear, at the moment when the lactic acid level was much higher than at the beginning of the experiments, the excess of lactic acid being due to the muscular contractions during the convulsions.

On this trend of thought, differing however in accepting that an alkalosis, localized at a motor area of the cerebral cortex would be the origin of convulsive attacks, were based the studies of Dusser de Barenne (17) and collaborators, to explain the mechanism of the action of strychnine on the nervous centres. They have admitted that the application of strychnine to the cerebral cortex produced a local alkalinization; the experimental results however showed that after the application of strychnine and the occurrence of the “spikes”, there was no local alkalinization responsible for the convulsive electrical activity which follows the application of strychnine. Jasper and Erickson (18) studied the importance of the pH on the appearance of convulsions caused by the injection of metrazol in cats. They took the pH of the blood simultaneously with an electrogram of the sigmoid gyrus, and concluded that the convulsions were independent of variations in the pH. LUBLIN and PRICE (19) also studied the influence of alkalosis and acidosis on the normal electrical activity of the cerebral cortex, and concluded that the electrical alterations observed by the increase or decrease in the carbon dioxide tension of the blood are due more to a
specific action of the gas rather than to an alteration in the pH of the interstitial fluid.

More recently Gellhorn and Heymans (20) studied the influence of anoxia, asphyxia and carbon dioxide on the normal and convulsive potentials of the cerebral cortex in dogs and cats; they found that carbon dioxide in a concentration of 10.6% has a predominantly excitant effect on the potentials in strychnine convulsions. It would be important if the authors had also studied the influence of higher concentrations, since these inhibit convulsions and reduce the amplitude of convulsive electrical potentials as was shown by, among others, Pollock (21) for chemical and electrical convulsions.

Even though some experiments led one to believe in the specific action of carbon dioxide, the problem today is debatable, more so because it is not clear what represents this specificity of action.

It was to clarify this point, whether carbon dioxide acts by a specific action or by modifying the pH of the blood or of the nervous centres, in its inhibition of cryepilepsy, that we did the experiments described in this paper. They consisted of perfusing dogs through the aorta with Ringers solution with various pH and recovering the perfusing fluid from the abdominal vein. The pH of this fluid was determined and taken as the index of the pH of the intercellular medium. The frogs perfused were subsequently subjected to cryoepilepsy.

**METHOD:** The experiments were done on frogs *L. ocellatus*. The frogs were perfused through one of the aortic arches with Ringers solution, oxygenated and acidified with hydrochloric acid. The pH of the Ringers solution, used to perfuse, was determined previously. The experiments were done with perfusing fluids with pHs of between 5.2 and 6.2. The solutions were prepared at the time of the experiment. The liquid was collected through a cannula introduced into the abdominal vein and the pH determined of the last portion. This last value we took to indicate the existing pH of the spinal cord. The determinations of the pH were done by colorimetric and electrometric methods, using in the latter case a quinidrone electrode in a Pehavi apparatus. The perfusion lasted 5 to 12 minutes. Ending it we rapidly prepared a hind-leg isolated-cord and tested the convulsive attack by cooling the spinal cord by the technique described previously (22). In another series of experiments to find the pH of the blood of normal frogs and the pH variations when these animals are given carbon dioxide, we used the technique of pulmonary insufflation, through a cannula inserted in the glottis of the frog. The insufflation was done with a gaseous mixture of 25% carbon dioxide in atmospheric air for 20 to 25 minutes. Ending it we punctured the ventricle and collected blood for the determination of the pH; the determination was done immediately the blood had been taken. The blood sample was not collected under oil. The pH was determined with a glass electrode in Beckman's apparatus.
RESULTS

In Table 1 we give the results of our experiments. Of the ten experiments done, in only one was the temperature in which the spinal cord was submerged not controlled. In all the others the temperature of the bath was known; we know from previous studies with normal frogs that at these temperatures the attacks are usually of great intensity. However the diminution in the intensity of the attacks, seen in parts of the experiments, must not be attributed solely to the influence of the pH of the perfusing fluid. Although the perfusing fluid had been oxygenated, the oxygenation could not be sufficient to maintain the necessary supply of oxygen to the tissues during the whole time of the perfusion; this diminution in the intensity of the attack could occur partly because of the relative anoxia. Preliminary control

<table>
<thead>
<tr>
<th>FROG NUMBER</th>
<th>Temperature of Ringers bath.</th>
<th>pH of perfusing fluid.</th>
<th>Result</th>
<th>pH of perfused fluid.</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.°</td>
<td>6.2</td>
<td>Strong attack</td>
<td>6.6</td>
<td>Colorimetric</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>5.8</td>
<td>Medium attack</td>
<td>6.5</td>
<td>Colorimetric</td>
</tr>
<tr>
<td>3</td>
<td>0.°</td>
<td>5.8</td>
<td>Medium attack</td>
<td>6.7</td>
<td>Colorimetric</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>5.8</td>
<td>Strong attack</td>
<td>6.6</td>
<td>Colorimetric</td>
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<tr>
<td>5</td>
<td>Ethyl chloride</td>
<td>5.6</td>
<td>Medium attack</td>
<td>6.2</td>
<td>Colorimetric</td>
</tr>
<tr>
<td>6</td>
<td>1.5</td>
<td>5.8</td>
<td>Weak attack</td>
<td>6.6</td>
<td>Electrometric</td>
</tr>
<tr>
<td>7</td>
<td>0.°</td>
<td>5.8</td>
<td>Medium attack</td>
<td>6.55</td>
<td>Electrometric</td>
</tr>
<tr>
<td>8</td>
<td>0.°</td>
<td>5.8</td>
<td>Weak attack</td>
<td>6.4</td>
<td>Electrometric</td>
</tr>
<tr>
<td>9</td>
<td>0.°</td>
<td>5.2</td>
<td>Medium attack</td>
<td>6.55</td>
<td>Electrometric</td>
</tr>
<tr>
<td>10</td>
<td>0.°</td>
<td>5.2</td>
<td>Medium attack</td>
<td>6.7</td>
<td>Electrometric</td>
</tr>
</tbody>
</table>

experiments showed that one can get an attack of cryoepilepsy 15 to 20 minutes after ligaturing the arterial bulb of the frog, or after ending the hind-leg isolated-cord preparation; but the attacks are less intense or may even fail, with the preparation of nearly 20 minutes or more of anoxia, this time of suppression of the attack depending on the existing surrounding temperature. These facts do not eliminate the influence of the pH on the diminution in intensity of the attacks but the diminution observed must be due to the two factors mentioned above. We obtained an attack, however, in all the frogs. The values of the pH found in the fluid obtained by the abdominal vein were always less than 7.0. In experiments N.° 5 and 8 we got an attack with the fluid of the abdominal vein having a pH of 6.4 and 6.2 respectively. If we consider that there is always a difference between the pH of the perfusing fluid and that of the perfused fluid which indicates an alkalization of the perfusing fluid, we must suppose that the pH
of the tissues is lower than that found in the fluid collected in the abdominal vein. These results clearly show that the perfusion of frogs with Ringers solution with an acid pH causes the pH of the tissues to fall to a level much lower than normal which is not in itself enough to prevent an attack of cryoepilepsy.

**pH values of the blood in normal frogs and in frogs under the influence of carbon dioxide. (§)** In three normal frogs we determined the pH of blood obtained through a cannula inserted in the aorta. The values found were: 7.15, 7.25 and 7.25. The frog in which we found the value of 7.15 had a cannula in its glottis for 20 minutes. The value of 7.25 found in the other two frogs agrees with that found in the European frog in the months of July to September and which is 7.25 to 7.30 (Oppenheimer-Pincussen. — Tab. Biol. Period. Bd. VII, pg. 284). During hibernation or starvation there is a rise of the pH to 7.36 to 7.51.

In the determinations done in frogs after the insufflation of a gaseous mixture containing 25% carbon dioxide, we found the following values: 1) frogs in which the blood was collected through a cannula inserted in the aorta: 7.2, 7.2 and 6.95. The first two values were found in the blood of the same frog, and the last in another frog whose blood stayed in an electrode in which we passed a jet of the same gaseous mixture as was used for the pulmonary insufflation of the frog. 2) frogs in which the blood was collected by direct puncture of the ventricle and in which we found the following values: 6.8, 6.7 7.5, and 7.05, the last two values being in the same frog.

These results show that even with a concentration of 25%, higher than is necessary to prevent an attack of cryoepilepsy, the pH of the blood does not reach values lower than those get with perfusion and which makes one consider that a fall in the pH of the tissues could cause an inhibition of the attack of cryoepilepsy by carbon dioxide.

**DISCUSSION**

The above results lead one to think that carbon dioxide has an inhibitory action on cryoepilepsy which would depend on a specific action of the gas on the nervous centres; the modification of the pH of the intercellular fluid which it causes, would not be enough to explain the inhibition seen.

It is fitting to enquire what one must understand by a specific action of carbon dioxide. This question was put by workers who defend the theory of a specific action of carbon dioxide in the regulation of respiration. The experiments which show it are naturally varied. We are not going to mention all the studies done on this problem; that would take us to another field not directly concerned with our work. We will, however, summarize the experiments and con-

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* The determination of the pH was done by Drs. R. Wurmsen and S. F. Wurmsen whom we thank.*
clusions to which Jacobs (23, 24) arrived, in view of the similarities with our own experiments.

Jacobs was anxious to known how carbon dioxide acted on living protoplasm: throughtions or by a specific action. To do this he investigated the influence of carbon dioxide on various phenomena. The action on the respiratory center would not be more than one particular example of the mechanism of the general action of the gas. In a variety of experiments Jacobs submitted tadpoles (Bufo americanus) to the influence of solutions of hydrochloric, oxalic, acetic, salicylic and carbonic acids, all having a pH of between 3.8 and 3.9. Whereas these experiments showed that the tadpoles that remained in the solutions of the other acids resisted for over an hour, those in the solution of carbonic acid scarcely resisted seconds. These experiments showed that it was not the pH of the solutions that determined the toxicity. If the solution saturated with carbon dioxide had sodium bicarbonate added until the solution had a pH of 6.9, that is nearly neutral, the results were the same. A solution of bicarbonate with the same concentration as that used before was not toxic. The experiment eliminated the possibility that carbon dioxide acted through the $-\text{HCO}_3$ ion, since one gets this in abundance in the solution of bicarbonate. Even more, the carbon dioxide acting in a medium buffered until almost neutral, has the same effect as the solution of carbonic acid. Similar results were obtained in experiments with protozoa of various species. It was not, consequently, the acidity of the medium which determined the toxicity of carbon dioxide. Carbon dioxide has a specific action. It was to clarify what constituted this specificity of action of carbon dioxide that Jacobs did experiments with flowers of Symphytum peregrinum, a plant of the family Borraginacea, which is purple in the pre-flowering phase and blue after the flower opens. This colour depends on the intracellular pigment and the change is connected with variations in pH, the purple colour appearing with high concentrations of hydrogen and blue with low concentrations. The pigment, thus, would act as an intracellular indicator.

Jacobs subjected the flowers in the blue phase to the influence of various pHs, obtained with distilled water, distilled water and carbon dioxide, solution M/2 of NaHCO₃ and CO₂, and M/2 of NaHCO₃. The flowers submerged in the solutions of M/2 NaHCO₃ and CO₂ and in distilled water and CO₂ changed to purple a few minutes afterwards, while in the other liquids there was no colour change or they became bluer, those treated with the solution of bicarbonate became in time greenish.

These experiments show that modifications of the intracellular pH are not strictly dependent on the pH of the external medium. The flowers submerged in the solution of M/2 NaHCO₃ and CO₂ with a pH of 7.4, turned purple much quicker than those in distilled water with a pH of 5.0 to 6.0 (acid due to the carbon dioxide dissolved in the air) This difference is explained by the greater diffusibility of carbon dioxide which, penetrating the cells much faster than the $+\text{H}$ ions or the
HCO₃ ions, modify the intracellular pH in a quite different direction to that of the external medium. Still working with flowers of S. peregrinum, Jacobs compared the diffusibility of carbon dioxide with that of other acids, such as benzoic, salicylic, acetic and hydrochloric, getting similar results to those found in the experiments with tadpoles.

The works on the penetration of acids in animal cells, even though numerous, have not compared the diffusibility of carbon dioxide in relation to other acids. The first studies using animal cells which possessed an intracellular pigment acting as an indicator, were done by Harvey (25) using gonad filaments of Stichopus ananus, which has purple pigment changing to orange red in an acid medium. Also Crozier (26) studied the penetration of acids in the cell using the skin of a mollusc, Chromodoris zebra, which possesses a blue pigment changing to pink at a pH of about 5.6. These authors studied the penetration of various acids and obtained the same results as Jacobs and Haas (27) for vegetable cells. Of more than ten acids studied, with small variations, salicylic, benzoic, formic, hydrochloric etc. were among those with the greater penetration, as were acetic, butyric acid etc. among those less penetrating.

These results show the similar behavior of animal and vegetable cells towards the acids tested and allow us to conclude that also in animal cells carbon dioxide is distinguished by its greater diffusibility.

The mechanism of inhibition of the convulsions by carbon dioxide could be explained by the rapid penetration of the gas in the neurone, lowering the internal pH; the pH value reached inside the neurone would be independent of the pH found in the intercellular medium or in the blood, and would depend on the intracellular conditions existing at the moment. The tension of carbon dioxide in the intracellular medium rapidly equalling that of the blood, due to the rapid diffusibility of the gas, would determine the pH in the neurone. This would make clear the reason why the existing experimental results which try to establish a correlation between the pH of the blood and the appearance or the inhibition of epileptiform convulsions, are so discordant. As was seen in Jacobs’s experiments with flowers, here also we could have a definitely alkaline reaction of the blood or of the intercellular medium and the pH of the intracellular medium could reach low levels.

**SUMMARY**

In this work we described experiments to show the influence of the pH on convulsions caused by cooling the spinal cord of frogs, and to study the mechanism of the inhibitory action of carbon dioxide on these convulsions. We found that carbon dioxide does not act by lowering the pH of the blood or intercellular medium. Carbon dioxide would have a specific action which would be connected with its great diffusibility. This property would enable it to penetrate rapidly into the interior of the neurone, appreciably lowering the intracellular pH, in the absence of a clear variation in the pH of the blood.
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