Inhibitory action of carbon dioxide on experimental convulsions

I. Experiments with strychnine convulsions on rabbits.

II. Experiments with convulsions produced by chemical excitation of the cerebral cortex on dogs.

by

H. Moussatché

(Division of Physiology of the Oswaldo Cruz Institute)

The inhalation of carbon dioxide in adequate concentration modifies the course of convulsions. Both stimulating and inhibiting actions of this gas on convulsions have been described. There have been many experimental and clinical studies describing the actions mentioned above. Brown-Sequard, about the middle of the last century, was one of the first to study the inhibiting action which the gas has on convulsions produced by section of the spinal cord in guinea-pigs. (1) He noted that if a current of sufficient intensity of the gas was given as soon as the convulsions began, they stopped, and he suggested that the inhibitory action was brought about by the irritation of the sensitive nerve endings of the larynx, trachea, etc. by the carbon dioxide which causes an inhibiting reflex. Previously Rosenthal had described also the inhibitory action of a current of oxygen on strychnine convulsions. He explained this effect as due to the superoxygenation of the blood. Brown-Sequard, however, considered oxygen as well as carbon dioxide acted by exciting the sensitive endings in the upper air passages. The conclusions of Brown-Sequard were based on the following experiments: 1) The inhibitory action disappears on section of the vagus or of the spinal cord above the origin of the phrenic. 2) Passing a current of carbon dioxide from the trachea to the nose also inhibited convulsions. These results left no doubt about the reflex nature of the inhibition produced by the current of gas. This was one particular case among other inhibitory reflex actions studied so well by this author (2).

The work of Brown-Sequard was followed by others confirming the inhibitory action of carbon dioxide on convulsions. Winterstein, on rats, (3), and
Ryan and Guthrie (4), on cats with strychnine convulsions, suppressed these by making the animals inhale the gas. In human epilepsy carbon dioxide has only been tried relatively recently after the introduction of the hyperpnoea test by Foerster (5) and Rosett (6) for the diagnosis of epilepsy. The first observations were made by Lennox (7) in an epileptic who breathed the air he exhaled: that is, he inhaled an atmosphere with an increasing percentage of carbon dioxide and a decreasing one of oxygen. He observed a sedative action on the frequent convulsive crises which the patient suffered. He also observed that the hyperpnoea test was negative when carbon dioxide was added to the inspired air. Fogg and Schmidt (8) conformed these results, preventing the occurrence of convulsions in epileptics who breathed air containing 5-15% carbon dioxide.

More recently the action of carbon dioxide on the central nervous system has been studied in more detail and in various experimental aspects. M. Osorio de Almeida (9) inhibited convulsions produced by severe chilling of the spinal cord in frogs by making them previously breathe mixtures of air containing 20% carbon dioxide. Gellhorn and collaborators (10, 11) also studied the inhibitory action of carbon dioxide on convulsions produced by various chemical substances, (strychnine, coramirtine, picrotoxin) and investigated the mechanism of this inhibition which they supposed is produced through inhibitory reflexes originating in the carotid sinus and aortic arch.

In related fields verifying the influence of carbon dioxide on the electric potentials of the cerebral cortex or on the motor response to stimulation of different segments of the central nervous system, the studies done relate to carbon dioxide, now as an excitant, now as an inhibitor, depending on the concentration of the gas and on the phenomenon studied. King Garrey and Bryan (12), showed that carbon dioxide inhibits the patellar reflex and this was confirmed by various other authors. There are a large number studies on the cortical potentials. Recently Gellhorn and Heymans (13) studied the question again, discriminating between the action of carbon dioxide on the normal potentials of the cerebral cortex and on convulsion potentials produced by strychnine.

This work is concerned with the results of our experiments using carbon dioxide as an inhibitor of convulsions observed in rabbits intoxicated with strychnine and of the epileptiform convulsions produced by the chemical excitation of the cerebral cortex in dogs. We also studied the influence of the reflexes originating from the carotid sinus and vagus on the inhibition of convulsions by carbon dioxide.
EXPERIMENTS ON RABBITS

In these experiments we proposed to verify the importance of the excitation of the upper air passages in the inhibition of strychnine convulsions. We carried out essentially three types of experiments 1) forcing a strong current of carbon dioxide through the upper air passages from the trachea to the nose 2) forcing a strong current of carbon dioxide through the trachea to the lungs. 3) making the animal breathe a mixture from a gasometer containing carbon dioxide and atmospheric air in varying percentages.

When rabbits are made to breathe gaseous mixtures containing high percentages of carbon dioxide in them it is necessary to consider the convulsive action of this gas. The occurrence of convulsions caused by carbon dioxide itself is a cause of error that must be avoided. In our experiments some animals, before being injected with strychnine, breathed the mixture of carbon dioxide that was being tried. As has been described by other authors, rabbits very often go into convulsions at the first inspiration of the gas if the concentration is about 25%: then follows a depressive phase. This convulsive action has already been verified by S. Fredericq in 1886 and even Brown-Sequard admitted carbon dioxide to be essentially an exciting agent when acting on the central nervous system. The convulsions are tonic in character and last a short time. They begin as soon as the gaseous mixture is inhaled, then the animals go into the phase of depression. Also when one stops the inhalation of the mixture and the animal returns to breathing atmospheric air, convulsions can be observed, a fact which has already been verified by Paul Bert on the dog and which we saw in some of our experiments.

To observe the inhibitory reflex effects of carbon dioxide passing through the upper air passages of rabbits intoxicated with strychnine, we connect the trachea of the animal to a cylinder containing the above gas with a pressure reduction valve that allows the regulation of the intensity of the jet. The intoxication of the animals with strychnine was of a varying intensity going from a clear hyper-reflexivity to frank convulsions. In none of the experiments where we passed the gas only through the upper air passages was there inhibition of the convulsions if these were of sufficient intensity. Throughout the whole convulsive period the gas passed through the trachea, larynx and nose of the animal without diminishing the intensity of the convulsions. In very mild convulsions the effects were doubtful. It could not be determined whether the convulsions would end at the moment of passing the current seeing that the duration of the convulsions was itself very variable.
When the current is passed in the direction of the lungs the effects are clear. The convulsions end after a few seconds. It may happen that convulsions due to the carbon dioxide itself appear. A current of sufficient intensity can alter the existing atmosphere in the lung to one of pure carbon dioxide which rapidly asphyxiates and kills the animal.

This disparity between the effects of carbon dioxide when passed through the upper air passages only, and when it reaches the alveoli, lead one to think that the inhibition of convulsions is not a consequence of the stimulation of the sensitive endings of the respiratory tract, or, at least, that this action is not the predominant one. If we substitute the strong and irritating jet of the gas for breathing atmospheres of known percentages of carbon dioxide, the results are striking and more reliable. In these cases, once started, one cannot inhibit the convulsive crisis by connecting the trachea of the animal to the gasometer because of the respiratory pause that occurs during the crisis, which prevents the desired carbon dioxide tension from reaching the alveoli and the central nervous system. After a few respirations the convulsions disappear completely.

The tensions necessary to prevent the convulsions vary with the degree to which the animal is strychninized, but they are generally high. Even for a mild degree of strychnine intoxication it was necessary to use a percentage above 20%. For the stronger degrees of intoxication only percentages much higher, between 30% and 40%, Kere efficacious (see Table I).

As we must see, comparing these results with those got in the inhibition of cortical convulsions in dogs, the spinal convulsions require a higher tension following account of an observation on a dog that was submitted to experiments with both varieties of convulsive attacks, confirms the observation made previously by other authors.

Experiment of 6-9-43. Dog, female, weight 5,700gr. Intraperitoneal injection of 0,14gr. of morphine. Right parietal bone trepanned. Exposure of motor zone. Determination of the centre of orbicularis muscle and application of strychnine to it. Appearance of typical epileptiform convulsions occurring at 3-4 minute intervals. Make breathe a gaseous mixture containing 15.2% carbon dioxide and 21.5% oxygen. The convulsion disappeared for 12 minutes and then, when the inhalation of the mixture was suspended, they reappeared. Intraperitoneal injection of 0,2cc. of a 1% solution of strychnine. Animal with typical strychnine convulsion. Inhalation of a mixture of 14.9% carbon dioxide and 20% oxygen: the convulsions continued. Substitution of the previous mixture for one containing 40% carbon dioxide. Clear diminution of the strychnine effects. On cutaneous stimulation some tonic contractions still appeared. Inhalation of a 50% carbon dioxide mixture made the strychnine effects disappear. Patellar reflexes weak. Respiration slow and deep.
In some of the observation on rabbits intoxicated with strychnine we previously sectioned the spinal cord and were able to observe intercurrently that in these animals the convulsions that appear when the animal is made to breathe a high percentage of carbon dioxide, or when this is stopped, only appear in their front limbs; the hind limbs which had been cut off from the higher nerve centres by the spinal section did not show convulsions, showing that the convulsions produced by high percentages of carbon dioxide are not of a spinal origin.

The analysis of the data given in Table I shows that to suppress the strychnine convulsions it is necessary to use high concentrations of carbon dioxide. If the inhalation of this mixture does not discard the interaction of an inhibition starting at the sensitive nerve endings of the air passages by the irritation which the high concentration of gas causes on them, the finding that the convulsions disappear in spinal animals shows that the gas acts directly on the centres. On the other hand the inhibitory reflex actions are generally transitory and the inhalation of carbon dioxide does not only prevent the appearance of convulsions during the whole period that the animal is breathing the gaseous mixture, but, as is often observed, the effect continues even after the inhalation has stopped, the convulsions not effect continues even after the inhalation has stopped, the convulsions not starting again or the animal showing a lower degree of hyper-excitability than it had before.
It is also found that the tensions necessary to prevent the convulsions are similar both in the spinal animals and in those with the spinal cord intact.

These facts lead to the supposition that the suppressing action of carbon dioxide on strychnine convulsions in rabbits acts fundamentally by a more direct intervention on the nervous centres, and the inhibitory reflex actions that act on the spinal convulsions play a part of minor importance.

EXPERIMENTS ON DOGS

These experiments were done on dogs with convulsions produced by the application of strychnine or acetylcholine on the sensory-motor points of the cerebral cortex. The animal was trepanned under light morphine narcosis and the sigmoid exposed, in which a motor centre was determined. On this a small square of filter paper soaked in the stimulant solution was placed. The paper with the stimulant was renewed at intervals depending on the experiment. To anaesthetize the animal we previously injected intra-peritoneally 0.01-0.02gr. of morphine per Kgr. of body weight. As was shown by Aman-tea (14) morphine predisposes to this type of convulsion, making it easy to obtain a state similar to epilepsy. When epileptic fits occur the convulsions follow one on top of the other with small and more or less regular intervals. The epileptogenic centre acquire a rhythm of discharge which keeps up for a long time and can kill the animal. This succession of attacks with more or less regular intervals is specially useful for observing factors which have an inhibitory or stimulating action on convulsions. To study the inhibitory action of carbon dioxide on this sort of convulsion we made the animal breathe the desired mixture through a tube connected to the trachea with Tissot’s inspiratory-expiratory valves. The inspiratory valve was connected to a gasometer like that of Tissot with a capacity of 100 litres and containing a gaseous mixture previously prepared. The expiratory valve opens to the exterior. The exact composition of the mixture in the gasometer was known by the analysis of a specimen with Orsat’s apparatus.

The animal breathed carbon dioxide for about 20 to 30 minutes, time enough in the majority of cases to show some action. We only began experiments after the animal had already had some spontaneous attacks at more or less fixed intervals.

We did not find a definite concentration of carbon dioxide inhibiting the convulsions. The concentration varied from animal to animal. There is a large margin of variation but even the higher concentrations attained are lower than those that were necessary for rabbits intoxicated with strychnine.
The concentrations over 12% began to show a certain inhibitory action. The most efficacious ones were about 15%. In one of the dogs it was necessary to use a concentration of 25%, which already has an anaesthetic action, in order to stop the attack. Table 2 summarizes the observations.

With low tensions, between 12% and 13% there may be an increase in the intervals between attacks which, being weaker in intensity and shorter in duration, sometimes are reduced to generalized clonic contractions. The tensions which are sufficient to inhibit a convulsive attack will not inhibit the clonic contractions of the muscles connected to the centre stimulated. To prevent the continuation of these contractions it is necessary to reach much higher concentrations of carbon dioxide. Carbon dioxide seems to act by suppressing the spread of the attack without modifying, in these concentrations, the state of the epileptogenic centre.

When the inspiration of carbon dioxide is suspended the attacks may reappear immediately or remain inhibited a long time. This inhibition which prolongs itself, even after the inhalation of carbon dioxide has been suspended, has already been described by Swingle, Wenner and Stanley (15) in the case of tetany caused by removal of the parathyroid. This fact still requires a satisfactory explanation. The following is a summary of the two observations which illustrate the facts mentioned above.

Experiment of 26-12-42. Dog. Weight 10.200 gr. Intra-peritoneal injection of 10 cc. of a 2% solution of morphine. Right side of skull trepanned and sensory-motor zone exposed. Centre of orbicularis palpebrum determined. 1.15 p.m. Beginning of the application of a 1% solution of strychnine on the centre of the orbicularis. Few seconds after appeared strong clonus and a spontaneous attack. New attack at 1.30 p.m. Application of strychnine repeated. Spontaneous attack which lasted 2 minutes. Two more attacks arose spontaneously at 1.38 p.m. and 1.42 p.m., both of great intensity. 1.44 p.m. began to breathe a mixture containing 17% carbon dioxide. Breathed until 2 p.m. without the development of new attacks. Began to breathe atmospheric air. 2.10 p.m. Strychnine renewed. Return of the attacks which succeeded each other spontaneously at 2.15 p.m., 2.20 p.m., 2.28 p.m., and 2.30 p.m. Observation ended.

Experiment of 29-12-42. Dog. Weight 5.300 gr. Intra-peritoneal injection of 5 cc. of 2% solution of morphine. Right side trepanned. Motor zone exposed and determination of the centre of orbicularis palpebrum. 2 p.m. Start of application of strychnine to centre of orbicularis. Strychnine renewed at 2.15, 2.20, 2.30, 2.45, and 2.55 p.m. when an attack was obtained by the mechanical stimulation of the orbicularis. 3.05 p.m. strychnine renewed. Attack by stimulation of the orbicularis. 3.25 p.m. strychnine renewed. Slight stimulation of the orbicularis resulted in a strong attack which lasted 1 minute 30 seconds. 3.30 p.m. new attack by slight stimulation of the orbicularis. Strong attack. 3.45 p.m. strychnine renewed. Stimulatio of the orbicularis. New attack. New convulsions were produced by stimulation of the orbicularis at 3.55 and 4.10 p.m. 4.10 p.m. made to breathe a mixture
of 17% carbon dioxide. 4.20 p.m. Strychnine renewed. Mechanical stimulation of the orbicularis. No attack. The animal breathed this mixture until 4.30 p.m. No attack produced by prolonged stimulation of the orbicularis. The paper with strychnin-2 was renewed and the orbicularis was stimulated after the animal had returned to breathing atmospheric air until 6 p.m. without having any new attacks.

Most of the experiments above were done using a 1% solution of strychnine as the stimulant. Of the experiments shown in the table only one

<table>
<thead>
<tr>
<th>DOG N.</th>
<th>SUBSTANCE USED</th>
<th>NUMBER OF ATTACKS BEFORE CO₂ (IN 20 MINUTES)</th>
<th>CONC. OF CO₂ IN THE INSPIRED AIR</th>
<th>EFFECT ON THE CONVULSIONS</th>
<th>DURATION OF INSPIRATION OF CO₂ (IN MINUTES)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Strychnine 1%</td>
<td>7</td>
<td>11.4</td>
<td>Weaker convulsions.</td>
<td>13</td>
</tr>
<tr>
<td>1</td>
<td>Strychnine 1%</td>
<td>—</td>
<td>18.0</td>
<td>Absence of convulsions.</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>Strychnine 1%</td>
<td>4</td>
<td>11.0</td>
<td>1 strong convulsion.</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>Strychnine 1%</td>
<td>4</td>
<td>12.0</td>
<td>3 strong convulsions.</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>Strychnine 1%</td>
<td>—</td>
<td>16.0</td>
<td>1 weak elonic.</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>Strychnine 1%</td>
<td>3</td>
<td>14.0</td>
<td>Absence of convulsions.</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>Strychnine 1%</td>
<td>3</td>
<td>18.2</td>
<td>Absence of convulsions.</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>Strychnine 1%</td>
<td>—</td>
<td>12.7</td>
<td>Convulsions continued absent.</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>Strychnine 1%</td>
<td>—</td>
<td>11.5</td>
<td>1 convulsive attack.</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Strychnine 1%</td>
<td>—</td>
<td>13.0</td>
<td>1 convulsive attack.</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>Strychnine 1%</td>
<td>—</td>
<td>16.0</td>
<td>1 weak attack.</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>Strychnine 1%</td>
<td>—</td>
<td>17.0</td>
<td>1 very weak.</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>Strychnine 1%</td>
<td>4</td>
<td>14.4</td>
<td>1 convulsive attack.</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>Strychnine 1%</td>
<td>—</td>
<td>15.2</td>
<td>1 convulsive attack.</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>Strychnine 1%</td>
<td>5</td>
<td>14.6</td>
<td>Absence of convulsions.</td>
<td>20</td>
</tr>
<tr>
<td>9</td>
<td>Strychnine 1%</td>
<td>3</td>
<td>19.0</td>
<td>Absence of convulsions.</td>
<td>20</td>
</tr>
<tr>
<td>9</td>
<td>Strychnine 1%</td>
<td>3</td>
<td>17.0</td>
<td>Absence of convulsions.</td>
<td>20</td>
</tr>
<tr>
<td>11</td>
<td>Strychnine 1%</td>
<td>4</td>
<td>17.0</td>
<td>Absence of convulsions.</td>
<td>20</td>
</tr>
<tr>
<td>12</td>
<td>Strychnine 1%</td>
<td>4</td>
<td>17.0</td>
<td>1 weak attack.</td>
<td>20</td>
</tr>
<tr>
<td>12</td>
<td>Strychnine 1%</td>
<td>—</td>
<td>18.0</td>
<td>Absence of convulsions.</td>
<td>20</td>
</tr>
<tr>
<td>13</td>
<td>Strychnine 1%</td>
<td>3</td>
<td>18.8</td>
<td>Absence of convulsions.</td>
<td>17</td>
</tr>
<tr>
<td>14</td>
<td>Strychnine 1%</td>
<td>3</td>
<td>19.0</td>
<td>Absence of convulsions.</td>
<td>20</td>
</tr>
<tr>
<td>15</td>
<td>Acetyl-choline. 10%</td>
<td>7</td>
<td>6.2</td>
<td>3 strong attacks.</td>
<td>6</td>
</tr>
<tr>
<td>15</td>
<td>Acetyl-choline. 10%</td>
<td>—</td>
<td>11.5</td>
<td>Weak convulsions.</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>Acetyl-choline. 10%</td>
<td>—</td>
<td>18.0</td>
<td>Absence of convulsions. (death of the animal a few minutes after).</td>
<td>—</td>
</tr>
</tbody>
</table>
was done using acetylcholine as the stimulant. Because of the greater ease in obtaining the epileptic state using strychnine we prefer this substance.

INFLUENCE OF THE VAGUS NERVE AND CAROTID SINUS IN THE INHIBITION BY CO₂

Previous experiments by various authors had shown the importance of the carotid sinuses and of the sensitive endings of the vagus in motor cortical phenomena. Gellhorn and Yesenick consider these organs of prime importance as factors in the inhibition of convulsions.

In our experiments we tried to find the importance of the reflexes starting in these zones on the inhibition by carbon dioxide of the convulsions produced by chemical excitation of the cerebral cortex in dogs. For this, in dogs in a state of epilepsy, whose convulsions had been suppressed by the inhalation of CO₂, we eliminated the interference of stimulations starting from the carotid sinus and the aortic arch. This suppression was done by section of the vagus and by surrounding the carotid sinus and carotid body with cotton wool soaked in a 10% solution of novocaine. Control experiments showed that by increasing the intra-sinus pressure the reflexes originating from the sinus were abolished.

We did a total of ten experiments, in seven of which the vagus was sectioned and the central end of this nerve stimulated; in the remaining three, besides sectioning, we eliminated the innervation of the carotid sinus on both sides. In none of these experiments was there evidence of a dominant influence of the vagus and carotid sinus on the inhibition of the attack. Section of the vagus when the animal was breathing an attack-inhibiting tension did not assist the development of attacks. An attack can develop right after section of the vagus, but this may occur because of the excitation produced by the section, since the electric stimulation of the central end of the vagus, in an animal in which the convulsions were inhibited by carbon dioxide, can make the convulsive attack reappear. The following is a summary of one of these experiments.

Experiment of 9-6-43. Dog. Weight 6,700gr. Intra-peritoneal injection of 6.7cc. of a 2% solution of morphine. Cranium trepanned and motor zone exposed. Determination of the centre of the orbicularis 3.20 p.m. Start of the application of strychnine to orbicularis. 3.24 p.m. Mechanical stimulation of the orbicularis. Attack lasting 1 minute 30 seconds. 3.28 p.m. Renew strychnine. 3.31 p.m. Spontaneous attack lasted 2 minutes 55 seconds. 3.55 p.m. Renew strychnine. 3.37 p.m. Spontaneous attack lasted 1 minute 40 seconds. 3.44 p.m. strong spontaneous attack lasted 2 minutes 45 seconds. 3.55 p.m. Renew strychnine. 3.56 p.m. Spontaneous attack lasted 2 minutes 30 seconds. At 4 p.m. began
to breathe a mixture containing 11.8% CO going on after to another mixture containing 12.1% and followed by another of 11.7% until 4:12 p.m. when a new attack occurred which lasted 2 minutes 24 seconds. Clonus continued very strongly and spread to the lips at 4:04 p.m. but there was no attack. Strycnine renewed at 4:03 p.m. and 4:15 p.m. Returned to breathing carbon dioxide in an 11.6% concentration 4:17 p.m. Section of the vaguses. 4:26 p.m. Spontaneous attack which lasted 1 minute 50 seconds. 4:29 p.m. Strycnine renewed. Weak clonus. The animal breathed the tension of carbon dioxide mentioned above until 4:40 p.m. when it returned to breathing atmospheric air. 4:43 p.m. Strycnine renewed. The animal had several spontaneous attacks.

One can see from this experiment, in which the tension of carbon dioxide is near the limit of inhibition, that section of the vagus does not influence the inhibition caused. On the other hand stimulation of the central end of the vagus, while the animal was breathing carbon dioxide in inhibitory concentrations, did not cause inhibition of the convulsions in any of the cases observed.

The experiments in which both the vagus and the carotid sinuses were eliminated showed identical results. The inhibitory effects of carbon dioxide remained clear. The following experiment illustrates this.

Experiment of 20-8-43. Dog. Weight 3700 gr. Intra-peritoneal injection of 7 cc. of a 2% solution of morphine. Trenpanation of the right side. Cerebral hemispere exposed. Determination of the centre of the orbicularis palpebrae. Carotid sinus exposed. Intubation of the trachea. Vaguses isolated. 3:20 p.m. Begin to strycninizze the centre. Clonus. 3:25 p.m. Strycnine renewed. 3:30 p.m. Strong clonus. Mechanical stimulation of the orbicularis. Attack which lasted 2 minutes. 3:36 p.m. Strycnine renewed. 3:40 p.m. Attack provoked by mild stimulation of the orbicularis — lasted 2 minutes 10 seconds. 3:45 p.m. Spontaneous attack which lasted 2 minutes 20 seconds. 3:48 p.m. Strycnine renewed. 3:51 p.m. Spontaneous attack which lasted 3 minutes 10 seconds. 3:55 p.m. Inhalation of carbon dioxide in a concentration of 18.8%. 3:58 p.m. Strycnine renewed. Turned to breathing a mixture from the other gasometer with 17% CO² During the period there was a spread of clonus up to the lips but no attack. 4:10 p.m. Inhalation of carbon dioxide stopped and strycnine renewed. 4:12 p.m. Spontaneous attack lasting 2 minutes. 4:15 p.m. Destruction of the nerve net round the carotid sinuses. Application of 10% novocaine. Section of the vagus. 4:17 p.m. Spontaneous attack which lasted 2 minutes. 4:20 p.m. section of the other vagus. Begin to breathe again 17% CO², 4:22 p.m. Strycnine renewed. 4:30 p.m. Breathing of carbon dioxide stopped. During this time it breathed the mixture from the gasometer there was once a spread of clonus to the muzzle but no attack. Shortly after the return to breathing atmospheric air it had a spontaneous attack followed by others at intervals of about 3 minutes.

This experiment shows that carbon dioxide inhibits the convulsions produced by chemical stimulation of the cerebral cortex in animals in which the sensitive endings in the aortic arch and carotid sinuses were eliminated. The
tensions of carbon dioxide effective were the same as those which acted when the sensory endings were still influenced by stimulated nerve centres.

DISCUSSION

The experiments described above confirmed the results obtained by other authors on the inhibitory action of carbon dioxide on experimental convulsions. We showed that epileptiform attacks caused by chemical excitation of the cerebral cortex in dogs can also be inhibited by carbon dioxide in adequate concentration.

The concentration of carbon dioxide necessary to suppress strychnine convulsions in rabbits or in dogs by the intoxication by this alkaloid differs from that which was shown sufficient to suppress the convulsions produced by chemical excitation of the cerebral cortex. In the convulsions of strychnine intoxication, which are of spinal origin, it was necessary to reach much higher tensions of carbon dioxide depending on the degree to which the animal was strychninized. We always used tensions higher than 20% because in cortical convulsions tensions between 15% and 18% are enough to suppress the convulsive attack. The spinal cord seems to be more resistant to high tensions of carbon dioxide than the cerebral cortex. These results are in agreement with experiments by other authors, who also showed that other spinal motor phenomena are more resistant to high tensions of carbon dioxide than the cortical.

In our results, however, the conclusions differ from some authors who interpret the inhibitory action of carbon dioxide as originating predominantly in reflexes which start in the sensitive nerve endings of the upper air passages or in the chemoreceptors of the carotid sinus and aortic arch. Our experiments show that carbon dioxide suppresses convulsions by a direct action on the nerve centres. The reflex influences, even though they may act, are not the only determining or the more important factors in the inhibitory action.

In Table I where the results are given of the use of carbon dioxide on convulsions produced by strychnine intoxication, we see in the experiments on animals with the spinal cord intact, that the concentration of carbon dioxide which is effective is found to be between 22% and 30%, near those which were effective in animals with the spinal cord sectioned. The inhibitory influence starting from the higher centres stimulated by carbon dioxide, as was observed by King and collaborators (12) for the patellar reflex or for the stimulation of sensitive endings, has been removed by the section of the
spinal cord. King and collaborators showed that the inhibition of the patellar reflex requires higher tensions of carbon dioxide in the spinal animal.

In the results of some authors mentioned at the beginning of this paper there is evidence of a reflex influence of the excitation in the convulsive action of the nervous centres. Other workers have found clear effects from the stimulation of the carotid sinus and vagus on the motor cortical phenomena. François-Franck (16) had already shown that the stimulation of the central end of the vagus can enhance convulsions. Vianna Dias, working on dogs (26), studied the effect of electrical excitation of the central end of the vagus on the convulsions by local strychnine application on the brain. He never observed inhibitory action in the gererallised convulsions. Sometimes, the excitation of the central end of the vagus inhibited the local muscular contractions induced by the strychnine on the central motor point. In our experiments in animals fully inhibited by carbon dioxide, section of the vagus or stimulation of the central end can cause an attack or spread clonic muscular contractions which still exist. The existence of a cortical centre of the vagus on the orbital surface of the frontal lobe, as was demonstrated by Bremer and Bailey (17) agrees with these experiments. These authors showed that the stimulation of the central end of the vagus increases the action potentials of the orbital surface of the frontal lobe.

The carotid sinus as a starting point of reflexes which enhance or inhibit the motor phenomena starting from various points of the central nervous system was also thoroughly investigated. Koch (18) inhibited spontaneous or reflex muscular contractions by suddenly increasing the intra-sinus pressure. The same influence was observed by Spychala (19) in dogs using a reflex with contraction of the quadriceps. Danielopolu (20) and collaborators found that convulsive attacks occurred more easily by electrical stimulation of the vagus or carotid sinuses, and they drew from these observations conclusions which were extended to the pathogenesis of convulsive attacks. These results were denied by Martino (21) and Baldacci (22). Also Heymans and Bouckaert (23) criticised the work of Danielopolu from the aspect of medical interest and pointed out that in physiological conditions excitation of the vascular sensitive zones has an inhibitory influence on motor cortical phenomena.

In our experiments the inhibitory influence starting in the vascular zones, sensitive to pressure by the increase in arterial pressure caused by carbon dioxide, can be discounted in virtue of the small variation, or none, which the gas produces in arterial pressure. An inhibitory influence acting through the chemo-receptors of the aortic arch and carotid sinus could be admitted. It
was Gellhorn and collaborators who studie most this possible mechanism of the action of carbon dioxide, and they showed that the inhalation of this gas in a concentration of 15% inhibited convulsions produced by strychnine, absinthe or coramirtine, though the anoxia had a slight inhibitory action or none. In the animals with the vagus sectioned and the carotid sinuses de innervated the action of carbon dioxide was no longer evident or was very slight, whereas the anoxia turned out to have a marked inhibitory action. Gellhorn and Yesenick found that in animals with the chemo-receptors removed, tensions of carbon dioxide up to 50% were still ineffective.

An explanation, though not definite, has been given by Gellhorn and collaborators (24), based on the work they did on the influence of anoxia and carbon dioxide on reflexes starting in the carotid sinus and aortic arch. These authors found that anoxia diminishes and carbon dioxide intensifies the depressive reflexes which start in these organs. On the other hand variations in arterial pressure can cause inhibitory effects on the convulsions and these authors extend these results to stimulation of the chemo-receptors. Anoxia causing a diminution in the efficacy of the inhibitory reflexes, these either are not felt or act weakly counteracting the direct depressive action which anoxia would exert on the nervous centres. The intensification caused by hypercapnia effects of carbon dioxide on the central nervous system. The destruction of these reflexogenic zones would release the somatic nervous system from the inhibitory reflex influences and allow the convulsions to occur even with high tensions of carbon dioxide. This is, essence, the explanation given by Gellhorn of the inhibitory action of carbon dioxide on convulsions.

Our experiments on dogs with convulsions caused by chemical stimulation of the cerebral cortex do not fit in with this explanation. In all the experiments we did, the de-innervation of the carotid sinuses and section of the vagus did not make the convulsions reappear when already inhibited by carbon dioxide. The tensions of carbon dioxide necessary to suppress the convulsions in both the de-innervated and the innervated animals was approximately the same. It was never necessary to reach the high concentrations which Gellhorn found. It should be stressed that in the sort of convulsion we produced in our experiments, because of their cortical origin, a marked reflex inhibitory effect was to be expected. Our results agree with those of McKail, Obrador and Wilson (25) in cats, in which the cerebral cortex was stimulated, muscular twitches were registered. In these experiments the authors observed a marked depression in the cortical motor response in the animal which breathed carbon dioxide in a concentration of above 5%. The
de-innervation of the carotid sinuses and section of the vaguses did not influence the results, which agree with our conclusions that the inhibitory action of carbon dioxide works predominantly by a direct action on the nerve centres.

CONCLUSIONS

1) Carbon dioxide in adequate concentration inhibits the convulsions produced by strychnine intoxication and by chemical excitation of the cerebral cortex in dogs with strychnine and acetylcholine.

2) The inhibition of spinal convulsions produced by strychnine intoxication needs a greater concentration of carbon dioxide than the convulsions of cortical origin.

3) In the spinal animals and with strychnine convulsions, the tensions of carbon dioxide needed to suppress the convulsions is about the same as in animals with the spinal cord sectioned.

4) The elimination of reflex actions starting in the chemo-receptors of the aortic arch and carotid sinus does not markedly influence the inhibition by carbon dioxide on convulsions produced by chemical excitation of the cerebral cortex in dogs.

5) These results show that carbon dioxide inhibits the convulsions produced by strychnine intoxication and by chemical excitation of the cerebral cortex acting directly on the nerve centres, the inhibitory reflex actions being of secondary importance.