On the Potential Changes in Spinal Cord During Administration of Carbon Dioxide

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Many investigations have been performed on the action of carbon dioxide on various excitable tissues. Most of these results agreed well upon the point that the excess of carbon dioxide causes the lowering of excisability. It is also mentioned that carbon dioxide exert an effect similar to the anelectrotonic on peripheral nerve fibers (8, 13, 14, 15)

However, any conclusive result on the mechanism of the action of carbon dioxide has been reported by *Lorente de Nó* (13), it has been regarded by others as a secondary effect due to the change of pH.

On the other hand, no investigation has been reported on the continuous action of carbon dioxide on the spinal cord, although there may be found in some papers the experiments on the potential change of spinal cord during asphyxia (5).

In contrast to the action of carbon dioxide, and enhancement of spinal reflex by strychnine is well-known (6, 12), and many studies on the changes of excitability of spinal motoneutrone by means of electrotonus have been carried out in details.

The present paper will describe the depressant action on spinal cord of carbon dioxide. It also aims to elucidate, by applying electrotonus to spinal cord together with carbon dioxide, that the depressant action is resulted by the same mechanism as in the peripheral nerve fibres.

METHOD

All the experiments were carried out on Japanese toads (bufo vulgaris japonicus) in winter. The spinal cord was excised from the animal body and immersed in Ringer solution in an air-tight acrylite chamber, into which oxygen or gas mixture consisting of oxygen and carbon dioxide was introduced through an air-tight rubber tube. The pressure of gas was controled by a Mariotte's bottle. At the beginning and the end of each experiment, the concentration of carbon dioxide in the gas mixture was examined with Haldane's gas analyser and its range was 6.6–29.8%. The gas mixture containing 95% oxgen and 5% carbon dioxide was usually adopted as a control. Switching of the test gas made by a three-way cock placed between the air-tight chamber and the gas containers (Fig. 1).

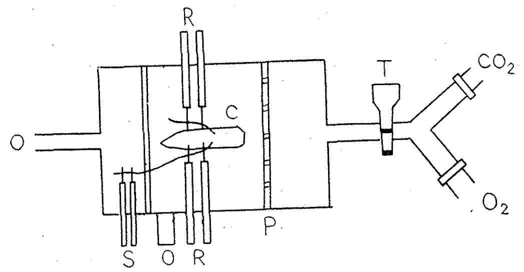

Fig. 1. Schematic diagram of the moist chamber.
C: spinal cord. O: gas outlet.
S: stimulating electrodes. T: three-way cock.
R: recording electrodes. P: partition to protect from movement due to bubbling.

The root potentials were evoked by single shocked delivered to the 9th or 10th dorsal root and recorded with a cathod ray oscilloscope and d.c. or a.c. amplifier (time constant: 300 m sec.). In odrer to give the effective stimulation throughout the whole stage of the experiment, supernormal stimuli were employed. The pH of Ringer solution was adjusted by M/500 phosphate buffer and it was measured potentiometrically by means of quinhydron electrode method before and after the experiment.

In the case of strychnization, the volume of Ringer solution in the moist chamber was carefully ascertained to be 20 ml and an appropriate amount of strychnine nitrate of $2\text{–}5 \times 10^{-5}$g/ml was dropped into it. The polarizing current for producing electrotonus was applied according to the method described by *Iwata* and *Otani* (10, 11).

RESULTS

The changes in VRP (abbreviation for ventral root potential) during the administration of 6.8% CO_2 are illustrated in Fig. 2. These records were obtained successively with intervals of 5 minutes between each. With the lapse of time, VRP decreases its height and the spike discharges superimposed on it diminish in number. But these changes could be recovered to the original state by replacing the test gas with the control gas or pure oxygen. This shows that the changes by carbon dioxide are entirely reversible in such concentration. It is noteworthy that VRP is rather increased after a transient decrease. This was frequently encountered in the experiment with CO_2 in relatively low concentration.

Carbon dioxide in higher concentration decreased VRP and diminished the spike discharges more rapidly and more remarkably. The records in Fig. 3 show the effects of 18.4% carbon dioxide. The changes mentioned above occurred promptly and no potential change in ventral root could be detected at all in the last stage

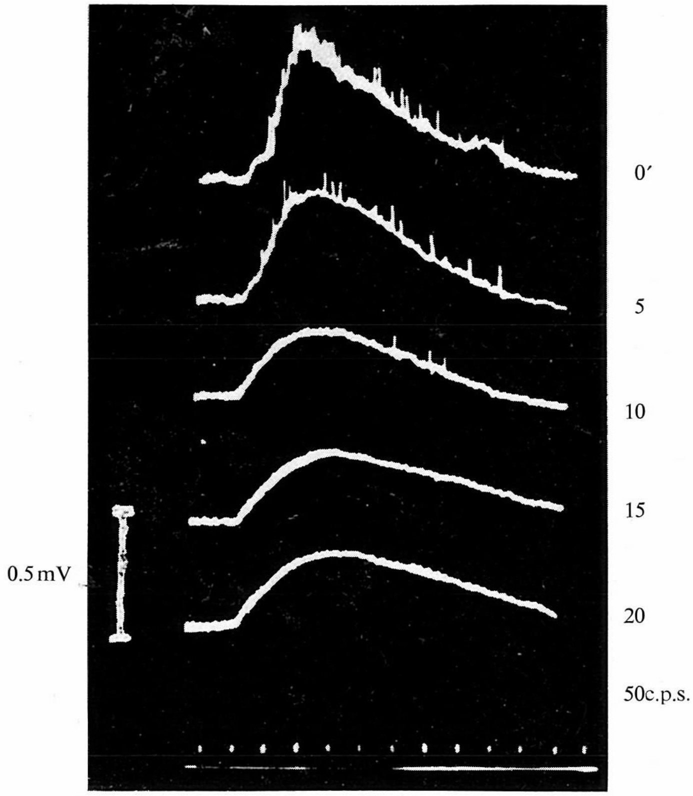

Fig. 2. Ventral root potentials and spike discharges superimposed on it were photographed sucessively with the time interval of 5 minutes.
Numbers to the right of figure show the time elapsed after the administration of CO_2, and that to the left is a calibration of the amplification. The last row is time scale.

even with a stimulus three times as intense as before. When the test gas was replaced quickly by the control gas or pure oxygen within several minutes after the disappearance of the responses, complete recovery could be ascertained. Similar results could be obtained about 60 min. after the administration of 10.8% CO_2, and for complete recovery it took 80–100 minutes. Below this concentration, VRP did not perfectly disappear even with long exposure (1–3 hours). The changes in DRP (dorsal root potential) caused by carbon dioxide tend to delay and to be less remarkable, when compared with those in VRP. In other words, the DRP could be obtained for several minutes after VRP had disappeared, and changes in the former exceed these in the latter at the same moment: e.g. 60 minutes after the administration of 10.8% CO_2, VRP was hardly observable, whereas the DRP could be recovered with a size of one fourth or one fifth of the control. These results were obtained not only with such a high concentration, but also with lower concen-

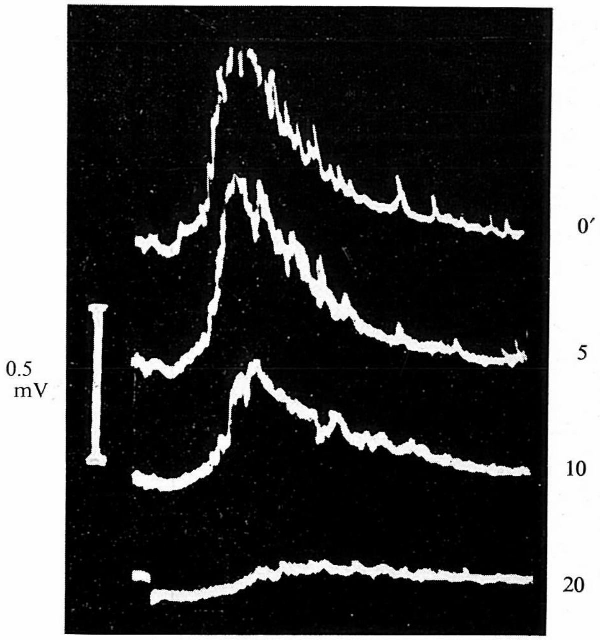

Fig. 3. Effects of 18.4% CO_2 on VRP. Notations are same as Fig. 2.

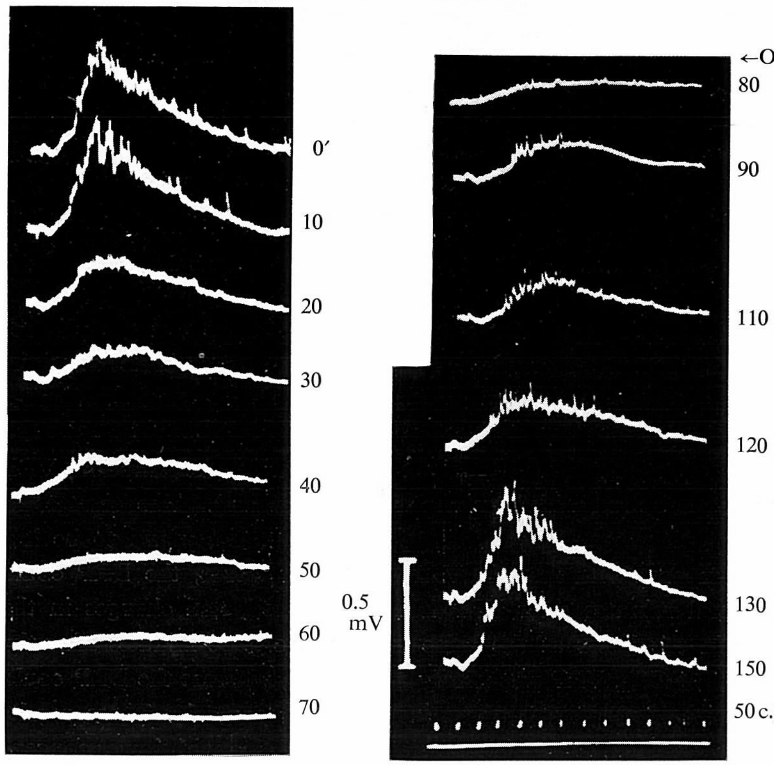

Fig. 4a. Effects of 10.8% CO_2 on VRP. Notations are same as before. At 70 minutes, gas mixture was switched to pure oxygen. Note the complete recovery of VRP and spike discharge.

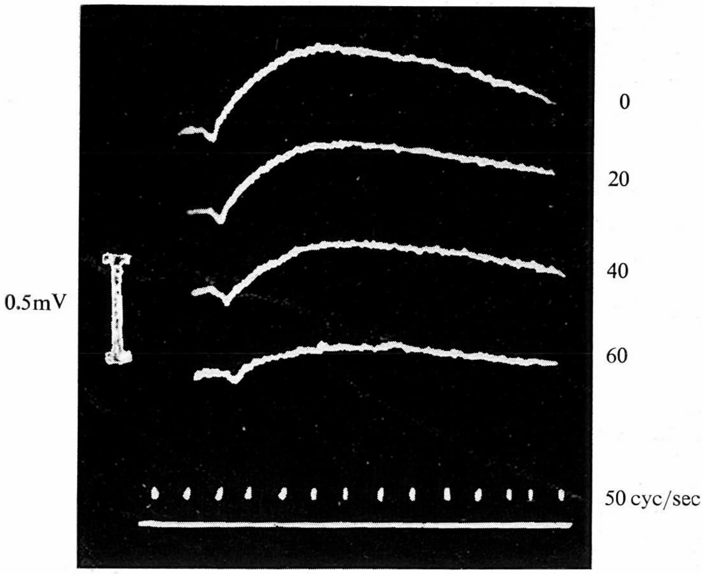

Fig. 4b. Effects of 11% CO_2 on DRP.
The preparation is same as Fig. 4a. Note the difference in height of potential level in the same period.

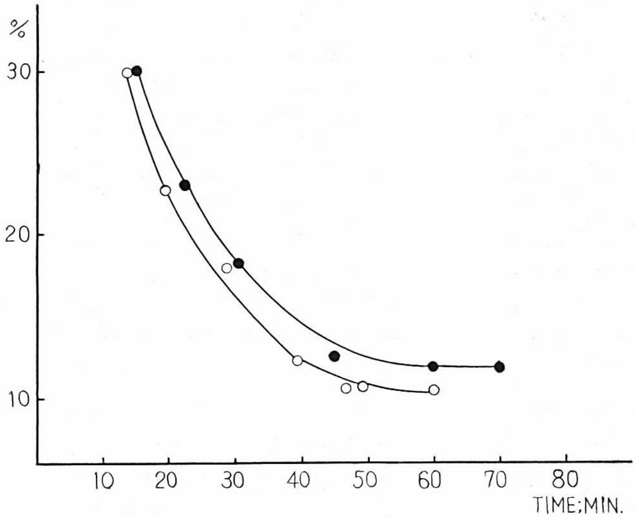

Fig. 5. Diagram showing the relationship between CO_2 concentration and the time require for dtotal disappearance of VRP and DRP. Open cicle: VRP., Solid Circle: DRP.

trations. (Fig. 4, Fig. 5).

When 2×10^{-5} g/ml strychnine was added to the Ringer bath after the root potentials became no more elicitable under the influence of CO_2 both VRP and DRP reappeared in response to single shock stimulation. In this case the amplitude of the reappeared VRP amounts only to 0.2 mV. at most. On the other hand, DRP was considerably enhanced. It reached to 0.2 mV within a few minutes after the administration of strychnine. But these changes did not proceed any further. With continued administration, the amplitudes of these potentials were slowly decreased (Fig. 6).

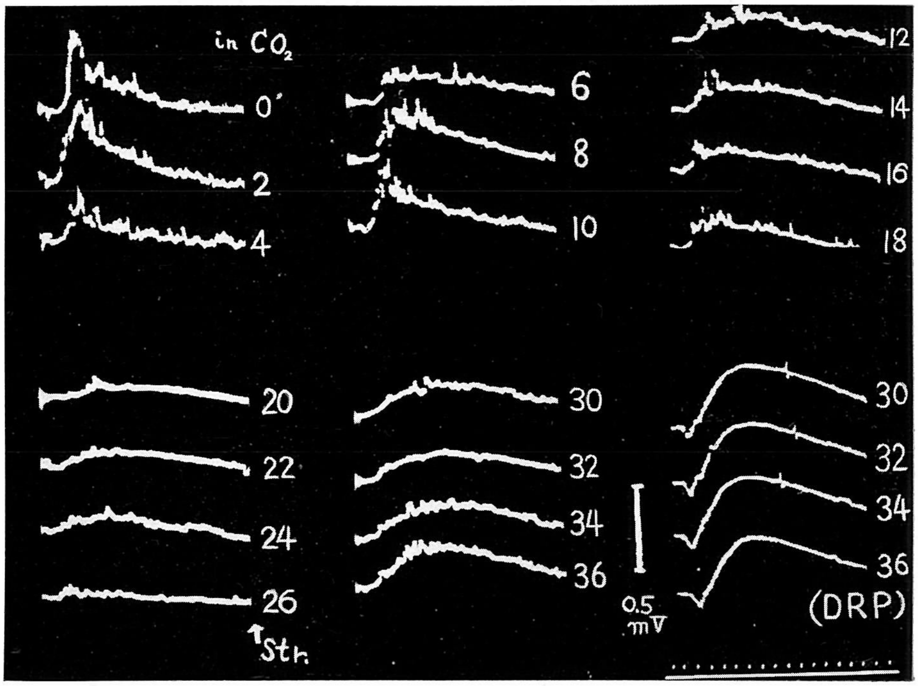

Fig. 6. Combined effects of CO_2 and strychnine.
Strychnine was administered between 26 and 30 minutes.
Lower right records show the enhancement of DRP.

When the test gas was switched to the control gas or pure oxygen, the enhancement by strychnization reappeared strikingly in several minutes. Within ten minutes after this procedure, the VRP surpassed the control value in size and the spike discharges also reappeared. It must be noted that the latent time for the first spike discharge became progressively shorter. In the begnning of switching the first spike was appeared at a point near the crest of VRP, but later it appeared on the rising phase of VRP and this potential level became lower with the lapse of time (Fig. 7a).

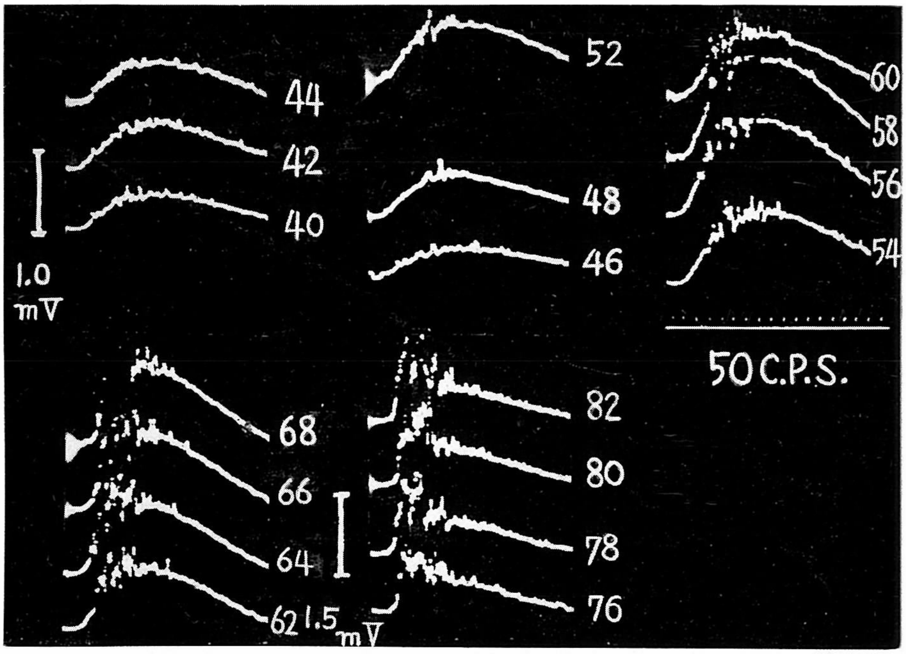

Fig. 7a. Successive changes in VRP after replacing CO_2 by control gas. Note the shift of spike discharge level on VRP. Numbers show time in minutes and are continued from Fig. 6.

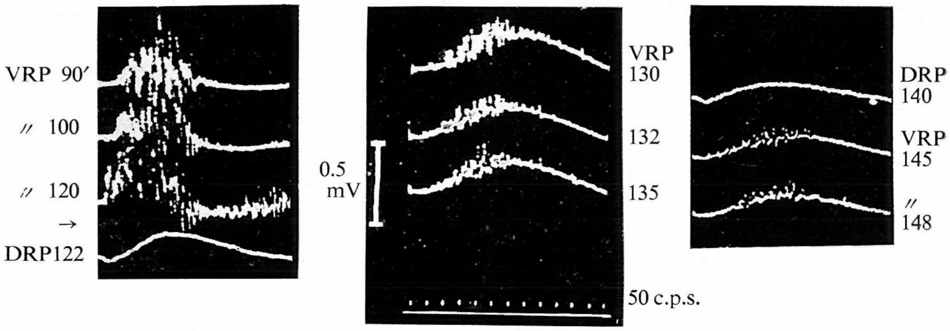

Fig. 7b. Effects of CO_2 (19,8%) on the spinal cord pretreated with strychnine. Numbers show time in minutes and are continued from Fig. 7a. CO_2 was introduced at the time shown by the arrow. (123 minutes after the first administration of the gas).

To such a preparation the test gas was readmitted and records were continued. (Fig 7b). At the stage just before CO_2 was introduced for the second time, it is likely that the preparation in the record may be considered to be caused by strychnization. Beside the features mentioned above, a marked positive deflection was recognized accompanying the burst of spikes. The so-called strychnine fluctuating potential was never evoked, probably because of the small dosage of the drug. In this period also, the DRP was increased in height to an extent exceedingly remarkable when compared with that of VRP.

When a gas containing over 25% CO_2 was used, the root potentials were abolished completely within twenty minutes. In the experiments with high concentrations of CO_2 (22–29%), three preparations among seven did not recover completely. Judging from these results and those of other workes (8, 13, 14, 15), it seems that the spinal cord are far more vulnerable than other excitable tissues of amphibia.

The effects of polarizing currents were examined with the preparations in the control gas and in the test gas (Fig. 8). With anelectrotonic current, VRP decreased

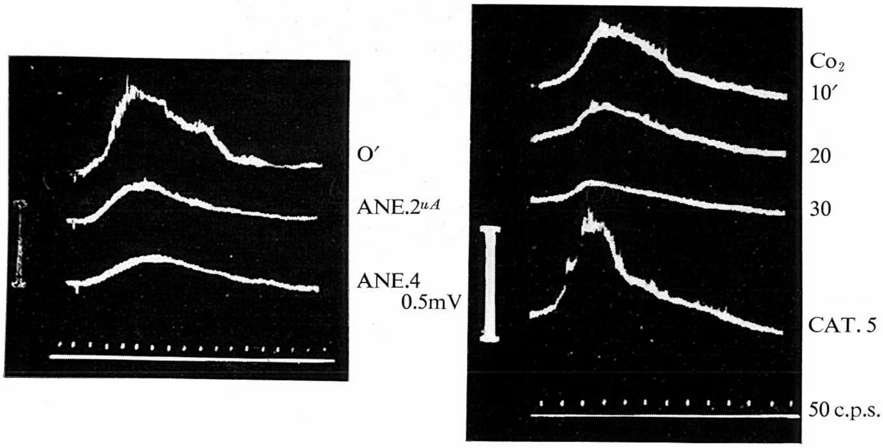

Fig. 8. Combined effects of 14.1% CO_2 and polarizing currents on VRP.
Left: Effects of anelectrotonus. Right: CO_2 and catelectrotonus.
Current intensity is shown in microampere. Note that the VRP was depressed by CO_2, but when catelectrotonus was applied this depression was removed.

in height and spike discharges decreased in number. These changes became more remarkable with increase in the intensity of current. And finally no potential change was elicited in the ventral root by whatever strong stimuli given to the dorsal root. These changes in the VRP resemble to those produced by administration of CO_2. On the other hand, catelectrotonic current applied to the spinal cord, which did'nt respond no more to any strong stimuli owing to the excess of CO_2, made a stimulus of usual intensity effective in eliciting VRP. As already reported by others (10, 11, 16), the polarizing currents have the effects to both and shortened by catelectrotonic current. It is interesting that there are many similar points between changes produced by CO_2 and anelectrotonus, and these similarity will be disccused below.

Fig. 9 shows the effect of changing the pH of Ringer solution. In this case also, the decrease of VRP is very similar to those by CO_2 excess.

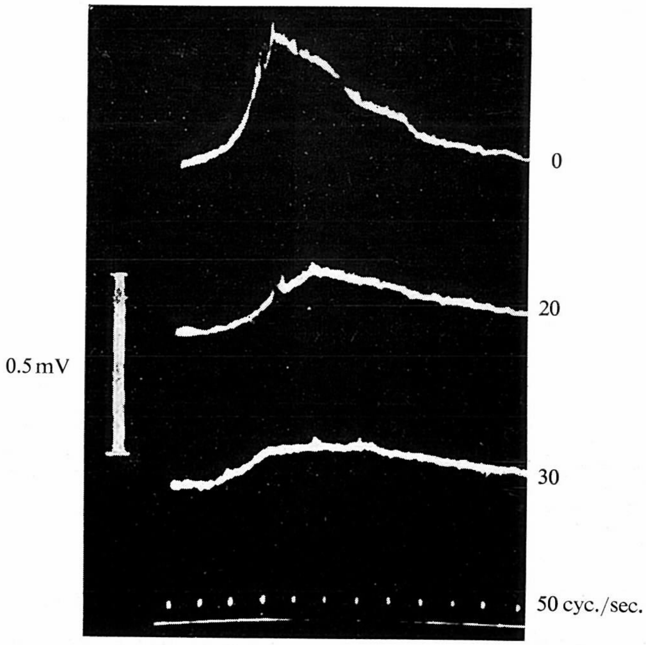

Fig. 9. Effect of acidified Ringer solution on VRP.
The preparation was immersed in Ringer solution of pH 6.0, and the VRPs were recorded successively. Numbers show time in minutes after the immersion.

DISCUSSION

As already pointed out by many authors, it is most likely that the ventral root potential is the electrotonic spread of e.p.s.p. (excitatory post synaptic potential) in moteneuron pool (2, 3, 4, 7, 11). Furthermore, it is well known that e.p.s.p. is decreased by catelectrotonus and increased by anelectrotonus.

The increase of VRP after a transient decrease, which was found in the present research by the administration of CO_2, may be a result of its anelectrotonic action. The fact that the VRP is elevated by anaesthetics was observed by *Brooks* et al. (5), and the narcotic action of CO_2 to the nervous system is also recognized. So, carbon dioxide may cause the hyperpolarization in motoneurons of spinal cord as well as in peripheral nerve fibres.

The disappearance of spike discharges in the later stage of CO_2 administration can be naturally expected, because the excitability of montoneuron is gradually lowered by the hyperpolarizing action of this gas. Hence, the dimished VRP in early stages with high concentration of CO_2 may be accounted for by the decrease of the bombardment from interneurons: in such stages it may be considered that the effects of carbon dioxide extend to interneurons. The fact that VRP once abolished can be restored by the administration of strychnine in small dosage makes this assumption very probable. As mentioned by some authors (6, 12), strychnine enhances bombardments from interneurons tremendously. It is shown in Figs. 6 and 7 that there were scarcely the spike discharges superimposed on VRP under the combined influence of CO_2 and strychnine, but the height of VRP was very large compared with that in the ordinary state. In this case, most of the motoneurons may have been hyperpolarized too much to discharge impulses in spite of the enhanced e.p.s.p., and so the spike discharges could scarcely be observed unless CO_2 was replaced by the control gas. Regarding the concentration of strychnine used in the present experiment, intense depolarization in the motoneurons can hardly be expected. Therefore, it is not unreasonable that fluctuating potential was not observed throughout the experiment. In addition, changes in VRP similar to those caused by CO_2 could ce produced by acidified Ringer solution. Hence, the assumption that the effect of CO_2 to VRP is the result of lowering the pH of surrounding solution may be possible.

SUMMARY

1. The changes of root potentials by the administration of carbon dioxide (6.6%–29.8%) was examined on the excised toad's spinal cord.
2. In low concentrations (below 8%) and in early stage of high concentrations of the gas, ventral root potential increases in height and spike (discharges) decrease in number, but the changes of dorsal root potential were hardly observable in these conditions.
3. In later stage of high concentrations (over 8%), ventral root potential decreases in height and spike discharges superimposed on it also decrease in number. The decrease of dorsal root potential was observed more later. In the experiment when the concentration was 11% ventral root potential abolished after 60 minutes.
4. The changes of root potentials were also examined by applying strychnine and polarizing currents to the spinal cord, combined with carbon dioxide or not. From these results, it may be concluded that the blocking action of carbon dioxide is attributable to the hyperpolarization of motoneurons and interneurons.

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