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THE MECHANISM OF THE INHIBITION BY CARBONIC ACID OF THE SMOOTH MUSCLE CONTRACTION PRODUCED BY HISTAMINE AND OXYTOCIN

BY

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Carbonic acid inhibited the stimulating action of histamine on guinea-pig intestine and of oxytocin on the uterus of many animal species. This effect was relatively specific. Under the same conditions the actions of acetylcholine, KCl, adrenaline (on rabbit uterus), and ergot alkaloids were not inhibited. The electrical excitability of smooth muscle was similarly not affected. The inhibitory action of carbonic acid is directly proportional to its concentration in the medium in which the isolated organ is maintained. The study of the activity of histamine and oxytocin at different *p*H in a medium free of $NaHCO_3$ and buffered with a mixture of sodium maleate and maleic acid suggested that the inhibitory action exerted by carbonic acid was specific and independent of the simultaneous modifications of the hydrogen ion concentration. The mechanism of these phenomena is discussed.

Evidence has been provided recently that carbon dioxide inhibits the excitability of peripheral nerves by a specific action (Lorente de Nó, 1947; Monnier, 1952; Coraboeuf, 1951, 1954). On the other hand, as early as 1923 Lovatt-Evans and Underhill had shown that CO_2 exerts an inhibitory action on the contractions of isolated intestine produced by different substances. Later, Garan (1938) showed that the activity of histamine on isolated guinea-pig intestine was a function of the partial pressure of CO_2 and attributed the effect to the formation of a carbamate compound, supposedly inactive, between histamine and CO_2. The conclusions of Garan (1938) were disproved by Kiese (1940), who also observed that CO_2 diminished the response of the intestine to histamine, although the action of acetylcholine was but little influenced and that of pilocarpine was enhanced. By studying the relationship between *p*H and the activity of histamine, Kiese (1940) concluded that the change in the polarity of the histamine molecule by modifications of *p*H could not satisfactorily explain the effect observed.

More recently, Halpern (1956a and b) studied the action of CO_2 on the contractions of the isolated guinea-pig ileum produced by histamine, acetylcholine, KCl and the antigen-antibody reaction. An inhibitory effect was found on contractions produced by histamine and in anaphylactic reactions, but under the same conditions the effect of acetylcholine remained unchanged or was increased. The effect of CO_2 was shown to be completely reversible. It could not be attributed to tissue anoxia, because it was produced by gas mixtures containing 20% CO_2 and 80% O_2: the partial pressure of oxygen was therefore higher than when air was used to oxygenate the medium.

Halpern, Mayer, and Bugnard (1956) have demonstrated that CO_2 also inhibits the action of oxytocin on the uterus of the rabbit, guinea-pig and rat. Here, too, the effect was specific for oxytocin and did not apply to the action of other substances which cause the uterus to contract.

The aim of the present work was to discover how carbon dioxide inhibits the action of histamine and oxytocin on smooth muscle. The results suggest that carbon dioxide modifies the reactivity of smooth muscle to these substances by a specific action which is not dependent upon the changes it produces in the *p*H of the medium.

METHODS

The investigations were performed either with isolated guinea-pig intestine or isolated uteri from guinea-pigs, rabbits, and rats. The organs were taken immediately after killing the animals by carotid artery section. Strips of 2 to 3 cm. were cut off and immersed in oxygenated Tyrode solution at 37° containing the following ingredients in 100 ml.: NaCl 0.8 g.; $CaCl_2$ 0.01 g.; KCl 0.02 g.; $MgCl_2$ 0.01 g.; NaH_2PO_4 0.005 g.; $NaHCO_3$ 0.1 g.; dextrose 0.1 g.; atropine 3 μg. When the concentration of $NaHCO_3$ was changed, a modification was made in the NaCl concentration to maintain the same molarity of the solution. In one series of experiments, a solution without $NaHCO_3$ was used and the *p*H was adjusted to the desired value with a buffer of sodium maleate and maleic acid. The composition of Tyrode solution was modified in this instance by replacing the $NaHCO_3$ by 0.07 g. of maleic acid (6 mM). The *p*H was then adjusted with N-NaOH (approximately 1.1 ml./l. to obtain *p*H=7.5). The solutions of histamine dihydrochloride, acetylcholine chloride, oxytocin, etc., were prepared in Tyrode solution at *p*H 7.4. The volume added to the bath was 0.1 to 0.5 ml. The total volume of the vessel in which the organ was immersed was 50 ml. The contractions were recorded on smoked paper, by frontal inscription, employing a magnification of about 20 times.

Electrical Stimulation.—The technique described by Paton (1956) was used, using the electronic stimulator of Ead (1951). The anode was platinum wire, 4 to 5 cm. long, introduced in the lumen of the intestine. The cathode was a similar wire in the solution. Stimulation was coaxial. A diagram of the apparatus is seen in Fig. 1.

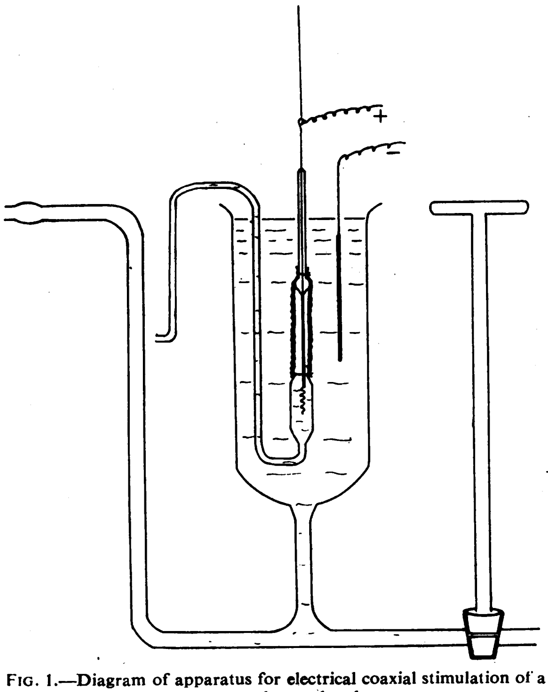

FIG. 1.—Diagram of apparatus for electrical coaxial stimulation of a smooth muscle tube.

Rectangular pulses of 20 msec. duration every 10 sec. were used to stimulate the gut. 20 V usually produced supramaximal stimulation.

pH Measurement.—The *p*H of the solution was measured instantaneously with a laboratory type *p*H meter (Cambridge Instrument Co.). The glass and calomel electrodes were immersed in the vessel in which the organ was studied.

Introduction of Gas Mixtures.—Air, oxygen, CO_2, or mixtures of CO_2 and O_2 were bubbled through the solution in each experiment. With air or oxygen, the *p*H of the Tyrode solution, initially about 7.5, slowly increased to 8.2 or even 8.5. A mixture rich in CO_2 produced a diminution of the *p*H. A typical experiment is illustrated in Fig. 2. By simultaneously bubbling air and a mixture of CO_2 and O_2, at different rates, the desired *p*H could be maintained for long enough to carry out the experiments. The H_2CO_3 content of the solution was always calculated from the measured value of *p*H according to the Henderson-Hasselbach equation:

$$pH = pK + \log \frac{(HCO_3^-)}{(H_2CO_3)}$$

where *p*K is the negative logarithm of the dissociation constant of carbonic acid. The value of *p*K, at 37°, is 6.31. In our calculations no allowance was made for activity coefficients and (HCO_3^-) was assumed to be equal to the $NaHCO_3$ content of the solution employed.

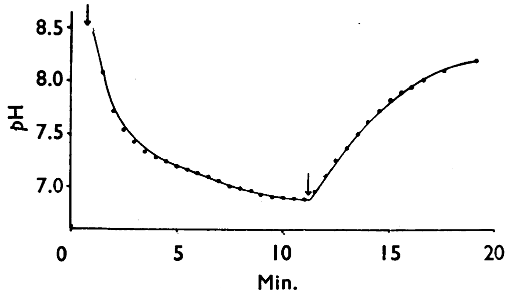

FIG. 2.—Modification of the *p*H of the normal Tyrode solution produced by the bubbling with 20% CO_2 from first to second arrow. At second arrow bubbling with air was commenced.

RESULTS

Relationship Between H_2CO_3 Concentration and Reactivity of the Isolated Guinea-pig Intestine to Histamine

Two methods were used to evaluate the effect of the H_2CO_3 on the action of histamine. With the first method, the height of the contraction of the isolated guinea-pig intestine produced by a fixed dose of histamine at different H_2CO_3 concentrations was determined. The results are shown in Fig. 3.

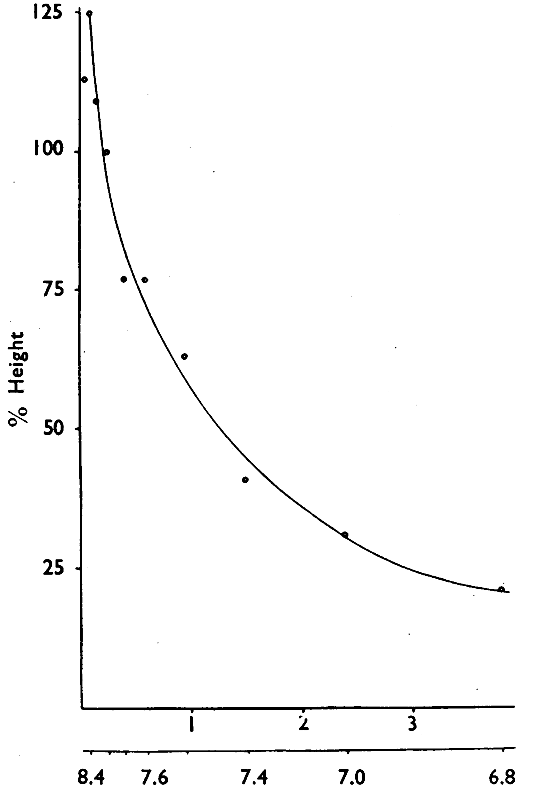

FIG. 3.—The height of the contraction of the isolated guinea-pig intestine preparation produced by a fixed quantity of histamine at various H_2CO_3 concentration in normal Tyrode solution expressed as % of that at *p*H 8·0 to 8·1 given the arbitrary value of 100. The *p*H values are indicated on the lower abscissa, while the H_2CO_3 concentration (mM/l.) is shown on the upper abscissa. Each point was based on at least 5 determinations with each of 4 strips of muscle.

In comparing the different contractions, the height of the one taking place at *p*H 8.0 to 8.1 was taken as 100%, and the heights at other *p*H values were referred to it. In every case, the same dose of histamine was used through the entire experiment and it was chosen so that vigorous but submaximal contractions were obtained. The dose of histamine base varied between 0.015 and 0.030 μg./ml., depending on the particular sensitivity of each intestinal strip.

The results show that the activity of histamine was diminished as the H_2CO_3 concentration was increased (Fig. 3). With a mixture containing 5% CO_2 and 95% O_2, the *p*H attained was 7.3 and the action of histamine was diminished by about 50% of that at *p*H 8.0. With a mixture containing 20% CO_2, equilibrium was reached at *p*H 6.8 and the diminution of activity was 80%.

It is impossible to measure precisely the degree of inhibition by this method because there was no direct relation between the height of the contraction and the concentration of histamine. Furthermore, at a *p*H of less than 6.8, the activity was so low that measurements could not be continued. To overcome these difficulties the second method was employed. This required the determination of the dose of histamine which produced the same response of the isolated intestine at different H_2CO_3 concentrations. The results in Fig. 4 show that, when equiactive doses of histamine are plotted against the H_2CO_3 concentration, a straight line was obtained. As the quantity of histamine necessary to produce the same effect was the reciprocal of its activity, it can be deduced from the observed relationship that the activity of the histamine was inversely proportional to the concentration of H_2CO_3.

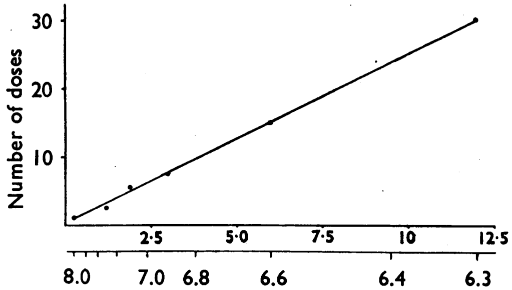

FIG. 4.—Dose of histamine (expressed in terms of the multiple of the dose required at *p*H 8·0) producing the same response of the isolated guinea-pig intestine preparation at various H_2CO_3 concentration, in normal Tyrode solution.

Role of pH

As the action of histamine on smooth muscle was modified by the H_2CO_3 content of the Tyrode solution, it was necessary to find whether this effect was due to changes in the *p*H or was produced specifically by H_2CO_3.

It is impossible to modify the H_2CO_3 concentration of Tyrode solution without simultaneously changing the *p*H, all the other conditions remaining the same. We approached this problem in two ways: (a) by modifying the $NaHCO_3$ of Tyrode solution; (b) by employing a buffer of different composition.

Influence of $NaHCO_3$ Concentration on the Reactivity of the Isolated Guinea-pig Intestine to Histamine and Acetylcholine.—By using Tyrode solutions which contained between 0.25 and 10 g./l. of $NaHCO_3$, it was possible to obtain a wide variation in *p*H with the same concentration of H_2CO_3. When air is bubbled through solutions containing 5 or 10 g./l. $NaHCO_3$, the *p*H reaches

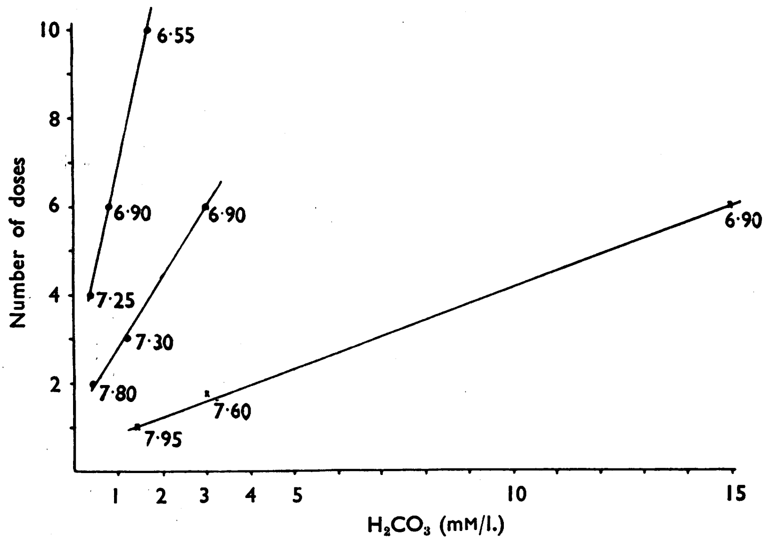

FIG. 5.—Dose of histamine (see Fig. 4) producing the same response of the isolated guinea-pig intestine preparation at various H_2CO_3 concentration and at various $NaHCO_3$ concentration. Upper curve $NaHCO_3$ 0·25 g./l.; middle curve $NaHCO_3$ 1 g./l.; lower curve $NaHCO_3$ 5 g./l. The numerals on the graph indicate the *p*H value at each point.

9 or more and the medium becomes quite unfavourable for the organ.

The results in Fig. 5 show that the effect of histamine diminished with increasing H_2CO_3 concentration at the same $NaHCO_3$ concentration and increased with increasing $NaHCO_3$ concentration at the same H_2CO_3 concentration. In addition, the effect of histamine on the guinea-pig intestine remained practically the same at constant *p*H and independent of the actual concentration of $NaHCO_3$ and H_2CO_3 (Fig. 6).

It was therefore impossible to decide, in this way, the exact rôle of *p*H.

Experiments with a Different Buffer System.—Many solutions were tried to find a convenient medium, free from $NaHCO_3$. Finally a solution containing maleic acid and sodium maleate proved quite suitable and, in it, organs responded regularly and reversibly for the periods of time essential for quantitative study. This was not so with solutions buffered with substances such as phosphate or citrate, which produced more or less irreversible modifications in the responses of the tissues.

Fig. 7 shows the results obtained when the activity of histamine was examined in the maleic acid/sodium maleate solution at different *p*H values, and, for comparison, the observations upon histamine activity in normal Tyrode solution of which the *p*H was modified by CO_2.

It appeared that the activity of histamine was not greatly affected by changes in *p*H, unless these were produced by increasing the H_2CO_3 concentration. In fact, at *p*H 6.8 the action of histamine was practically unchanged in the maleate medium, while it was very much reduced in normal Tyrode solution. It may be concluded, therefore, that carbonic acid exerts some more or less specific action.

Influence of H_2CO_3 on the Smooth Muscle Contractions Produced by Electrical Stimulation or by Substances Other than Histamine

It was of interest to investigate the specificity of the action of H_2CO_3 on the response to histamine. The results were as follows: The action of a standard dose of acetylcholine in guinea-pig intestine was un-

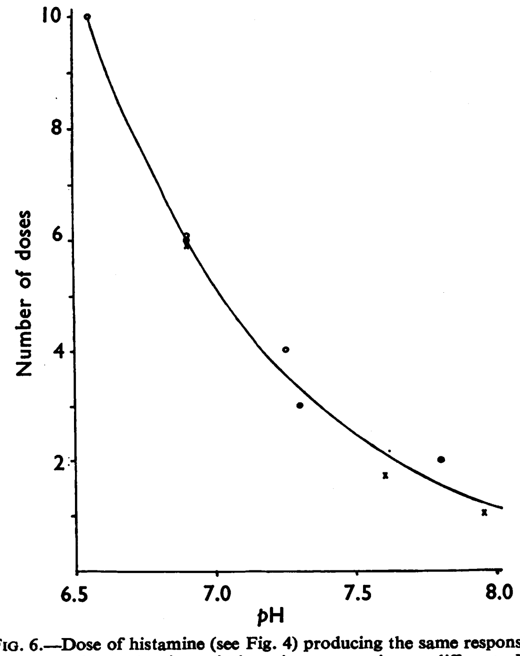

FIG. 6.—Dose of histamine (see Fig. 4) producing the same response of the isolated guinea-pig intestine preparation at different *p*H values, using Tyrode solution with different concentrations of $NaHCO_3$ (O=0.25 g./l.; ●=1 g./l.; X=5 g./l.).

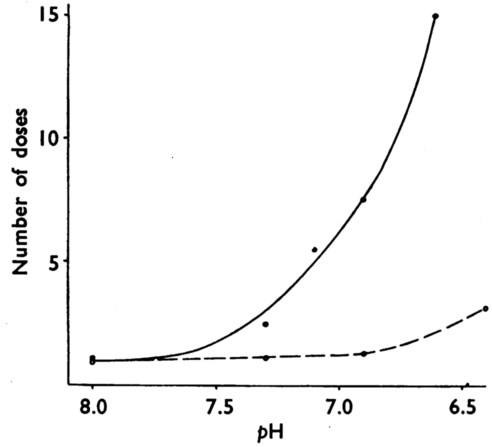

FIG. 7.—Dose of histamine (see Fig. 4) producing the same response of the isolated guinea-pig intestine preparation at different *p*H values. Full line, in normal Tyrode solution (bicarbonate buffer); broken line, in Tyrode solution buffered with sodium maleate and maleic acid.

affected or enhanced by the H_2CO_3 within the limits studied (Fig. 8). The action of KCl was not modified between *p*H 8.1 and 6.9. The contraction of the intestine produced by electrical excitation was not modified by H_2CO_3 between *p*H 8.1 and 6.9 (Fig. 9).

Influence of H_2CO_3 Concentration on the Response of the Isolated Uterus to Oxytocin

Halpern *et al.* (1956) showed that H_2CO_3 can inhibit the action of oxytocin on the isolated uterus as illustrated in Fig. 10. Experiments with KCl, adrenaline (rabbit uterus) and methylergometrine (Methergen) showed that H_2CO_3 only modified the oxytocin response (Fig. 11). The relationship of the activity of oxytocin to *p*H

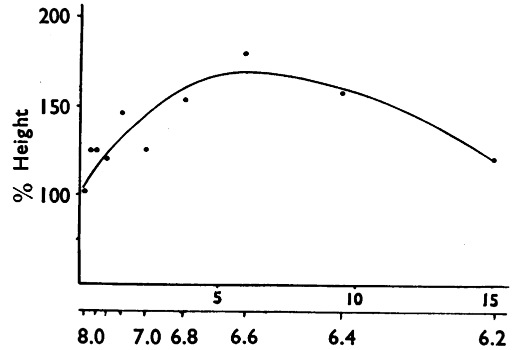

FIG. 8.—Height of the contraction (see Fig. 3) of the isolated guinea-pig intestine preparation produced by a fixed quantity of acetylcholine at different H_2CO_3 concentration, in normal Tyrode solution. Upper abscissa, H_2CO_3 mM/l.; lower abscissa, *p*H.

showed that it was greatly inhibited at *p*H 6.8 in Tyrode solution (bicarbonate buffer) but that it was not changed at the same *p*H in the maleate buffered medium (Fig. 12). The observations upon histamine and oxytocin thus resemble each other.

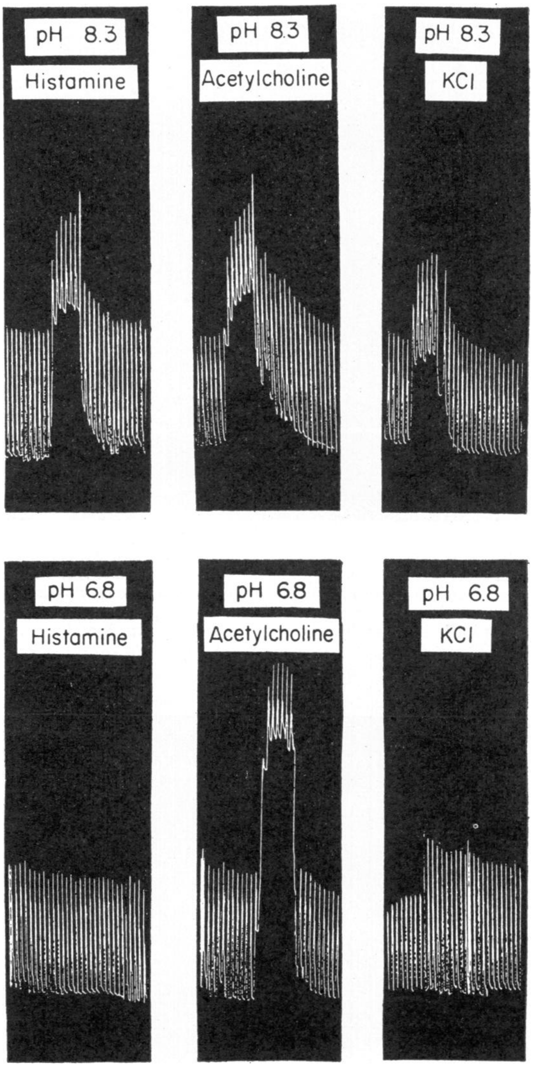

FIG. 9.—Contractions of the isolated guinea-pig ileum preparation in response to periodic electric stimulation. Pulse width, 20 msec.; frequency, 6/min.; 20 V. Upper records: effects of histamine (1.5×10^{-8}), of acetylcholine (0.5×10^{-8}) and KCl (1×10^{-3}) on the responses of the intestine in standard Tyrode solution (buffer, $NaHCO_3$ 1 g./l.), bubbled with pure oxygen. Lower records: effects of identical concentrations of the same substances on the intestine in the same Tyrode solution, the *p*H of which has been lowered to 6·8 by bubbling with a gaseous mixture containing 75% O_2 and 25% CO_2.

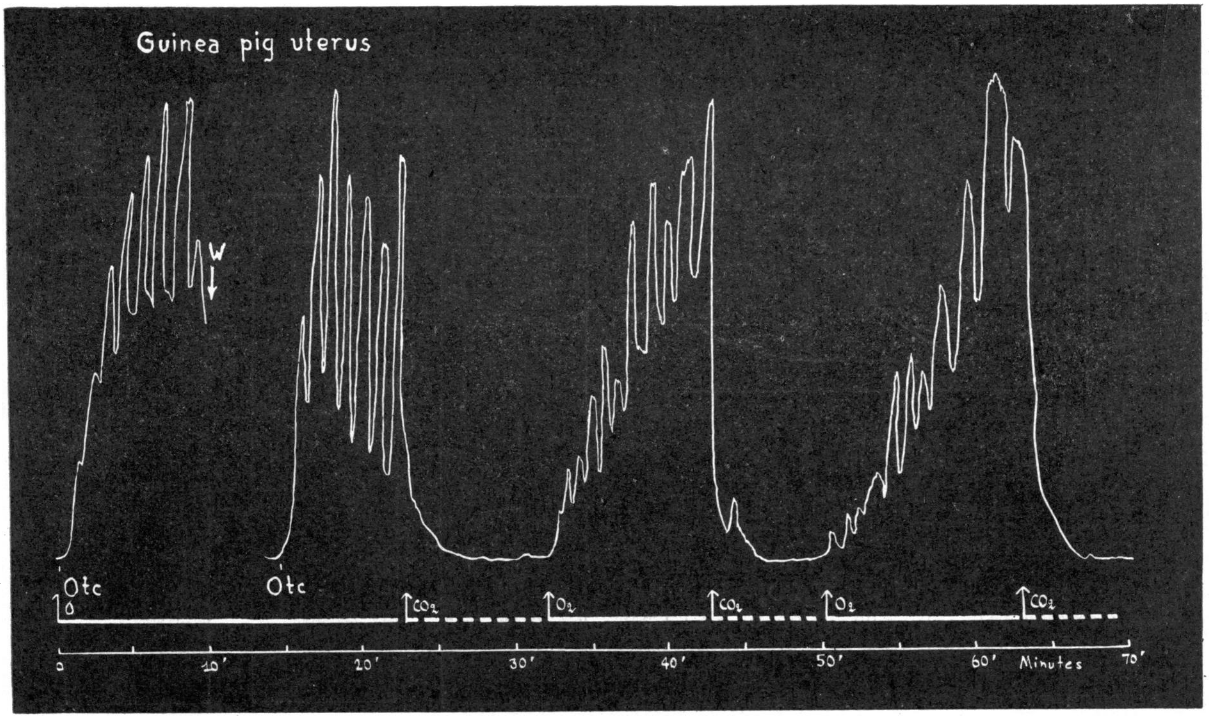

FIG. 10.—Isolated guinea-pig uterus preparation in standard Tyrode solution (buffer, $NaHCO_3$ 1 g./l.). From left to right: Effect of a standard dose of oxytocin (1×10^{-4} I.U.) on the uterus in Tyrode solution oxygenated with pure oxygen. After washing, addition of the same dose of hormone produces similar effect. Without removing the hormone, a gaseous mixture containing 75% O_2 and 25% CO_2 was substituted for oxygen at the point marked CO_2. At the point O_2, pure oxygen was substituted for the gaseous mixture. The same procedure is repeated twice. Otc=Oxytocin; W=wash. Full line=pure oxygen; broken line=gaseous mixture containing 75% O_2 and 25% CO_2. Time, 5 min.

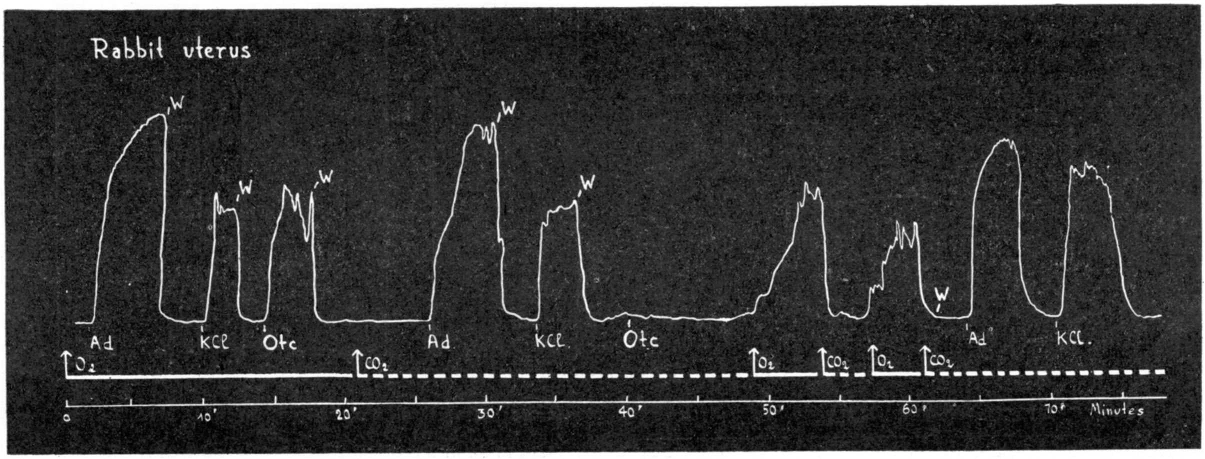

FIG. 11.—Isolated rabbit uterus preparation in standard Tyrode solution (buffer, $NaHCO_3$ 1 g./l.). From left to right: Effects of a standard dose of adrenaline (2×10^{-6}), KCl (5×10^{-4}) and of oxytocin (1×10^{-4} I.U.) on the uterus in Tyrode solution oxygenated with pure oxygen (full line). After washing, a gaseous mixture containing 75% O_2 and 25% CO_2 was substituted at the point CO_2 and the responses to the drugs again tested. Without washing out the oxytocin, pure oxygen was substituted for the gaseous mixture at O_2. Ad=adrenaline; Otc=oxytocin; W=wash. Full line=pure oxygen; broken line=gaseous mixture containing 75% O_2 and 25% CO_2. Time, 5 min.

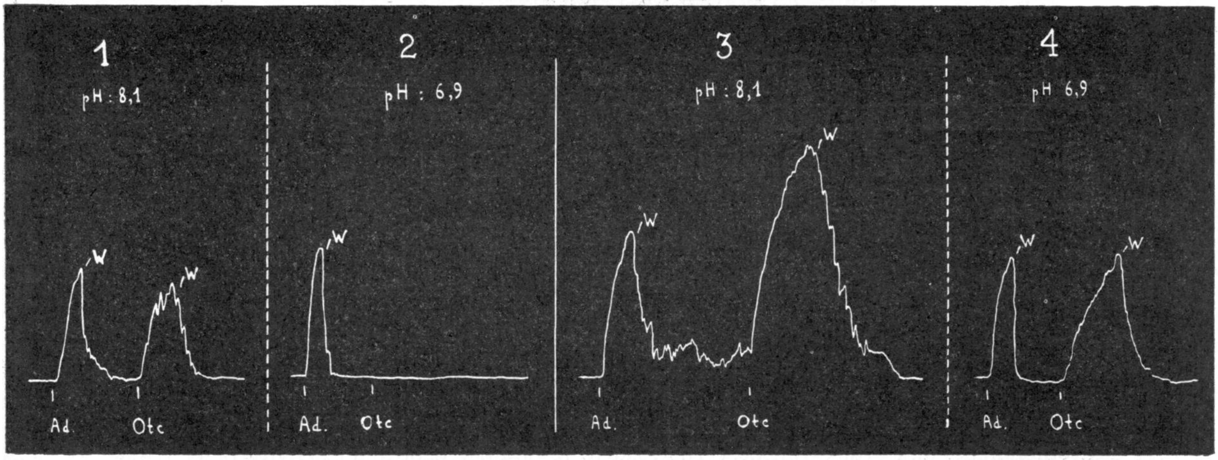

FIG. 12.—Isolated rabbit uterus horn preparation. On the left side (**1** and **2**) in standard Tyrode solution (buffer, $NaHCO_3$ 1 g./l.); on the right side (**3** and **4**) in Tyrode solution buffered with maleic acid/sodium maleate. **Ad**=adrenaline; **Otc**=oxytocin; **W**=wash. In **1**, effects of a standard dose of adrenaline (2×10^{-6}) and of oxytocin (2×10^{-4} I.U.) in normal Tyrode solution oxygenated with pure oxygen. In **2**, a gaseous mixture containing 75% O_2 and 25% CO_2 was substituted for oxygen and the *p*H fell to 6.9. Note response to adrenaline but the absence of response to oxytocin in the same dosages as in **1**. In **3**, Tyrode solution buffered with maleic acid/sodium maleate. Effects of the same doses of adrenaline and of oxytocin. In **4**, the *p*H of the Tyrode solution has been lowered to 6.9 by change of the buffer constituents (maleic acid/sodium maleate) while the medium is oxygenated with pure oxygen and the effects of the same doses of adrenaline and oxytocin are shown.

DISCUSSION

The results indicate that carbonic acid inhibited the action of histamine and oxytocin on smooth muscle. As has been previously reported, the effect was not due to tissue anoxia. It is crucial to know if the inhibition was produced primarily by reduction in the *p*H of the medium due to modifications in the H_2CO_3 concentration. The introduction of CO_2 into Tyrode solution, buffered with $NaHCO_3$, decreased the *p*H, and it seems logical to relate this fact to the inhibitory action. This supposition is supported by the results of experiments at various $NaHCO_3$ concentrations.

But this hypothesis is invalidated by the fact that when the bicarbonate buffer was replaced by a mixture of sodium maleate and maleic acid, the activity of histamine remained the same in spite of the variations of *p*H. In the presence of H_2CO_3, the action of histamine is diminished about 7 times at *p*H 6.8 in comparison with that at *p*H 8.1, yet it is hardly affected in the maleate medium (Fig. 8). This leads to the conclusions that the inhibition of histamine activity was independent of *p*H and that it was related to the presence of carbonic acid and its salt. The same appears to be true for oxytocin. In some experiments (unpublished observations), similar results were obtained employing a bicarbonate-free Tyrode solution buffered with phosphate.

We can then postulate that the inhibition of the action of histamine and oxytocin is not due to the decrease of *p*H, but that it is determined by a qualitative effect of carbonic acid. What is the nature of the effect? The formation of an inactive compound between histamine and H_2CO_3 seems unlikely. Possibly H_2CO_3 modifies the sensitivity of the specific cellular receptors for certain substances.

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