Effects of pH on Blood Flow and Peripheral Resistance in Muscular and Cutaneous Vascular Beds in the Hind Limb of the Pentobarbitalized Dog

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The responses of the vascular beds in skeletal muscle and skin to changes in blood pH on the acid and alkaline sides of the physiologic range were compared. Dilatation occurred in both cutaneous and muscular beds when the blood pH was shifted to the acid range. Dilatation also occurred in the muscle beds when the blood pH was shifted to the alkaline range but such change in blood pH caused vasoconstriction in skin.

The vasomotor effects of injections of varying hydrogen ion concentration have been an age-old problem. Recently Kester, Richardson and Green have reviewed the literature and reported that in the innervated whole hind limb of the dog a shift in pH either way from the normal causes vasodilation. In 1953, Lanier and co-workers reported that vascular beds of the skin gave, on the whole, lesser dilator responses to methacholine injection and to periods of ischemia than did those of muscle. In view of these differences we felt that it would be desirable to study the effects of progressive alterations in the hydrogen ion concentration of the blood above and below physiologic range on the blood flow in muscular and cutaneous vascular beds separately.

In addition to the references in the paper by Kester and associates, the following should be noted. Kety and associates in 1948, and Lange and associates in 1951 described a generally augmented blood flow during diabetic and artificially induced acidosis. Fleisch and Sibul found that a pH change in the blood of 0.05 pH unit caused by carbonic acid produces a vascular dilatation of the order of 100 per cent in the posterior extremities of urethane-narcotized cats. They also found that acetocetic, pyroracemic and numerous fatty acids result in a dilatation. Isserlitz and colleagues reported that the infusion of iodoacetic acid and fluoroacetic acid into the femoral artery caused a marked increase in the blood flow. The barbiturates are strongly alkaline buffer solutions and have a transient or even more prolonged dilator effect, but the role of the low hydrogen-ion concentration of these drugs as a possible factor in this action has not been investigated.

METHOD

Arterial pressure and the rate of blood flow were measured in the normally innervated muscle and cutaneous beds in five dogs each; a total of 325 injections were made, with a median of 33 injections per animal. All dogs were anesthetized with 30 mg. per kilogram of sodium pentobarbital intravenously. When necessary, additional pentobarbital was administered during the experiments to maintain as uniform a depth of anesthesia as possible. The dogs received initially 40 mg. per kilogram of Treburon plus 20 mg. per kilogram every half hour throughout the experiment to prevent coagulation. No experimental procedures were performed until at least one hour after the introduction of the initial doses of pentobarbital and Treburon in order to prevent complications due to their actions on the circulatory system.

The rate of flow in the femoral artery was measured...
ured throughout each experiment with an improved model of the electromagnetic flowmeter of Richardson, Denison and Green. Initial and final calibrations of the flowmeter were made in each experiment and when compared revealed an average error of ±9 per cent, except in one case in which there was a ±28 per cent error. The pulsatile flow was damped electronically to facilitate the recording and measurement of the mean flow. The pressure drop across the cannula and connecting tubing was 0.179 mm Hg per milliliter per minute. The rate of flow was recorded with an Esterline-Angus direct current milliammeter, Model AW. The usual sensitivity was 72 ml. per minute for full scale deflection. Checks of "zero" flow were made about every 20 minutes throughout each experiment. The mean rate of drift was 2.98 ml per minute per hour.

Lateral pressure in the cannula system immediately distal to the flowmeter was recorded by means of a Statham strain gauge, Model P 23 A, used with a Brush strain analyzer and another Esterline-Angus recorder. "Zero" pressure was referred to a level 4 cm. posterior to the anterior surface of the sternum and was taken once every hour throughout the experiment.

In all flow studies 1 ml. injections of the solution to be tested were made by way of a needle inserted through a segment of rubber tubing distal to the flowmeter and strain gauge while the rate of flow and the lateral pressure in the cannula system was being recorded continuously. All injections were given within 10 seconds ± 2 seconds. For the position of the flowmeter and pressure gauge and the site of injections with respect to the dog's hind leg see Lanier and colleagues.

For testing the muscular vascular beds, the femoral artery was severed, the two ends cannulated and connected to the flowmeter. All cutaneous branches of the saphenous, anterior tibial, and perforating cutaneous arteries were ligated. For the cutaneous beds the saphenous artery was cannulated to admit the distal end of the tubing from the flowmeter; the proximal end of the flowmeter was connected to the cannulated contralateral femoral artery. Either the saphenous artery was cannulated sufficiently distal from its bifurcation with the femoral artery to escape the branches supplying muscle or else all muscle branches were ligated. For further details on the dissection see Lanier and colleagues. At the conclusion of each experiment the leg was injected with India ink and postmortem examination revealed the extent to which the specific area under observation had been supplied during the experiment. In no case was there a significant amount of muscle supplied during a skin experiment, and vice versa.

A buffer solution containing 0.1 mole per liter each of monobasic sodium phosphate, sodium citrate, glycine, and alpha-alanine was prepared. This solution had a pH of 6.18. Immediately prior to each experiment 10 ml. portions of this solution were adjusted to the desired pH with 1.0 N HCl or 1.0 N NaOH. This was then diluted to 20 ml. with physiologic saline. Values for the two extremes of the pH range used were 3.75 milliequivalents of 1.0 N HCl and 1.0 milliequivalents of 1.0 N NaOH for pH 2.0 and 10.0, respectively. All pH measurements were made with a Coleman model 19 line-operated electrometer, rated by the manufacturer to be accurate to within 0.05 pH unit when careful technic is employed.

RESULTS

I. Effects on Vessels of Muscle. Figure 1 shows typical records of flow responses to solutions of pH 2.0, 7.4 and 10.0. The solid line of figure 2 portrays the results of the in-
EFFECT OF pH ON MUSCULAR AND CUTANEOUS VESSELS

150

Injections expressed as per cent control peripheral resistance unit and shows that a deviation in pH of the buffer solutions amounting to ±1.5 pH units or more from that of physiologic range, results in a significant increase in the rate of blood flow, and that this increase becomes progressively greater the further pH deviates from that of physiologic range.

Injections of the buffered solution on the alkaline side of physiologic range resulted in momentarily greater increases in flow than solutions deviating on the acid side by an equivalent amount. For example, the average response for a pH of 10.0 was 33.5 per cent of the control peripheral resistance unit while that for pH 5.0 was only 58 per cent. The probability of the difference between pH 5.0 and pH 10.0 is less than 0.0001. Nevertheless, the flow responses to the acid injections were of longer duration than those to the alkaline injections, and tended to be more permanent, in that they very often would not return to control until after an injection of either pH 7.4 or an alkaline solution.

Control flows represented by the 100 ordinate in figure 2 ranged from 14 ml. per minute to 82 ml. per minute, with an average of approximately 50 ml. per minute. The dead space from the site of injection to the vessel was 3.4 ml. At this rate of flow the measured reaction could occur in 15 to 2½ seconds after the time of injection, with an average of 4 seconds. On this basis we can safely say that there was practically no residual latency to the response.

During these experiments various animals possessed an inherent difference in their reactivity to the solutions. For example, when plotted according to per cent control peripheral resistance units there was a space corresponding to 10 or 20 per cent separating their curves. The dip present in the solid line at pH 5.0 (figure 2) resulted in part from the fact that only three experiments were done using this pH compared to five performed at all other pH values, and the responses at this pH as well as the control pH were higher in these three cases than in the other dogs. For this reason a second set of calculations were made.
Since we found that the least reaction resulted from an injection of pH 8.0, we used that point as 1.0, and compared all other points with it by means of a ratio. This we believe gives a truer picture of the relative differences in the actions of these solutions on the muscular vasomotor tone (see figure 3). When plotted in this manner the dip in the curve at pH 5.0 was reduced.

Lateral pressure in the cannula system measured distally to the flowmeter declined approximately 6.0 mm Hg for a decrease in resistance to 15 per cent of control and approximately 3.0 mm Hg for a decrease in resistance to 88 per cent of control. Measurements of the pressure drop through the flowmeter cannula and connecting tubing at various rates of flow fully accounts for this drop.

The data given in figures 2 and 3 are based on the pH of the injected fluid. Since the volume injected was small relative to the volume of blood flowing into the vessel the actual change in blood pH was much smaller. Kester and associates calculated that the pH of the blood would be changed to about pH 6.5 by the solution at pH 3.0 concentration and to about pH 8.1 by the buffer solution at pH 10. When the blood flow responses were replotted using as the abscissal scale the probable pH of the blood as determined by Kester and associates, the curve still clearly showed that a given change in the pH of the blood toward the alkaline side of the physiologic range results in a greater dilator response than an equivalent change in the blood pH toward the acid side.

The addition of the NaOH or the HCl to the buffer solution changed its osmotic pressure and might have had other effects. Therefore in each experiment, in order to test the possible effects of titrating the buffer, samples of the buffered solution were carried either to pH 10.0 with 1.0 N NaOH and then mixed with 1.0 N HCl until a final solution of pH 7.4 was obtained, or to pH 2.0 with 1.0 N HCl and then mixed with 1.0 N NaOH to get a solution of pH 7.4. The results of injecting these solutions are shown in figures 2 and 3 under the abscissal scale of pH 7.4. There was no difference between the solution carried to pH 2.0 and retitrated to pH 7.4 or the one carried to pH 10.0 and retitrated to pH 7.4. Neither was
there a significant difference in the responses to these solutions as compared with the standard solution which was simply adjusted from pH 6.18 to pH 7.4.

The effect of the adrenergic blocking drug, Ilidar, on the response to the injections was noted by injecting a dose, 1 mg. per kilogram, prior to the injection of the buffer solution. It was found to have no effect on the response in muscle to the acid or alkaline solutions.

II. Effects on Vessels of Skin. Records of the flow responses to pH 2.0, 7.4, and 10.0, are reproduced in figure 4. As a rule, the flow responses to acid injections were of longer duration than those to the alkaline injections, and in several instances failed to return to control.

The dotted line in figure 2 is a plot of all the observations expressed as per cent control peripheral resistance units (PRU). It will be seen that in the cutaneous vascular beds a deviation of the pH toward the acid side of physiologic range results in a vasodilatation, but, unlike the muscle vascular beds, any deviation in the pH toward the alkaline side brought on vasoconstriction. This response occurs with any deviation in the pH of ±1.5 pH units or more, and increases progressively with deviation from physiologic range. When plotted on semilogarithmic paper, the responses to alkaline injections were more nearly symmetric to those on the acid side, and the plot approached a straight line relationship.

Unlike the experiments on blood vessels of muscle, these animals failed to demonstrate an inherent difference in their reactivity to the injections; the curves plotted for each animal could easily be superimposed. However, the data were also compared using pH 8.0 as 1.0. The results are plotted as the dotted line in figure 3.

The "dead space" from injection site to blood vessel was approximately 3.4 ml. Control cutaneous flows, represented by the 100 ordinate in figure 2, ranged from 3.8 ml. per minute to 28 ml. per minute, with an average of 14 ml. per minute. At these rates of flow the measured reaction could occur in 8.2 to 52 seconds after injection. Typical records in figure 4 show that on this basis there was no measurable residual latency to the response.

The lateral pressure in the cannula system measured distally to the flowmeter declined approximately 3 mm. Hg for a decrease in peripheral resistance to 53 per cent of the control and increased approximately 4 mm. Hg for an increase in resistance to 140 per cent of control. Again measurements of the resistance to flow through the cannula and connecting tubing fully accounted for the difference.

When the blood flow responses were replotted logarithmically using as an abscissal scale the probable pH of the blood as determined by Kester and co-workers, we found, in contrast to muscle flow, that change in pH of the blood toward the acid side of physiologic range produced a plot which was practically a linear extension of the plot on the alkaline side.

The effects of osmolarity, etc., were tested with pH 7.4 x solutions as described above. The average response to this solution is found in figures 2 and 3 under the abscissal label of pH 7.4 x. There appears to be no significant difference in the response to this solution as compared with pH 7.4.

The adrenergic blocking drug, Ilidar, had a minimal effect on the responses to the acid...
solutions, but augmented the response to the alkaline solutions. Average values, in per cent of control peripheral resistance units, before and after introduction of Ilidar were:

<table>
<thead>
<tr>
<th>pH</th>
<th>Before</th>
<th>After</th>
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</thead>
<tbody>
<tr>
<td>2.0</td>
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<td>40</td>
</tr>
<tr>
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<tr>
<td>10.0</td>
<td>141</td>
<td>500</td>
</tr>
<tr>
<td>11.0</td>
<td>107</td>
<td>800</td>
</tr>
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**DISCUSSION**

**I. Muscle.** The responses obtained in this series agree with those of Fleisch and Sibul,6 Lange and co-workers,4 Issekutz and co-workers5,7 and Kester and co-workers,1 in that they were all able to demonstrate a generally augmented blood flow after the injection of acid and/or alkaline solutions.

The solid line in figure 2 reveals that at pH 7.4 the resistance equals 74 per cent of control resistance, denoting a dilatation even with a solution of physiologic pH. This suggests that a factor other than pH has been introduced constantly to produce the vasodilator response. Since the response to saline was not significantly different from that of a solution at pH 7.4 this dilatation probably resulted from lowered blood viscosity due to the injection. Since the 7.4 x and 7.4 solutions gave the same response, the additional increase in flow obtained with solutions of pH other than 7.4 must be due to a change in the pH since any other factors such as dissociation of the buffer components were also present in the solution at pH 7.4 x and at pH 7.4. The dilator response with the buffer solution adjusted to pH 8.0 was less than that at pH 7.4. This suggests that a blood pH of approximately 7.45 (produced by the buffer at pH 8.0) may be more physiologic than pH 7.4 for muscle.

As stated above, injections of buffer at pH 10.0 result in greater increases in flow than solutions of pH 5.0, both differing from pH 7.4 by an equivalent amount. Kester and colleagues have reproduced a curve which shows that the buffer solution at pH 10.0 produces a greater change in the blood pH (to pH 8.12) than one at pH 5.0 (to pH 6.98). However, even when correction is made for this, a given change in the pH of the blood toward the alkaline side still results in a greater increase in flow than an equal change in blood pH toward the acid side. The longer and more permanent responses to the injections of solutions of high hydrogen-ion concentration, as compared with those of low concentration, may indicate that, perhaps, a different mechanism effects the response.

The decrease in the peripheral vascular tone and increase in blood flow, dependent upon the pH of the solution and also upon the ability of the blood to buffer this solution, indicates the existence of a highly effective mechanism in muscle designed to restore the pH of the blood to normality when the hydrogen ion concentration deviates significantly from that level in either direction. Whether this mechanism is a chemical agent stimulated into action by the direct effect of the pH on the wall of the vessel or on an element in the blood, or whether it is a neural agent stimulated by the irritation of the pH on the vessel wall has not been determined. The apparent absence of a measurable latency in the response to the injections tends to rule out any theory that it might be caused by a neural agent.

**II. Skin.** Our results do not agree with those of the many authors who found a dilatation to any pH change.1,3,4,5,6,8

Observation of the dotted line in figure 2 reveals that at pH 7.4 there was a dilatation corresponding to 96 per cent of the control peripheral resistance units. This, no doubt, is due to the lowered viscosity of the blood, although there might be some inherent dilator agent in the buffer solution used. In this regard it should be noted that muscle (solid line in same figure) responded with a dilatation equal to 74 per cent of the control peripheral resistance units. Any agent that was present in the muscle was also present in the skin, and the difference in the figures may confirm that skin vascular beds, in general, are not as reactive as muscle beds. This was also noted by Lanier and co-workers2 with regard to the dilator response to methacholine and to a period of ischemia. This result is even more pertinent since, because of the lower rate of control flow, the concentration of the buffer was relatively higher in the skin than in the muscle.

Without further experimentation the results
obtained with a blocking dose of lidar cannot
be explained.

III. General. Kester and colleagues who per-
formed the experiment on the whole leg, ob-
tained a greater dilatation on the acid side,
and less dilatation on the alkaline side of
physiologic range, than we got in muscle alone.
Therefore, our results would be in agreement;
the dilatation of the skin on the acid side would
tend to augment the dilatation of the muscle,
while the constriction on the alkaline side in
the skin would tend to lessen their dilatation
on the whole leg with increased pH values.
The vasomotor tone varies continuously in
the same degree throughout the range in pH
values for skin, whereas the muscular beds di-
late on both sides of the physiologic pH value.
There would seem, therefore, to be no normal
pH for the skin vascular beds; and pH prob-
ably does not play a significant role in the
control of the blood flow through the skin;
whereas it probably does in muscle.

SUMMARY

1. The blood flow and the arterial pressure
supplying either the muscular or the cutaneous
vascular beds was measured continuously in
the innervated hind limb of the dog, using an
electromagnetic flowmeter.
2. The pH of the blood was altered from the
physiologic range by intra-arterial injections
of 1 ml buffered solution adjusted to various
hydrogen-ion concentrations from 2.0 to 11.0.
3. With solutions on the acid side of physio-
logic range, blood flow was augmented in both
muscle and skin.
4. Solutions on the alkaline side of physio-
logic range elicited a dilatation in muscle and a
constriction in skin.
5. In muscle a given change of blood pH
from 7.4 on the alkaline side resulted in greater
response than did an equivalent change in the
acid range.
6. The greater responses with alkaline solu-
tions were accentuated by the poorer buffering
power of the blood on the alkaline side.
7. Our results suggest another fundamental
difference between the reactivity of muscle and
skin vascular beds in addition to those pre-
viously described by Lanier and co-workers
who demonstrated that muscle beds show a
greater dilator response, than do the cutaneous
beds, to methacholine, to a period of ischemia,
and to epinephrine after adrenergic blocking
drugs.

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