CXXXVI. THE INFLUENCE OF TEMPERATURE ON THE p_H OF BLOOD.

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THE effect of temperature on the p_H of blood has been the subject of controversy. The discrepancy has arisen from the fact that in the particular technique employed by an observer, sometimes the pressure of CO_2 , sometimes the concentration and sometimes neither was maintained at the same level at the two temperatures.

The easiest observation to make is one in which the CO_2 pressure is the same whilst the temperature is varied. This was the case in Hasselbalch's [1917] experiments upon ox blood. He found, somewhat to his surprise, that the p_H calculated both from electrometric observations and from the proportion of CO_2 in the hydrogen used and the combined CO_2 in the blood, was the same at 18° as at 38° . Michaelis and Davidoff [1912], on the other hand, in their early observations, found the p_H 0.21 higher at 38° than at $18\text{--}20^\circ$. Corral [1921] obtained results similar to Michaelis, viz. 0.23 difference in p_H .

In Michaelis and Davidoff's method the blood is enclosed in a stoppered U-shaped electrode vessel and shaken with a bubble of hydrogen. If the bubble of hydrogen be small compared with the volume of the blood, the content of CO_2 in the latter remains approximately constant whatever the temperature, whereas in Hasselbalch's later technique the blood is equilibrated with the same CO_2 pressure at different temperatures and the content varies in accordance with the solubility of CO_2 at those temperatures.

As a matter of fact, the content of CO_2 is not constant in Michaelis' method of procedure because the volume of hydrogen is not reduced to negligible proportions but, as the buffering effect of the NaHCO_3 and haemoglobin is considerable, nearly the same p_H is found as if it were.

The conditions in Corral's experiments were intermediate. He used Hasselbalch's older form of electrode vessel and, if we understand him aright, the volume of hydrogen was about equal to the volume of blood.

EFFECT OF TEMPERATURE ON p_H OF BLOOD WHEN THE PRESSURE OF CO_2 IS MAINTAINED CONSTANT.

We agree with Hasselbalch that, when the CO_2 pressure is constant and the CO_2 content varies with the changing solubility of CO_2 in water, the effect of temperature on the p_H of blood is very slight.

In the following observations the details of the electric measurements were those outlined in a previous communication to this *Journal* [Lepper and Martin, 1926]. The blood was reduced and equilibrated with H_2 containing about 5.8 % CO_2 and the same mixture of hydrogen and CO_2 was used in the electrode vessel. The arrangements for doing this in a thermostat were similar to those described by Warburg [1922]. An abundant supply of hydrogen with the desired mixture of CO_2 was compressed into a steel bottle and could be turned by a three-way cock either into the rotating bottle in which the blood was brought into equilibrium with the gaseous mixture or into the electrode. Blood could be drawn from the bottle into the electrode or *vice versa* without coming into contact with air.

	p_H at 18°	p_H at 38.5°
Rabbit's blood containing 54 % haemoglobin, sodium fluoride 0.02 % and potassium oxalate 0.04 %	7.420	7.427
Do. Do.	7.391	7.385
Human blood with same amount of oxalate and fluoride	7.370	7.364

The difference in p_H at 18° and 38° is within the error of our experiments. When freshly drawn blood was used we found it to be essential to prevent glycolysis occurring as this happens sufficiently rapidly at 38° to vitiate the results. Hasselbalch, we believe, avoided this source of error by using blood which had been whipped and had stood some time. We did so by adding sodium fluoride, which Evans [1922] found to inhibit glycolysis.

As, with constant CO_2 pressure, the p_H of blood remains nearly the same notwithstanding the rise in temperature, the fall in acidity due to diminished CO_2 concentration in the liquid at the higher temperature must be compensated by the increase in the dissociation of the weak acids, H_2CO_3 (first H), H_2PO_4 (second H), and proteins. Raising the temperature of 0.03 *M* $NaHCO_3$ solution in equilibrium with 5 % CO_2 from 18° to 38° decreased the concentration of CO_2 dissolved to 0.6 of its former value. This, by its effect on the $CO_2/NaHCO_3$ ratio would raise the p_H by 0.22. As a matter of fact, the p_H is raised by only about half this amount, half being compensated by the increase in the dissociation of H_2CO_3 .

In the case of blood, with its other weak acids, phosphates and proteins, the influence of temperature upon their various acid dissociation constants comes also into play. The problem is a complicated one and cannot be entered into here, but it is obvious that the net effect, on warming the solution, on the different dissociation coefficients, approximately balances the effect of diminished CO_2 content, provided the excess of CO_2 dissolved and the amount driven out of the $NaHCO_3$ by the increased acidity of the proteins, etc., can leave the system.

This may not be merely a chance happening but a provision against alteration of p_H with change of temperature of the blood in different parts of the circulation.

EFFECT OF TEMPERATURE ON THE p_H OF BLOOD WHEN THE CONCENTRATION OF TOTAL CO_2 IS MAINTAINED CONSTANT.

The effect of raising the temperature, the total CO_2 concentration remaining constant, is to lower the p_H of solutions containing a mixture of CO_2 and $NaHCO_3$. Hastings and Sendroy [1924] found for 0.033 M $NaHCO_3$ a fall of 0.055 for 18° rise in temperature in the presence of 0.1 M $NaCl$. We have found about the same fall, 0.057 for 20° , for 0.02 M $NaHCO_3$ in presence of 0.13 M $NaCl$.

The fall in p_H is considerably greater with plasma or blood. In both cases, the influence of temperature on the dissociation of the proteins is also manifested and this effect should, from Meyerhof's [1922] determination of the heat of ionisation of serum-albumin, be considerable.

We have not been able to arrange a satisfactory experiment to determine the effect of temperature under the above conditions by means of the hydrogen electrode. Our efforts with the early method of Michaelis and Davidoff [1912] afforded discordant results as the blood is not de-oxidised. Various attempts to obviate this were not very successful. On one occasion we determined the p_H of a sample of blood electrometrically at 38° and then drew it off from the electrode vessel over mercury and allowed it to cool to 18° and subsequently expelled it into a tube under paraffin and stoppered the tube. We then sedimented the corpuscles by centrifuging and determined the p_H of the plasma with the quinhydrone electrode. For the latter determination, we used the method described by Cullen and Büllmann [1925]. The p_H at 38° was found to be 7.24; at 18° it was 7.44 and 7.48 respectively in two different samples, a mean difference of 0.22. The agreement between the duplicate observations is not good but the quinhydrone electrode is not suitable for accurate determinations of c_H in solutions so alkaline as p_H 7.4.

It then occurred to us that the problem could be solved by using the modified colorimetric method of Cullen [1922] described in our previous paper [Martin and Lepper, 1926], in which the diluted plasma or blood is hermetically sealed in a glass tube so that the CO_2 cannot escape.

Cullen found a difference of about 0.2 between the p_H of plasma at 20° and 38° . The p_H was arrived at electrometrically at 38° and colorimetrically at 20° so that any discrepancy between these two methods falls upon the determination.

As in our micro-method the plasma or blood is in hermetically sealed tubes with only a minute amount of gas in the capillary to permit of sealing, the effect of temperature on the colour of the phenol red in the diluted plasma can be observed very simply by warming or cooling it. Determinations at both temperatures are in this case made on the identical sample and by the same method. It is necessary to apply the appropriate corrections for the influence of temperature on the dissociation of the indicator to arrive at the real p_H at the higher temperature.

Observations on five samples of plasma are set out in Table I below. The procedure was that already described [Martin and Lepper, 1926, p. 38]. Each tube was immersed in a test-tube of water with a slip of filter paper in it as a background. The apparent p_H of the diluted plasma was determined firstly at 18°, then at 38° and lastly again at 18°.

Table I.

Apparent p_H of plasma at			Mean difference between 18° and 38°
18°	38°	18°	
7.54	7.47	7.52	0.06
7.55	7.50	7.56	0.06
7.58	7.50	7.57	0.07
7.60	7.52	7.60	0.08
7.58	7.52	7.58	0.06
Mean			0.066

Hastings and Sendroy [1924] found that the p_H for phenol red increased 0.13 between 20° and 38°, so that 0.14 should be deducted from the apparent p_H at 38°. This gives 0.206 as the amount to be subtracted from the p_H found at 18° to arrive at the p_H of human plasma at 38°. This is in close agreement with Cullen's figures [Cullen, 1922].

The effect of temperature on the p_H of diluted blood can be determined in the same way. Hasselbalch [1917] found that when the CO_2 pressure was constant the influence of temperature upon the p_H of blood was quite different from that on serum. He found serum behaved as NaHCO_3 sol. We do not quite agree with this statement, but grant that it reacts more like a weak NaHCO_3 sol than like blood, so it would appear unlikely that the plasma and blood would behave alike when the total CO_2 content is maintained constant. Indeed, considering the high concentration of haemoglobin in blood, this seemed to us improbable.

The direct measurements of Brown and Hill [1923] give - 10,500 cal. as the heat of ionisation of haemoglobin, which is of the same order of magnitude as Meyerhof [1922] arrived at from their heats of neutralisation for serum-albumin and serum-globulin. The acid dissociation constants of haemoglobin should, therefore, according to the van 't Hoff isochore

$$\frac{d}{dT}(\log k) = \frac{10,500}{RT^2} \text{ or its integral}$$

$$k_{18}/k_{37} = e^{\frac{10,500(T_{18}-T_{37})}{1.98 \times T_{18}T_{37}}}$$

be doubled when the temperature rises from 18° to 38°.

We have ascertained the effect of temperature on the p_H of blood by studying the influence of the temperature at which the corpuscles are separated upon the p_H of the resulting plasma. We employed the micro-method described above. Fluoride is essential for this experiment. Without it the tubes become progressively acid at 37°. Six tubes of blood diluted 1 in 15 with saline containing sodium fluoride and indicator were prepared and hermetically sealed.

Two of them were centrifuged in a room the temperature of which was 19° and the p_H of the plasma determined at 19° . Four others were placed in water at 37° for half an hour and centrifuged in a room, the temperature of which was 37° . They were replaced in a bath at 37° and their p_H determined whilst at this temperature. These four tubes were now allowed to cool and the corpuscles were redistributed throughout the tubes by shaking. After 15 minutes at 19° they were centrifuged at 19° and their p_H determined at 19° . The results are shown in Table II below.

Table II.

	Apparent p_H at 37° . Corpuscles separated at 37°	p_H at 19° . Corpuscles separated at 19°	Difference
1	—	7.58	—
2	—	7.60	—
3	7.54	7.60	0.06
4	7.51	7.58	0.07
5	7.51	7.58	0.07
6	7.53	7.59	0.06
			<hr/> 0.065

When 0.13 is added for the dissociation of the indicator to the mean difference of 0.065, the real difference of p_H of the diluted blood for 18° increase in temperature is 0.195. This, for 20° difference, would be 0.216. This is a little more than we obtained with plasma, which was 0.206 for 20° . The difference is not, however, outside the experimental errors.

When CO_2 cannot escape from the system, the effect of temperature on the p_H of plasma and corpuscles seems to be very nearly the same, viz. a fall of about 0.2 for a rise of temperature from 18° to 38° . This, to us, unexpected result means that an adjustment of electrolytes between corpuscles and plasma is not necessitated as the temperature of the blood alters in circulating through the body.

CONCLUSIONS.

1. As Hasselbalch found to be the case with ox-blood, if the *pressure* of CO_2 remains constant, the p_H of human and rabbit blood does not alter significantly when the temperature varies from 18° to 38° .

2. With *concentration* of total CO_2 constant, the p_H of human blood falls about 0.2 when the temperature is raised from 18° to 38° .

3. The effect of temperature, with concentration of total CO_2 constant, is the same upon the c_H of the corpuscles and the corresponding plasma.

4. If 0.2 be deducted from the p_H of the plasma determined by the colorimetric method of Cullen or Hawkins [1923] or by the authors' microcolorimetric method at 18° , it will give the p_H of the corresponding blood at 38° .

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