THE BICARBONATE RESERVE AND THE DISSOCIATION CURVE OF OXYHEMOGLOBIN IN FEBRILE CONDITIONS.*

By A. C. WHITE.

(From the Department of Therapeutics of the University of Edinburgh, Edinburgh, Scotland.)

(Received for publication, November 12, 1924.)

The effect of increased temperature in causing a more rapid dissociation of oxygen from the blood was noted by Paul Bert (1) and later by Hüfner (2, 3). Barcroft and King (4) in 1909 also demonstrated this, giving a series of dissociation curves at various temperatures for blood and hemoglobin solutions. Barcroft and Hill (5) working with dialysed hemoglobin found that \( K \), the oxygen dissociation constant for hemoglobin, varied with temperature, obeying the van't Hoff isochore.

\[
\frac{d}{dt} \left( \log K \right) = \frac{-q}{RT^2}
\]

where \( q \) is the heat given out by 1 gram molecule of hemoglobin on union with oxygen. They used this method to obtain a minimum value for the molecular weight. Then Hill (6) brought forward the modified equation

\[
\frac{y}{100} = \frac{Kx^n}{1 + Kx^n}
\]

in which \( y \) = the percentage saturation of hemoglobin with oxygen, \( x \) = oxygen pressure, \( K \) is the equilibrium constant, and \( n \), a constant greater than unity. This was shown by Barcroft (7) to apply to whole blood with the same accuracy as it does to hemoglobin solutions. Later Brown and Hill (8) and Stadie and Martin (9)

* The greater part of the work was carried out during the tenure of the Stark Scholarship in Clinical Medicine, Edinburgh University, aided by a grant from the Moray Fund for Research.
have investigated and fully discussed the thermodynamics involved in the dissociation of oxygen from hemoglobin. It is only, however, in the papers of Barcroft and King (4) and Krogh and Leitch (10) that we find the biological significance of these changes discussed. Much more work has been done on the bicarbonate reserve in fevers and this will be dealt with in the discussion of the results observed.

In view of the foregoing facts, it seemed of interest to determine some of the relationships of oxygen and hemoglobin during pyrexia. The two conditions investigated in this series were (a) the bicarbonate reserve and (b) $\frac{1}{K}$, the reciprocal of the dissociation constant, at the temperature of the patient. Were there sufficient material, $\frac{1}{K}$ was determined on the same blood at 37°C., which was assumed to be an average normal temperature. Rectal temperature during pyrexia was taken as the most accurate index of the patient's temperature, unless it is otherwise stated. Axillary temperature was never used. Indeed during certain fevers, especially lobar pneumonia, it may be very fallacious owing to its often being higher in the affected side. The observations, whenever possible, were carried out both during and after the pyrexial period.

**Methods.**—The blood was drawn, without stasis, kept corked on ice until required, a little powdered neutral potassium oxalate being added to prevent coagulation, and a little sodium fluoride to prevent acid production (Evans (11)). In all cases the bicarbonate reserve was determined first, the blood being exposed to definite concentrations of CO$_2$ as outlined by Christiansen, Douglas, and Haldane (12), the CO$_2$ of the gaseous phase being determined by the small Haldane apparatus, and the CO$_2$ content of the blood by the new Haldane apparatus (13). $\frac{1}{K}$ was determined as follows: About 5 cc. of the blood sample were put in a cylindrical saturator (about 400 cc. capacity) and the oxyhemoglobin was reduced by passing a stream of nitrogen through for 3 to 5 minutes, the atmospheric air being thus displaced. The saturator was corked, and measured volumes of CO$_2$ and O$_2$, saturated with water vapour and calculated to approximately the desired pressures, were displaced into it by means of a mercury burette. The saturator then was equilibrated at the desired temperature in a water bath for 15 to 20 minutes, excess pressure being released at the 5th and 10th minutes. The gaseous phase was analysed as above, and the percentage saturation was determined by the new Haldane apparatus. $\frac{1}{K}$
A. C. WHITE

was determined by a nomogram and corrected to 40 mm. Hg. CO₂ by the Adair-Henderson line (14, 15),¹ and this is the value of \( \frac{1}{K} \) recorded. In all cases the altered solubilities of the gases were allowed for.

The value of \( \frac{1}{K} \) given, unless otherwise stated, is that derived from a single concordant duplicate analysis.

As a preliminary, the effect of rise in temperature on normal human blood was observed at 37°C. \( \frac{1}{K} \) was 2,400 (mean of fourteen duplicate analyses), at 41°C. 4,000 (mean of nine duplicate analyses). The total shift of the dissociation curve to the right, consequent on the increased \( \frac{1}{K} \), was about equivalent to that figured by Brown and Hill (8). The effect of increased temperature (37–43°C.) on the bicarbonate reserve of the blood in vitro was mainly a reduction of the dissolved CO₂, altering, therefore, the \( \text{H}_2\text{CO}_3: \text{NaHCO}_3 \) ratio and lowering the CH₄ as Haggard (16) and Van Slyke (17) had found. According to Stadie and Martin the molecular buffer value of hemoglobin itself is independent of the temperature.

The series of cases reported include some six cases of lobar pneumonia, two cases of tonsillitis in the course of hyperthyroid disease, a case of pulmonary tuberculosis, and a case of rheumatic fever.

Case 1.—A. F. Age 31 years; fisherman. Lobar pneumonia; right lower lobe. Previous history unimportant. Blood first examined the 7th day of the disease. Temperature 39.5°C.; pulse 110; respirations 40. Bicarbonate reserve at 39.5°C. and 41.8 mm. Hg. CO₂ pressure was 53.9 volumes per cent CO₂. \( \frac{1}{K} \) at 39.5°C. 5,300, at 37.0°C. 2,500.

8 days later, temperature normal for 4 days. Pulse 74; respirations 22. Bicarbonate reserve at 37°C. and 36.2 mm. Hg. CO₂ was 49.3 volumes per cent CO₂. \( \frac{1}{K} \) at 39.5°C. 4,700, at 37.0°C. 3,000.

Case 2.—W. T. G. Age 24 years. Lobar pneumonia; right lower lobe. History of previous respiratory trouble; general condition poor. Temperature

¹The work of Barcroft and others (19) has shown that the Adair-Henderson line is not perfectly straight but shows a slight S inflection. This inflection, however, is not great enough to invalidate the results obtained by the method outlined above.
BICARBONATE RESERVE AND OXYHEMOGLOBIN

39.4°C.; pulse 104; respirations 32. Blood first examined 5th day of the disease. Bicarbonate reserve at 39.4°C. and 38.9 mm. Hg. CO₂ was 51.4 volumes per cent CO₂. \[ \frac{1}{K} \] at 39.4°C. 6,100, at 37.0°C. 1,600.

When temperature had been a week normal, pulse 64; respirations 20. Bicarbonate reserve at 37°C. and 37.4 mm. Hg. CO₂ was 56.3 volumes per cent CO₂. \[ \frac{1}{K} \] at 39.4°C. 5,800, at 37.0°C. 2,400.

Case 3.—J. B. Age 31 years; picture house attendant. Lobar pneumonia; right lower lobe, with pleurisy; several rigors and temporary pyuria while in hospital. Blood examined 5th day of the disease. Pulse 104; respirations 50; temperature 40°C. Bicarbonate reserve at 40°C. and 38.1 mm. Hg. CO₂ was 44.7 volumes per cent CO₂. \[ \frac{1}{K} \] at 40.0°C. 4,100, at 37.0°C. 3,600.

Case 4.—A. H. Age 65 years; no fixed occupation. Found collapsed on the street; had probably been ill 5 days; died 2 days after admission. In p.m. showed grey hepatisation of lower part of left upper lobe; general edema of lung and acute bronchitis. Temperature 38.0°C. (oral); pulse 100; respirations 30. Bicarbonate reserve at 38.0°C. and 48.8 mm. Hg. CO₂ was 52.9 volumes per cent CO₂. \[ \frac{1}{K} \] at 38.0°C. 7,200, at 37.0°C. 4,200.

Case 5.—J. H. Age 42 years; ploughman. Influenza, 14 days previous to admission; 2 days previous to admission stabbing pain in left chest, sudden dyspnea, and fever; no rigors; slight cyanosis. Blood examined day after admission. Pulse 105; respirations 32; temperature 39.6°C. (oral). Bicarbonate reserve at 39.6°C. and 44.6 mm. Hg. CO₂ was 56.3 volumes per cent CO₂. \[ \frac{1}{K} \] at 39.6°C. 4,400, at 37.0°C. 3,150. Transferred to surgical side for operation—empyema.

Case 6.—J. F. Age 48 years; porter. Lobar pneumonia; left lower lobe. Blood examined 5th day of disease; at time showed some cyanosis and twitching of the muscles of the face; Trousseau's sign and Chvostek were negative. Temperature 38.9°C.; pulse 90; respirations 40. Bicarbonate reserve at 38.9°C. and 40.4 mm. Hg. CO₂ was 41.1 volumes per cent CO₂. \[ \frac{1}{K} \] at 38.9°C. 4,100 (double duplicate analyses), at 37.0°C. 4,300. 5 days later temperature normal; pulse 70; respirations 36. Bicarbonate reserve at 37°C. and 37.6 mm. Hg. CO₂ was 54.8 volumes per cent CO₂. \[ \frac{1}{K} \] at 38.9°C. 4,000 (double duplicate analyses), at 37.0°C. 4,100 (double duplicate analyses).

Case 7.—M. B. Age 20 years. Exophthalmic goiter; while in hospital patient had a slight pyrexia after removal of tonsils. Temperature 39.1°C. Bicarbonate reserve at 39.1°C. and 36.0 mm. Hg. CO₂ 48.6 volumes per cent CO₂; at 37.0°C. and 35 mm. Hg. CO₂ 48.4 volumes per cent CO₂. \[ \frac{1}{K} \] at 39.1°C. 6,400,
A. C. WHITE

at 37.0°C. 2,400. After temperature had been 7 days normal $\frac{1}{K}$ at 39.1°C. was 5,200, at 37.0°C. 3,200 (double duplicate analyses).

Two cases of exophthalmic goiter with normal temperature and normal bicarbonate reserve showed for $\frac{1}{K}$ at 37.0°C. values of 3,000 and 3,200 (double duplicate analyses).

Case 8.—Mrs. M. Age 35 years. Exophthalmic goiter; of a very severe type, at one time metabolism was 109 per cent above normal; follicular tonsillitis. At time of blood examination pyrexia had lasted 36 hours. Temperature 38.8°C.; pulse 100; respirations 40. Bicarbonate reserve at 38.8°C. and 38.4 mm. Hg. CO₂ was 48.5 volumes per cent CO₂ at 38.8°C. was 4,700 (triple duplicate analyses). 12 days later, temperature again normal. $\frac{1}{K}$ at 38.8°C. 4,800, at 37.0°C. 2,400 (double duplicate analyses).

Case 9.—J. G. Age 33 years. Pulmonary tuberculosis; both apices involved; sputum +++ for tubercle bacilli; constant hectic temperature. Blood examined during pyrexia. Oral temperature 38.3°C.; pulse 104; respirations 24. Bicarbonate reserve at 38.3°C. and 39.6 mm. Hg. CO₂ was 55.2 volumes per cent CO₂. $\frac{1}{K}$ at 38.3°C. 3,600.

Case 10.—W. M. Age 31 years; overman. Rheumatic fever; first signs of illness 6 days prior to admission. History of previous rheumatic fever with slight cardiac damage. Temperature 38.8°C. (oral); pulse 105; respirations 24. Bicarbonate reserve at 38.8°C. and 41.3 mm. Hg. CO₂ was 54.9 volumes per cent CO₂. $\frac{1}{K}$ at 38.8°C. 4,040. 6 days later temperature and pulse were normal; bicarbonate reserve at 37°C. and 38.8 mm. Hg. CO₂ was 51.1 volumes per cent CO₂. $\frac{1}{K}$ at 38.8°C. 3,200, at 37.0°C. 3,500. 5 days later bicarbonate reserve at 37.0°C. and 42.2 mm. Hg. CO₂ was 53.4 volumes per cent CO₂.

Tables I and II summarize the data obtained.

DISCUSSION AND SUMMARY.

From Fig. 1 it may be seen that the effect of elevated temperature during the pyrexial period upon $\frac{1}{K}$ and therefore on the dissociation curve of oxyhemoglobin was, on the average, greater than would have been expected from experiments on normal blood in vitro, and greater than would be expected in view of the alkalosis occurring
### TABLE I.

**1/K**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>During pyrexia</th>
<th>After pyrexia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Body temperatupe</td>
<td>1/K at 37.0°C</td>
</tr>
<tr>
<td>1</td>
<td>Lobar pneumonia.</td>
<td>39.5</td>
<td>5,300</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>39.4</td>
<td>6,100</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>40.0</td>
<td>4,100</td>
</tr>
<tr>
<td>4</td>
<td>&quot; (fatal).</td>
<td>38.5</td>
<td>7,200</td>
</tr>
<tr>
<td>5</td>
<td>&quot; (empyema).</td>
<td>39.6</td>
<td>4,400</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>38.9</td>
<td>4,100</td>
</tr>
<tr>
<td>7</td>
<td>Tonsillitis.</td>
<td>39.1</td>
<td>6,400</td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>38.8</td>
<td>4,700</td>
</tr>
<tr>
<td>9</td>
<td>Pulmonary tuberculosis.</td>
<td>38.3</td>
<td>3,600</td>
</tr>
<tr>
<td>10</td>
<td>Rheumatic fever.</td>
<td>38.8</td>
<td>4,040</td>
</tr>
</tbody>
</table>

### TABLE II.

**Bicarbonate Reserve (Whole Blood).**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Temperature</th>
<th>CO₂ pressure</th>
<th>CO₂</th>
<th>Haldane curve figure at 37.0°C</th>
<th>Interval after first observation</th>
<th>CO₂ pressure at 37.0°C</th>
<th>CO₂</th>
<th>Haldane curve figure at 37.0°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39.5</td>
<td>41.8</td>
<td>53.9</td>
<td>51.9</td>
<td>8</td>
<td>36.2</td>
<td>49.3</td>
<td>49.0</td>
</tr>
<tr>
<td>2</td>
<td>39.4</td>
<td>38.9</td>
<td>51.4</td>
<td>50.4</td>
<td>-</td>
<td>37.4</td>
<td>56.3</td>
<td>49.8</td>
</tr>
<tr>
<td>3</td>
<td>40.0</td>
<td>48.8</td>
<td>52.9</td>
<td>55.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>39.6</td>
<td>44.6</td>
<td>56.8</td>
<td>53.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>38.9</td>
<td>40.4</td>
<td>51.1</td>
<td>51.2</td>
<td>5</td>
<td>37.6</td>
<td>54.8</td>
<td>49.9</td>
</tr>
<tr>
<td>6</td>
<td>39.1</td>
<td>36.0</td>
<td>48.9</td>
<td>48.95</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>38.8</td>
<td>39.6</td>
<td>55.2</td>
<td>50.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>38.8</td>
<td>41.3</td>
<td>54.9</td>
<td>51.7</td>
<td>6</td>
<td>38.3</td>
<td>51.1</td>
<td>50.1</td>
</tr>
<tr>
<td>9</td>
<td>38.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>42.3</td>
<td>53.4</td>
<td>52.1</td>
</tr>
</tbody>
</table>
Fig. 1. Variation of $\frac{1}{K}$ with temperature during the febrile state. Numerals are the case numbers. Roman numerals indicate $\frac{1}{K}$ at $37^\circ$. Arabic numerals indicate $\frac{1}{K}$ at pyrexial temperature.
during fever. Temperature rise, and excess hydroxyl ion acting 
*in vitro* in the opposite directions, seemed to indicate a more stable 
state of affairs than was found. Apparently other factors have 
come into play, as, for example, alterations in the proportions and 
centrations of the various electrolytes. In pneumonia, for in-
stance, there is a retention of chloride during the febrile period with 
excessive loss of phosphates. The variations were not due to vari-
tions in the hemoglobin molecule itself since from the work of Adair, 
Barcroft, and Bock (18) hemoglobin must apparently be reckoned 
as having identical properties in normal individuals of the same 
Species. If Barcroft's (19) hypothesis be right, namely that the 
CH within the corpuscle is higher than that of the plasma, the observed 
variations of $\frac{1}{K}$ may not be so surprising. In view of the fact that 
the hemoglobin inside the corpuscle is enclosed within a semiper-
meable membrane, the possibility arises of the setting up of mem-
brane equilibria which will protect the respiratory pigment from 
excessive changes of reaction that may occur in the plasma, and thus 
the optimum conditions for the carriage of oxygen to the tissues may 
be maintained. Krogh and Leitch (10) in 1919 also drew attention 
to the protected situation of hemoglobin inside the corpuscle. In 
Case 6 it seems as if the alkalosis consequent on the febrile state had 
gained the upper hand and had extinguished the normal tempera-
ture reaction. This is rather confirmed by the fact that clinically 
the case showed one of the earlier signs of an alkalosis; namely, 
twitching of the facial muscles. Case 10, who had been on sali-
cylate, also showed an analogous effect, when 6 days after the first 
observation the temperature shift was practically nil. The rela-
tionship between pH, $\frac{1}{K}$, and the febrile temperature still 
awaits investigation. The extent of the shift of the dissociation 
curve was not by any means uniform; in neither Fig. 1 nor Fig. 2 
is the highest value of $\frac{1}{K}$ at the highest temperature recorded. 

Fig. 2 shows the effect of temperature rise upon $\frac{1}{K}$ after cessation of 
the pyrexia; the effect is not so marked. Some cases, however, 
showed a variation in excess of the normal as if there was not yet 
complete return to normal.
Fig. 2. Variation of $\frac{1}{K}$ with temperature after the period of pyrexia. Numerals are the case numbers. Roman numerals indicate $\frac{1}{K}$ at 37°. Arabic numerals indicate $\frac{1}{K}$ at previous pyrexial temperature.

--- enclose variations of $\frac{1}{K}$ at 37–41°C of a normal blood.
Biologically these changes are of importance in that this shifting of the dissociation curve to the right in fever means that there is more oxygen available for the tissues than normally, more especially at higher pressures. The tension of unloading is raised. This, in addition to the accelerated circulation and the probable increased velocity of reaction, means that even in a localized area of inflammation, if there is increased temperature, the tissues are placed in a better position for resisting infection as a result of their better oxygenation. That there is increased metabolism during fever has been conclusively shown by Du Bois (20) and others using large bed calorigmeters. Du Bois has shown that the increase in metabolism obeys van't Hoff's law, increasing 13 per cent for each 1°C. rise. This shifting of the curve then falls into line with these observations as an adaptive response to the febrile condition, and the febrile temperature, if not too great, would seem to be a purposive attempt to aid the combating of infection. This shifting of the curve probably explains Uyeno's (21) observation on the effect of increased temperature on the circulation in the cat; namely, increased coefficient of utilization, and increased fall in the saturation of the mixed venous blood.

Turning now to Table II, we find that, if the CO₂ dissociation curve of Haldane (12) is accepted as normal, the bicarbonate reserve of five of the cases was above normal, of one normal, and of the rest below normal. Gastric secretion was not the cause of the varying curves, since the time of drawing the blood in all cases was during intestinal digestion. No observations having been made on the blood pH or the alveolar CO₂, we cannot be absolutely certain as to the actual reactions, more especially in the last cases; Case 6, however, was probably one showing a partially compensated CO₂ deficit in view of the absolute lowering of the total bicarbonate and evidence clinically of a tendency to alkalosis. Case 3, which had a lowered reserve, was probably similar. Kochler (22) gives a series of blood pH determinations in acute fevers in which ten out of twelve cases showed an uncompensated alkalosis when the temperature was 103°F. (39.4°C.) or over. Pemberton and Crouter (23) in a study on the response to the therapeutic application of external heat also observed a tendency for the reaction to shift to the alkaline side as shown by
the alteration in the pH of the sweat. Hill and Flack (24) and Bazett and Haldane (25) observed that in thermal fever there was an excessive loss of CO$_2$ comparable to the effect of hyperpnea. These facts are of importance in view of the above results regarding the bicarbonate reserve. That there was a definite alkalosis in some of the cases is at least shown by the value of $\frac{1}{K}$ at 37.0°C. during the pyrexial period in Cases 1, 2, and 7. The upper limits of $\frac{1}{K}$ at 37.0°C. both during pyrexia and after were similar. Of other factors that might be considered, reference may be made to the work of Barbour and his associates (26–29), who showed that in hyperthermia and fever there is an alteration in the concentration of the blood. But the changes were hardly of such magnitude as to cause the variations above detailed.

Addendum.—Recently Bock, Field, and Adair (Bock, A. V., Field, H., Jr., and Adair, G. S., J. Biol. Chem., 1924, lix, 353) have published dissociation curves for normal blood, which do not obey Hill's equation. In view, however, of the mass of data based on methods giving results satisfying this equation the above facts are presented.

In concluding, the writer desires to express his thanks to Professor J. C. Meakins for suggesting the subject and for his helpful criticism and advice.

BIBLIOGRAPHY.
11. Evans, C. L., J. Physiol., 1922, lvi, 146.