LXXXVI. THE BICARBONATE OF THE PLASMA AND THE HYDROGEN ION CONCENTRATION OF THE BLOOD OF GUINEA-PIGS SUFFERING FROM SCURVY.

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IN 1900 Wright examined the blood of six patients suffering from scurvy and found a decrease in the alkalinity of the blood. Some of these cases improved rapidly subsequent to treatment with alkalis [1900]. He obtained similar results with infantile scurvy [1908]. From these observations Wright concluded that scurvy was caused by a condition of acidosis brought about by a diet deficient in organic salts of sodium and potassium. Lamb [1902] examined scorbutic prisoners in an Indian jail, but was unable to detect any decrease in the alkalinity of their blood. The prisoners did not improve after the administration of alkalis.

Hess and Killian [1918] estimated the bicarbonate in the blood of a number of cases of infantile scurvy by Van Slyke's method and found somewhat less than the normal amount. The amount was represented by 40–45 vols. % CO_2 .

Evidence of alteration in c_{H} is lacking.

Some diminution in the quantity of bicarbonates in scurvy is not unlikely for a scorbutic diet is generally one which affords an acid ash.

The hypothesis that acidosis is the cause of scurvy was not in accordance with early observations. It was apparent 200 years ago that fresh vegetables and fruits, especially those of the citrus variety, readily prevented and cured scurvy, whereas dried vegetables were ineffective. The organic salts were presumably still present after drying.

Cockburn (1696) concluded "that no moisture whatever could restore the natural juices of the plant lost by evaporation and altered by a fermentation which they underwent in drying." Lind [1772] quotes instances in which dried vegetable foods had been found to be useless in the treatment of scurvy. The work of Holst and Frölich on the experimental production of scurvy in guinea-pigs greatly facilitated the study of its pathology and enabled the antiscorbutic value of different food stuffs to be biologically

assayed [1907, 1908]. Latterly, Zilva [1924] has concentrated the active fraction of lemon juice and found that less than half a milligram daily was sufficient to protect guinea-pigs fed on a diet which otherwise led to fatal scurvy. The very minute amount of the substance necessary to bring about this anti-scorbutic effect completes the evidence of its specific properties.

A scurvy-producing diet is, as Wright pointed out, generally one which on combustion affords insufficient base and it seemed worth while to find out whether experimentally produced scurvy in guinea-pigs is always associated with a diminution in the bicarbonate in the blood and whether any alteration in the reaction of the blood occurred.

EXPERIMENTAL METHODS.

The blood was obtained by cardiac puncture and collected under paraffin in centrifuge tubes containing a little powdered potassium oxalate.

The bicarbonate of the plasma was titrated by the micro-method described in the foregoing paper [Lepper and Martin, 1925]. The results are expressed as vols. CO_2 %, because most workers are in the habit of thinking of "alkali reserve" in these terms.

This method we found to agree with the gasometric method of Van Slyke to within ± 5 %.

The hydrogen ion concentration of the blood was estimated by the method of Dale and Evans [1920]. The dialysis was carried out at room temperature which varied from 16–22°. The temperature of the dialysate varied from 18–22°.

The collodion sacs used required 3 cc. of blood to fill them, but it was found immaterial whether this amount of blood was used or 1 cc. introduced under oxalated saline. Only the smaller amount of blood was available in some cases.

We have not adjusted our observations of p_{H} at room temperature to the corresponding values for blood at 38° as the amount to be subtracted is still uncertain, nor has a correction for the effect of variation of temperature on the standard phosphates been made as this would be within the error of our observation.

We are indebted to Prof. Martin for the following note on the correction for the effect of temperature on the dialysate:

"The p_{H} of the corresponding blood at 38° would be about 0.14 lower than the figures ascribed to the dialysates, if we assume that Hasselbalch's observation, that ox blood at 38° has the same p_{H} as at 18° when in equilibrium with the same pressure of CO_2 , is applicable to human blood. To produce Hasselbalch's result the diminished acidity due to the lesser CO_2 concentration in the liquid at the higher temperature must be compensated by the increase in the dissociation constants of the weak acids, H_2CO_3 , serum protein and oxy-haemoglobin.

Raising the temperature from 18–38° diminishes the concentration of CO_2 in water which is in equilibrium with CO_2 at constant pressure to 0.59 of the original amount. The result of this on the $\frac{\text{CO}_2}{\text{Bicarb.}}$ ratio would raise the p_{H} of a bicarbonate solution about 0.23. The effect on blood would be less owing to the buffering action of the serum proteins and haemoglobin. According to Hasselbalch's [1916, p. 126, Fig. 4], and Parson's [1920, p. 346, Fig. 1], observations on human blood, the rise would be 0.13 supposing the pressure of CO_2 were 40 mm. As the p_{H} was found to be the same at 18° and 38° with pressure of CO_2 constant, the increase in the dissociation constants of some or all of the weak acids with rise in temperature must produce an opposite effect of the same magnitude.

The effect of temperature upon the K of serum protein and haemoglobin has not, as far as I am aware, been determined, but the dissociation constant of the first H^+ in H_2CO_3 does indeed increase by this amount on raising the temperature from 18–38°, p_{K} at 38° being 0.13 less than at 18°.

In Dale and Evans' technique, loss of CO_2 is avoided as much as possible and only the effect of a rise in K is manifested.

It is by no means certain, however, that Hasselbalch's observations on ox blood are quantitatively applicable to human blood, for Cullen found a considerable variation in the differences in p_{H} at 20° and 38° in the case of horse, dog and human plasma and even between plasma and serum of the same animal."

The diet given to each control guinea-pig was about 60 g. of cabbage, 40 g. of hay and 95 g. of mixed oats and bran per day.

The diet of the guinea-pigs on which scurvy was produced consisted of oats and bran (two volumes of bran to one of oats) and about 40 cc. of autoclaved milk. On this diet the animals developed scurvy after about 15–21 days and succumbed to the disease after a month.

We have examined guinea-pigs (250–300 g.) which have subsisted on such a scorbutic diet for 10–25 days and have assessed the severity of the disease as "slight" when only a few haemorrhages were observed, as "moderate" when definite enlargement of the costochondral junctions, moderately haemorrhagic zones and brittleness of bones occurred, and as "severe" when the haemorrhages, the enlargement of the costochondral junctions, and the brittleness of the bones were very marked.

I. THE BICARBONATE OF THE PLASMA OF NORMAL GUINEA-PIGS.

The results are shown in Table I.

It will be seen that there is a large difference in the bicarbonate content between the extreme observations both for blood drawn from the right and from the left heart. For arterial blood this amounted to as much as 33 vols. %, for venous 32 vols. %. Such wide variations in bicarbonate have not been observed in man even in diabetes.

Table I. *The bicarbonate of the plasma of normal guinea-pigs.*

Number of guinea-pig	Vols. % CO ₂ in plasma from left heart	Number of guinea-pig	Vols. % CO ₂ in plasma from right heart
1	37.0	17	36.0
2	23.25	18	60.5
3	56.5	19	65.25
4	45.7	20	48.5
5	29.5	21	61.0
6	50.0	22	65.75
7	25.4	23	68.25
8	48.25	24	56.25
9	48.0	25	57.5
10	48.0	26	53.5
11	28.5	27	48.0
12	42.0		
	38.0		
13	38.0		
14	42.75		
15	49.75		
16	34.75		
Total 17	Arterial plasma, mean 40.3 vols. %	Total 11	Venous plasma, mean 56.4 vols. %

Observations by Cullen and Howard Robinson [1923] on human normal venous plasma showed extremes of 59.6 and 71 vols. %, a difference of only 11.9 %. The venous blood was drawn from the arm after a period of rest and with precautions against stasis. Buell [1919] found that in guinea-pigs the bicarbonate was low when the animal struggled during the experiment. No such relation could be found in our observations. The guinea-pigs were firmly held by a skilled assistant and the movements of the animal were limited and of short duration.

The values obtained when "slight struggling" was noted were indeed about 8 % higher for both arterial and venous blood. In three guinea-pigs in which "considerable struggling" occurred the results were, venous blood 61 vols. %, arterial blood 28.5 and 34.75 vols. % respectively.

As such wide variations were found in these different guinea-pigs, samples of arterial and venous blood were collected from the same animal. The results are shown in Table II.

Table II. *The bicarbonate of the plasma of arterial and venous blood of the same guinea-pig.*

No. of animal	Vols. % CO ₂ in plasma from left heart	Vols. % CO ₂ in plasma from right heart
1	48.0	68.25
2	28.5	57.5
3	42.75	53.5
4	34.75	48.0

The arterial and venous blood of the guinea-pigs collected under the conditions of our experiment showed a difference in the bicarbonate content of 10–20 vols. % CO₂. It is probable that the relation to food and the stage of digestion reached in the alimentary tract is of importance, but unfortunately

no special precautions were taken to control this factor as its possible influence was not realised at first. In almost all cases the blood was collected before the animals had been fed, but no note was made as to whether food remained in the cage, so that some animals may have eaten much more recently than others.

II. THE BICARBONATE OF THE PLASMA OF GUINEA-PIGS SUFFERING FROM SCURVY.

The results are shown in Table III.

Table III. *The bicarbonate of the plasma of scorbutic guinea-pigs.*

Number of guinea-pig	Vols. % CO ₂ in plasma from left heart	Degree of scurvy	Number of guinea-pig	Vols. % CO ₂ in plasma from right heart	Degree of scurvy
1	27.5	Slight	18	44.0	Slight
2	29.0	"	19	56.75	"
3	38.5	"	20	45.5	Severe
4	42.5	"	21	50.0	"
5	33.25	"	22	55.5	"
6	33.0	"			
7	29.5	"			
8	42.0	"			
9	39.0	"			
10	39.75	Moderate			
11	38.0	"			
12	32.25	"			
13	36.0	"			
14	40.0	"			
15	37.5	Severe			
16	32.25	"			
17	37.5	"			
Total 17 Arterial plasma, mean 36 vols. %			Total 5 Venous plasma, mean 50.3 vols. %		

It will be seen that the scorbutic guinea-pigs had on the average a slightly lower amount of bicarbonate in both arterial and venous plasma than the normal animals, the difference being about 5 vols. % CO₂. This difference is much less than that between the extreme values found in normal animals and might be considered to be without significance, were it not for the fact that the bicarbonate of all these scorbutic guinea-pigs is consistently low.

In order therefore to find out whether this slight alteration in the "alkali reserve" was due to the disease or to a deficiency of sodium and potassium salts in the diet, a number of guinea-pigs were put on a scurvy-producing diet with the addition of 0.8 g. of sodium citrate. The citrate was given by the mouth through a tube daily but was omitted on the day the blood was taken except in three cases recorded in Table V which will be referred to later.

The animals developed well-marked scurvy.

The results are shown in Table IV.

It will be seen that the bicarbonate of the plasma of scorbutic guinea-pigs 24 hours after the dose of citrate is still on the average slightly less than that

of normal animals, the variation from the mean is however much greater than that found in scorbutic animals which are not having citrate and approximates to the normal variation.

Table IV. *The bicarbonate of the plasma of scorbutic guinea-pigs 24 hours after the last dose of sodium citrate.*

Number of guinea-pig	Vols. % CO ₂ in plasma from left heart	Vols. % CO ₂ in plasma from right heart	Degree of scurvy
1	32.5	47.0	Slight
2	45.5	55.5	"
3	22.7	54.5	Severe
4	39.75	—	"
5	34.0	—	"
6	42.75	—	"
7	50.75	—	"
Total 7	% CO ₂ arterial plasma 38.2	% CO ₂ venous plasma 51.3	

Table V. *The bicarbonate of the plasma of scorbutic guinea-pigs 3 hours after the last dose of sodium citrate.*

Number of guinea-pig	Vols. % CO ₂ in blood from left heart	Vols. % CO ₂ in blood from right heart	Degree of scurvy
1	52.5	66.5	Slight
2	55.5	—	"
3	52.25	86.5	"
Total 3	% CO ₂ arterial plasma 53.46 (normal 40.7)	% CO ₂ venous plasma 76 (normal 56.3)	

Table V shows that if the blood of the scorbutic guinea-pigs is collected 3 hours after the last dose of citrate then the bicarbonate content of both the arterial and venous blood is higher than the normal average.

These observations indicate that:

1. The low mean level of the bicarbonate in the blood of scorbutic guinea-pigs is associated with a deficiency of sodium and potassium salts of organic acids in the scurvy-producing diet.
2. The addition of sodium citrate to the diet restored the normal bicarbonate content of the blood, but did not influence the onset of scurvy.

III. THE HYDROGEN ION CONCENTRATION OF THE BLOOD OF GUINEA-PIGS SUFFERING FROM SCURVY.

The p_H found in the blood of normal guinea-pigs is shown in Table VI. The two chief experimental errors in Dale and Evans' method are:

1. Loss of CO₂ from the blood during dialysis.
2. Access of CO₂ to the standard phosphate solutions.

Care was taken to avoid these sources of error as far as possible but as they both give too high p_H readings, observations made by this method tend to be too alkaline rather than too acid.

The p_H of the blood at 38° would probably be about 0.15 lower than that of the dialysates at room temperature (see Prof. Martin's note on p. 582).

Table VI. *The p_H of the dialysate of the blood of normal guinea-pigs. Observations at room temperature.*

Number of guinea-pig	p_H of blood from left heart	p_H of blood from right heart
1	7.61	—
2	7.61	—
3	7.645	—
4	7.58	—
5	—	7.46
6	7.57	—
7	7.59	—
8	—	7.53
9	7.56	—
10	7.6	—
11	7.62	—
Total 9. Arterial blood, mean 7.59		Total 2. Venous blood, mean 7.49

The blood was taken before the animals had been fed and no marked struggling was noted in any case.

The scorbutic animals were examined under similar conditions. Samples of blood were taken between the 15th and 24th day after the diet was started. One of the guinea-pigs was killed at each experiment and examined post-mortem so that an estimate of the degree of scurvy present could be arrived at; the other guinea-pigs were kept alive and re-examined some days later when the scorbutic condition had become very severe.

The results are shown in Table VII.

Table VII. *The p_H of the dialysate of the blood of guinea-pigs suffering from scurvy. Observations at room temperature.*

No. of guinea-pig	No. of days on scorbutic diet	p_H of blood from left heart	p_H of blood from right heart	Degree of scurvy
1	15	—	7.54	
2	15	—	7.44	Slight
3	18	7.55	—	
4	18	—	7.56	Severe
5	18	7.67	—	
6	20	—	7.53	
7	20	7.65	—	
8	20	7.52	—	Moderate
9	20	7.64	—	
10	22	—	7.59	Moderate
1	22	7.51	—	Slight
3	24	7.67	—	Severe
6	24	7.64	—	"
7	24	7.58	—	"
9	23	7.71	—	"
Total 10. Arterial blood, mean 7.61		Total 5. Venous blood, mean 7.53		

No evidence of an increased hydrogen ion concentration of the blood was found in any of the guinea-pigs.

SUMMARY AND CONCLUSIONS.

There are large variations in the amount of bicarbonate in the plasma of normal guinea-pigs; the hydrogen ion concentration, on the other hand, shows only slight variations.

The titratable alkali of the plasma of guinea-pigs suffering from scurvy is on the average at a lower level than that of normal guinea-pigs. This is due to the deficiency of sodium and potassium salts of organic acids in the scurvy-producing diet. The addition of sodium citrate to the diet restores the normal bicarbonate content of the plasma but does not influence the onset of scurvy.

An increase in the hydrogen ion concentration of the blood of guinea-pigs suffering from scurvy was not observed.

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