THE OXYGEN TENSION IN THE SUBMAXILLARY GLANDS AND CERTAIN OTHER TISSUES

By JOSEPH L. BARCROFT, M.A.

From the Physiological Laboratory, Cambridge

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At first sight it is perhaps not surprising that the various secretions of the body should be credited with a certain quantity of oxygen as well as of nitrogen. These gases are, no doubt, in a condition of simple solution and were they in the proportions in which they occur in serum their presence, on casual consideration, would not be surprising. On further investigation, however, it becomes evident that these very secretions are formed by the passage of fluid through the cells of secreting glands, these cells take up oxygen more rapidly than any other structures in the body are known to do, and therefore it is much more remarkable that such a secretion as saliva should contain as much oxygen as does the serum, than it would be if the saliva contained none. It is, however, still more remarkable that saliva and milk should be credited with containing more oxygen in solution than there is in the same volume of serum.

The present paper consists of (1) a critical discussion of the analysis of the gases in saliva, which have been obtained by previous workers, and from which it will appear that in the opinion of the author these analyses are not seriously amiss; (2) a description of some experiments which confirm them; (3) a discussion of the light thrown by the facts cited upon the physiology of certain tissues.

I. THE OXYGEN IN SALIVA

Two physiologists have published analyses of the oxygen in saliva. The following data are given by Pflüger¹ :—

Quantity of saliva	Oxygen per cent.	Nitrogen per cent.
36·56 c.c.	·4	·7
33·03 c.c.	·6	·8

1. Pflüger's *Archiv*, vol. I., p. 688.

Pflüger, in discussing these figures, notices firstly that the quantity of oxygen is as great as (in the second analysis greater than) that of the plasma ; for the latter he gives the figure .5 per cent. This value for the oxygen in plasma is a purely conjectural one, for Pflüger's analysis of plasma yielded an oxygen value of .1-2 per cent. These figures were considered to be too low, since the blood when it was shed, and before the corpuscles were separated, deprived the plasma of some oxygen.

Exact determinations of the percentage of oxygen in plasma have been made by Bohr.¹ The maximum value given by him for the oxygen in plasma (*i.e.*, the value corresponding to a tension of 120 mm. of mercury) is .364 per cent. This we may take provisionally as being correct.

It appears, therefore, that both of Pflüger's analyses give an oxygen percentage in saliva which is higher than the value given by Bohr for his oxygen percentage in plasma.

An examination of Pflüger's figures does not indicate that they err on the side of being too high. Reference to his values for nitrogen shows that these are very low (.7 per cent. and .8 per cent. respectively). There does not, therefore, appear to be any leakage of air into his apparatus. Indeed, his figures rather suggest that incomplete exhaustion has taken place, for 100 cubic centimetres² of water at 38° exposed to a partial pressure of nitrogen of 610 mm. (*i.e.*, 79 per cent. of an atmosphere) absorb .90 c.c. of the gas.

Passing from the figures of Pflüger to those of Külz³, it appears that the analyses performed by this author shew even higher values for the oxygen in saliva than do those of Pflüger.

The following table gives the values for oxygen and nitrogen respectively as found by Külz :—

1. Article entitled *Blutgase und Respiratorische Gaswechsel* in Nagel's *Handbuch der Physiologie des Menschen*, p. 199.

2. Bohr, *ibid.*, p. 116.

3. *Zeitschrift für Biologie*, vol. XXIII, p. 326.

OXYGEN TENSION IN THE SUBMAXILLARY GLANDS 3

Quantity of saliva analysed in c.c.	Oxygen per cent.	Nitrogen per cent.	Quantity of saliva analysed in c.c.	Oxygen per cent.	Nitrogen per cent.
100·0	1·01	2·67	24·49	1·39	3·77
24·49	1·33	2·52	25·95	0·84	2·49
25·95	1·46	3·10	100·0	0·84	2·37
25·95	1·38	3·34	100	1·25	3·20
24·49	1·19	2·65	100	1·04	2·88
25·95	1·17	2·75			

It seems probable that the above values are too high. This may be judged from the percentages of nitrogen which are given, the apparent quantity of nitrogen varies from 2·37 to 3·77 volumes per cent.

If we suppose that the percentage of nitrogen in the saliva is the same as that of the blood (1·20 according to the mean of sixteen estimations by Bohr) we can correct the figures given above. In the first column Külz gives 2·67 per cent. of nitrogen; of this 1·20 may be taken as representing the nitrogen of the saliva, and the remainder 1·47 per cent. as accidental. If, however, 1·47 per cent. of nitrogen is due to air in the pump the approximate quantity of oxygen from the same source will be one quarter of this amount, namely, ·37 per cent. The amount of oxygen actually found by Külz was 1·01 per cent. It would therefore appear that the corrected oxygen in the saliva is ·64 per cent (1·01—·37). This figure at all events is not likely to be too low. By applying the same correction to all Külz's figures, the following table is arrived at:—

Nitrogen recorded by Külz	Less 1·2 per cent.	Oxygen from air	Oxygen in saliva	Nitrogen recorded by Külz	Less 1·2 per cent.	Oxygen from air	Oxygen in saliva
2·67	1·47	·37	·64	3·77	2·87	·64	·75
2·52	1·32	·33	1·00	2·49	1·29	·32	·52
3·10	1·90	·48	·98	2·37	1·17	·29	·55
3·34	2·14	·54	·84	3·20	2·00	·50	·75
2·65	1·45	·36	·83	2·88	1·68	·42	·62
2·75	1·55	·39	·76				

The percentages given in the fourth and eighth columns of the above table may be regarded as minimum percentages. Two things are clear, they are of the same order as Pflügers analyses, and they all exceed the value ·364 which we have taken as representing the oxygen in plasma at a tension of 120 mm.

II. EXPERIMENTS ON THE EFFECT OF THIONIN INJECTIONS.

At a demonstration before the Physiological Congress in 1898, O. F. Grünbaum showed that if thionin were injected into the circulation and the chorda tympani subsequently stimulated the saliva was tinged with the bluish colour of the pigment. This observation I have repeated on three occasions, and not only so but I have found that if the submaxillary gland be cut in two while the stimulation is proceeding, the whole cut surface of the gland is a uniform blue colour. The unstimulated gland on the other side is not necessarily the same. The cut surface of the gland was almost normal in colour in two of the three experiments, whilst in the third there was just appreciably less colour than in the stimulated gland, it turns blue, however, on exposure to air. The presumption is that the thionin becomes reduced to a greater or less degree in the resting gland (as Ehrlich shewed with alizarin blue in the tissues generally). It does not get reduced either by the active gland or by the saliva. To reach the saliva, however, it must pass actually through the cells of the submaxillary gland.

In respect of its action on thionin, the submaxillary differs very obviously from muscle. During Experiment II, shortly after observing the glands, I stripped the skin off the thighs of the cat. The muscles were a little, but only a little, coloured and the same on both legs. On stimulation of the right anterior crural nerve the muscles on the anterior surface of the right thigh became obviously less coloured than those of the left side, which showed that the thionin became reduced more actively by the contracting than by the resting muscle.

The pancreas, like the salivary glands, was blue superficially and on section (Experiments II and III).

The mucous membrane of the stomach was more blue than the unstriped muscle (Experiments II and III).

The thionin was injected in normal saline (40 c.c.). The injection acted as a violent diuretic, there was a copious discharge of blue urine, after which the bladder rapidly filled and became tense with an additional secretion of a similar fluid. The kidney on section was

not blue. The natural colour, however, made it difficult to judge of any artificial pigmentation. Three hours afterwards, the section which had been cut out and which was exposed to air in the meantime, was somewhat blue in the medullary region (Experiment II).

Neither the heart nor the lungs appeared abnormal in colour (Experiments II and III).

The fat of the omentum was its usual pinkish-white colour (Experiments II and III); cut into small pieces and allowed to stand, it became slightly tinged with blue. In the case of such a tissue as fat the colouration would not be great however, as the tissue from its nature would not readily become permeated with a dye contained in aqueous solution.

The following is the protocol of the second experiment in which my original observations on the submaxillary gland were confirmed and the observation on other organs were made.

Dec. 24. Cat. A. C. E. mixture given through trachea tube cannula in femoral vein. Chorda tympani and anterior crural nerves exposed for stimulation on right side.

12.14—12.19. 20 c.c. of solution of thionin in normal saline injected into r. femoral vein.

12.19. Stimulation of chorda. Good flow of saliva, blue colouration.

12.22. 13 more c.c. of thionin injected, chorda stimulated trace of colour in saliva.

12.24. 10 more c.c. of thionin injected.

The colour was obvious in the saliva, chorda stimulated.

12.26. Right submaxillary cut out and cut open, whilst the stimulus was maintained.

The gland was blue throughout.

12.28. Left gland (unstimulated) taken out and cut open, blue on surface, section not blue except at edge.

12.35. Profuse micturition, urine blue.

12.40. Pancreas blue on surface—stomach mucous membrane bluer than muscle.

12.45. Took skin off legs, same colour. Stimulated right anterior crural nerve—muscles became less blue than on left side, neither side was more than tinged.

12.54. Fat in omentum not coloured.

12.55. Bladder full of blue urine.

4. Kidney scarcely coloured.

12.57. Lung and heart not coloured.

4.0. Specimens of left submaxillary blue, fat faintly blue, lung not coloured, kidney not obviously coloured in cortex, coloured in medulla.

Dec. 26, 11 a.m. Specimen of fat much more obviously blue.

THEORETICAL DEDUCTIONS

After Ehrlich demonstrated the fact that alizarine blue and other stains were reduced by certain tissues, Bernstein¹ published a scale of the reducing powers of certain tissues. His data were derived from placing these tissues, *post-mortem*, in the pigment and studying the time necessary for its reduction. On this scale muscle appeared to have the greatest reducing power of all the organs he tested.

If the results of the experiment which I have detailed above were translated into the language of Bernstein, the following results would be arrived at :—(1) That the resting submaxillary gland had a greater reducing power than the active submaxillary ; (2) That the reducing power of the pancreas was relatively very low ; (3) That the reducing power of active glands (the submaxillary and the kidney) was very low, since the pigment which actually goes through the cells of these glands appears unreduced in the urine.

If, however, the reducing power be defined as the amount of oxygen used up per minute by one gramme of the various structures it will be seen that the following are the approximate reducing powers of the various structures mentioned above :—

Active submaxillary ²	·1	...	Active kidneys	·07
Resting submaxillary	·03	...	Heart beating feebly ⁵	·006
Resting pancreas ³	·05	...	Muscles of leg resting ⁶	·002
Resting kidneys ⁴	·03	...	Muscles of leg tetanus	·018

There is, therefore, an apparent divergence between the reducing power of the tissues as calculated from their oxygen-consumption and the reducing power given by Bernstein. The reason of this difference is not far to seek. Bernstein had been studying the power of reduction which *post-mortem* tissues, deprived of oxygen, possess, whilst the above figures apply to living tissues in their own environment and supplied with oxygen at the rate, and by the means, which normally satisfies their needs.

1. Schäfer's Text Book of Physiology, Vol. 1, p. 782.

2. Barcroft, *Journal of Physiology*, Vol. XXVII, p. 43.

3. Barcroft and Starling, *Journal of Physiology*, Vol. XXX, p. 496, 1904.

4. Barcroft and Brodie, *Journal of Physiology*, Vol. XXXII, p. 52.

5. Taken from a research now being carried on by Dr. W. E. Dixon and myself.

6. An approximation computed from the results obtained by V. H. Mottram A. E. Stansfield, Miss Tweedie, and myself.

The colour of the tissues in my experiments with thionin cannot therefore be compared in any way with Bernstein's, nor can it be taken as a measure of the reducing power of the tissues in Bernstein's sense. It is rather an indication of the normal conditions, in respect of oxygen tension, under which these organs exist in their varying states of rest or activity. In the muscles of the leg, as in the submaxillary gland, there is an increased consumption of oxygen in the condition of activity. The active muscles become less blue, the active gland becomes more blue; the deduction is that activity in the gland is associated with an increased oxygen tension, activity in the muscle with a decreased oxygen tension. In other words, whilst in both tissues active reduction is taking place, this active reduction is more than counterbalanced in the case of the gland by some other process which keeps up the tension of oxygen.

This conclusion is borne out by the analyses of Pflüger and of Külz, who have shewn that the fluids which have passed through the gland cells have an appreciable oxygen tension. This oxygen tension differs in the secretions of different glands, but in all it exists. The view that secreting glands carry on their work with a much more copious environment of oxygen than is given to active muscles is not a new one. It was essentially bound up with Bernard's observation that the blood from the active submaxillary was arterial in colour; this point has been accentuated by Bohr,¹ who has calculated the concentration of oxygen in the submaxillary vein, during the conditions of rest and activity of the gland, from my own figures; his calculation shews the following data for the venous blood from the submaxillary gland:—

Condition				Percentage saturation of O ₂	Concentration of O ₂ in plasma
Rest	...	...	...	52·8	·089 per cent.
Chorda stimulation	...	...	...	73·3	·132 per cent.

Neither of these authors, however, have pointed out that this rise of oxygen tension is associated with an enormous oxygen consumption.

The following calculation will show the magnitude of this phenomenon.

1. *Ibid*, p. 200.

In the dog, as the result of nine experiments,¹ 1.4 c.c. of saliva per minute leave the gland during chorda stimulation; this is equivalent to about 1.6 c.c. of water leaving the vessels.² If the oxygen which the gland uses were transferred to the gland in the water, at the same velocity as the water of the secretion, we may ask ourselves what would be the concentration of the oxygen in the water on its journey between the blood and the cell.

The quantity of oxygen leaving the blood is (1) that used by the glands—.86 c.c.; (2) that contained in 1.4 c.c. of saliva, $1.4 \times .007$ (mean of Külz reduced results) = .01; (3) that contained in the lymph—an unknown factor which must be neglected. The total, therefore, is .87 c.c. per minute. If this were dissolved in 1.6 c.c. of water the concentration would be 55 per cent., which would correspond to a tension of about twenty-three atmospheres of pure oxygen.

Somewhat similar calculations might be made in the cases of the kidney and the pancreas.

There are several lines of thought along which a solution of the problem before us may be sought.

In the first place, it is conceivable that, as was once supposed, the respiration of the gland takes place in the blood, *i.e.*, that the gland thrusts a reducing substance into the capillaries which directly and instantly reduces the haemoglobin. This view is opposed to all we know about tissue respiration. Further, such a substance would have to possess the property of reducing the haemoglobin in an inappreciable time without being able to break up the molecules of oxygen in the stream through which it is thrust, and thrust at a velocity greater than that of the saliva. The reason of this last statement depends upon the fact that there is free oxygen in the saliva. A body possessing a reducing power of this nature is not very remarkable, for ferrous sulphate reduces potassium permanganate instantly, whilst it only reduces free oxygen slowly. But if such a body existed, it might be expected to find its way to some extent into the saliva, and there, and also in the gland, to reduce the thionin.

1. Barcroft, *Journal of Physiology*, Vol. XXVII, p. 40.

2. Barcroft, *Journal of Physiology*, Vol. XXV, p. 457.

Another consideration which yields a more likely solution of the problem is, that the oxygen diffuses from the blood to the gland very much more rapidly than the stream of water travels. For the passage of a given quantity of oxygen from the capillary wall to the cell, the difference in oxygen tension in the two positions necessary to produce the flux would be inversely as the specific velocity of which the molecules were capable. By supposing that the molecules passed very rapidly we might postulate a small difference of tension. But a difference of tension must exist. This difference of tension might be maintained in either of two ways: (1) by a low oxygen tension in the gland; (2) By a high oxygen tension in the capillary wall. The experimental evidence which has been before us in this paper indicates that the former of these conditions does not exist; we are, therefore, bound to fall back upon the conception that there is something active, some oxygen secreting quality in the capillary wall, unless we can find some other means of raising the oxygen tension in the saliva above the calculated value in the plasma.

A third possibility is that the calculated value of the tension of oxygen in the capillaries is too low—we have taken it at 120 mm. of Hg. According to the computation of Haldane this figure is much lower than the oxygen tension of the arterial blood. But the conception of oxygen tension set forth by Haldane is of no avail. For, suppose the oxygen tension of the arterial blood to be 160 mm.—true the concentration of oxygen in the plasma would rise to a value approaching that given by the analysis of saliva—but the dissociation-curve of oxy-haemoglobin is such that the tension would at once fall when the first vestige of oxygen left the haemoglobin.

Adhering to the conception that the calculated oxygen tension in the capillary is too low, we might suppose that some constituent of the blood played the role of actually turning the oxygen out of the haemoglobin in the capillaries.

Bohr has brought forward evidence of CO_2 performing such a function, but on far too small a scale to account for the phenomenon which we are considering. We know of no other substance which can be held to perform this function in the blood.

CONCLUSIONS

1. The analysis of Pflüger and Kulz show that the oxygen tension in saliva is greater than that in plasma, and that there is a definite oxygen tension in other secretions, milk, urine, etc.
2. Injection of thionin shews that the active submaxillary gland does not reduce the dye sufficiently to prevent the gland becoming blue throughout. The saliva and the urine are also blue.
3. The salivary gland becomes bluer during activity, the muscles of the leg less blue.
4. In view of the great consumption of oxygen in the submaxillary gland, the high oxygen tension of saliva is not easy to account for, the most probable of the explanations discussed is that the capillary walls have the power of raising the tension of the gas as it passes through them.