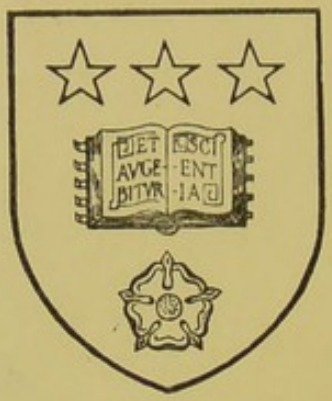
The respiratory function of the blood.



THE RESPIRATORY FUNCTION OF THE BLOOD

CAMBRIDGE UNIVERSITY PRESS
London: FETTER LANE, E.C.
C. F. CLAY, MANAGER

Edinburgh: 100, PRINCES STREET
London: H. K. LEWIS, 136, GOWER STREET, W.C.
WILLIAM WESLEY & SON, 28, ESSEX STREET, STRAND
Berlin: A. ASHER AND CO.
Leipzig: F. A. BROCKHAUS
New York: G. P. PUTNAM'S SONS
Bombay and Calcutta: MACMILLAN AND CO., LTD.
Toronto: J. M. DENT AND SONS, LTD.
Tokyo: THE MARUZEN-KABUSHIKI-KAISHA

THE RESPIRATORY FUNCTION OF THE BLOOD

BY

JOSEPH BARCROFT, M.A., B.Sc., F.R.S.

FELLOW OF KING'S COLLEGE, CAMBRIDGE

Cambridge:
at the University Press
1914

Cambridge
PRINTED BY JOHN CLAY, M.A.
AT THE UNIVERSITY PRESS

TO THE

PROVOST AND FELLOWS OF KING'S COLLEGE
CAMBRIDGE

I DEDICATE THIS VOLUME

PREFACE

AT one time, which seems too long ago, most of my leisure was spent in boats. In them I learned what little I know of research, not of technique or of physiology, but of the qualities essential to those who would venture beyond the visible horizon.

The story of my physiological "ventures" will be found in the following pages. Sometimes I have sailed single handed, sometimes I have been one of a crew, sometimes I have sent the ship's boat on some expedition without me. Any merit which attaches to my narrative lies in the fact that it is in some sense at first hand. I have refrained from discussing subjects which I have not actually touched, but which might fittingly have been included in a modern account of the blood as a vehicle for oxygen. Such are the relation of narcosis to oxygen-want and the properties of intracellular oxidative enzymes. The omission of these and other important subjects has made the choice of a title somewhat difficult. I should like to have called the book, what it frankly is—a log; did not such a title involve an air of flippancy quite out of place in the description of the serious work of a man's life. I have therefore chosen a less exact, though more comprehensive title.

After all, the pleasantest memories of a cruise are those of the men with whom one has sailed. The debt which I owe to my colleagues, whether older or younger than myself, will be evident enough to any reader of the book. It leaves me well-nigh bankrupt —a condition well-known to most sailors. But I owe another large debt of gratitude to those who, as teachers, showed me the fascination of physiology, to Dr Kimmins*, and especially to Dr Anderson†. At a later stage I learned much from Dr Gaskell, Professor Langley and Dr Haldane.

* Formerly science master at the Leys School now Chief Inspector of the Educational Department of the London County Council.

† Formerly supervisor in physiology to King's College, now Master of Gonville and Caius College.

There are occasions on which every sailor of the deep sea has to ship a pilot. Mr A. V. Hill has brought me into those harbours which are best approached through the, to me, unknown channels of mathematics. I have to thank him and also Miss Dale for much help with my proofs.

In the preparation of this book my acknowledgments are due to many friends and others for allowing me to reproduce their photographs or illustrations—Mr C. G. Douglas (Figs. 115—119), Prof. Durig (Figs. 127 *a*, 131 and 133), Dr Aggazzotti (Figs. 127 *b* and 132), Dr Haldane and the Council of the Royal Society (Figs. 71, 72 and 73), Mr Hartridge and the Council of the Royal Society (Fig. 104), Dr Lewis and the publishers of *Heart* (Fig. 105), Dr Krogh and the publishers of the *Skandinavisches Arch. für Physiologie* (Figs. 98 and 99), Dr Rohde and the publishers of the *Archiv für experimentelle Pathologie und Pharmakologie* (Figs. 52—55), Prof. Nuttall and the publishers of the *Journal of Parasitology* (Fig. 2), and Dr Warburg ("Ueber Hemmung der Blausäurewirkung in lebenden Zellen," *H. S. Zeits. für phys. Chemie*, Band 76, S. 331, Strassburg, Karl J. Trübner), and to the Editor of the *Journal of Physiology* for permitting the reproduction of many figures of my own.

Lastly, my thanks are due to certain Corporations who have given me grants for research—the Royal Society, the British Association, the Fellows of King's College and the Commission for the Study of the Biochemical Effects of High Climates and Solar Radiation presided over by Prof. Pannwitz of Berlin.

J. B.

CAMBRIDGE,
December 1913.

CONTENTS

PART I

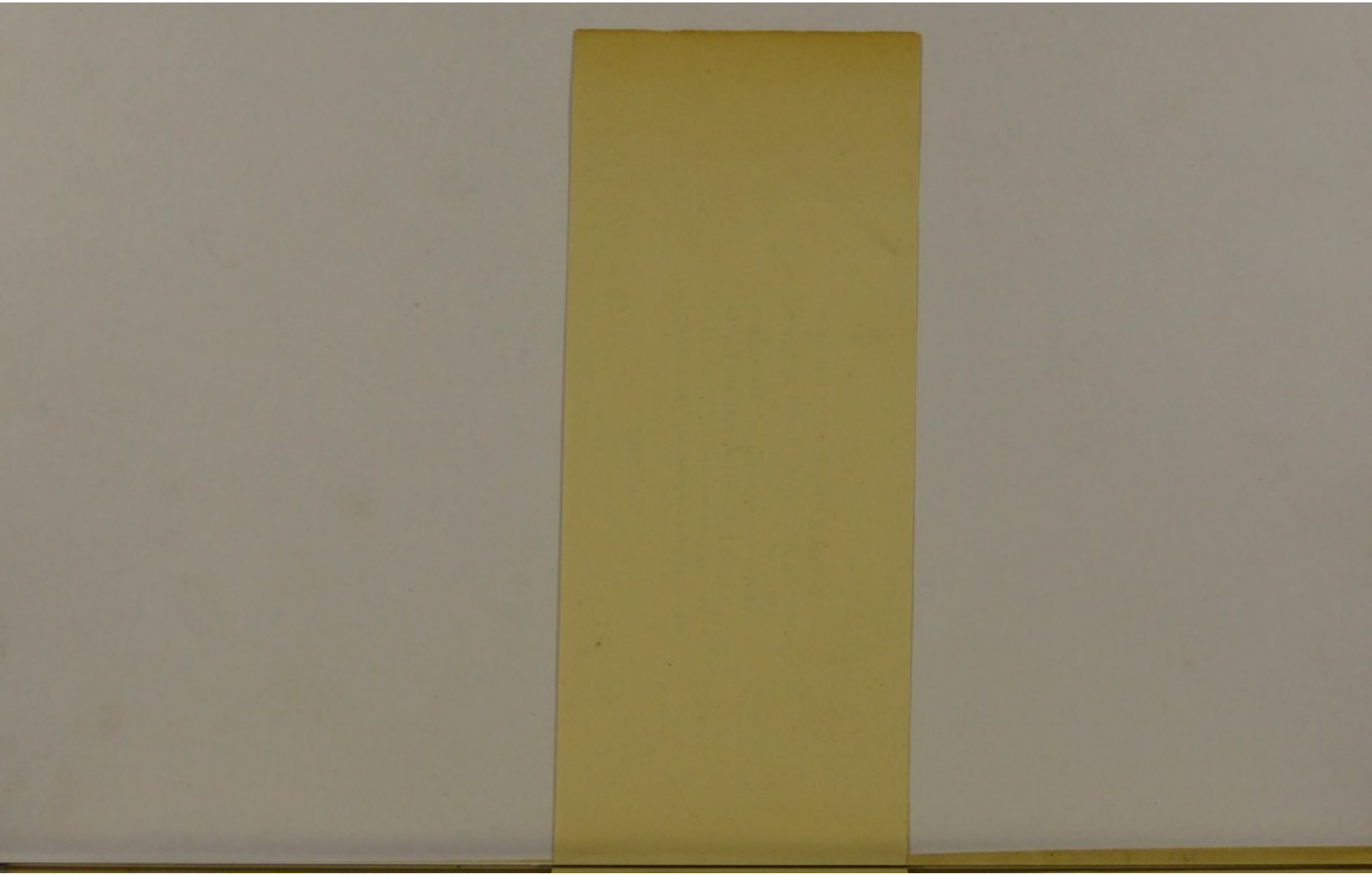
THE CHEMISTRY OF HAEMOGLOBIN

PART II

THE PASSAGE OF OXYGEN TO AND FROM THE BLOOD

PART III

THE DISSOCIATION CURVE CONSIDERED AS AN "INDICATOR" OF THE "REACTION" OF THE BLOOD



ERRATA.

p. 67 last line, *for* preparing *read* preferring.

p. 97 line 27, *for* Neumann *read* Neuman.

pp. 130 and 131, *for* Pikes *read* Pike's.

p. 259 *et seq.*, *for* Chisholm *read* Chisolm.

p. 316, *for* $\frac{y}{100}=\frac{Kx^n}{1+Kx^2}$ *read* $\frac{y}{100}=\frac{Kx^n}{1+Kx^n}$.

p. 316 line 17, *for* K^x, *read* $K=10^x$.

PART I

THE CHEMISTRY OF HAEMOGLOBIN

CHAPTER I

THE SPECIFIC OXYGEN CAPACITY OF BLOOD

Writers on historical subjects frequently allow themselves to speculate upon the momentous issues which follow from trivial circumstances. A general stays to drink the health of his king in some tavern, and the delay determines the result of a battle and therewith also the reigning dynasty and the State religion. In like manner I have sometimes indulged in the luxury of inquiring what would have happened to the animal kingdom had it not been for the accidental occurrence of the chemical substance haemoglobin. Consider the respiration of muscle. It is among the most efficient of machines. In warm-blooded animals muscle when working at its full power uses up its own volume of oxygen in about ten minutes[1]. This oxygen is carried to it by the haemoglobin of the blood—a substance so rich in oxygen that a relatively small quantity of blood satisfies the need of the muscle. Were it not for this red pigment some 200 c.c. of fluid would have to be circulated through every gram of muscle in ten minutes of time.

To put the matter in another way; blood carries about 40 times as much oxygen as the same volume of plasma. Therefore to convey as much oxygen round the body as is carried by the blood, would in the absence of haemoglobin demand 150 kilos of plasma, or perhaps more. The contents of the vascular system would therefore amount to twice the present weight of the body. The body would in short be unable to cope with the weight of its own blood. The whole basis of its economy therefore hinges upon the accidental possibility of the occurrence and properties of haemoglobin.

Nor is there any chemical substance which exactly resembles haemoglobin, though in some of the lower animals there are poor imitations of it. But for its existence man might never have attained any activity which the lobster does not possess, or had he done so, it would have been with a body as minute as the fly's.

Consider the question from the standpoint of evolution. If the wing or the beak of a bird is too short for the highest efficiency it can be modified in successive generations by a process of natural selection until it attains the most suitable proportions. But in dealing with chemical substances nature is set a very different task: either they occur as stable compounds or they do not occur. Unless we are to suppose that in times gone past there has been an infinite variety of possible and impossible chemical combinations, we must suppose that nature so far from adapting chemical substances to herself has had to adapt herself to the chemical substances which exist. And therefore I will adopt as a basis for the consideration of respiration an investigation into the chemical nature of the substance haemoglobin on which respiration, in the higher animals at least, depends. I will begin therefore by considering the hard facts with which we have to do in the chemistry and physics of the subject.

The first question which I would answer is, Is haemoglobin a single substance? In books on Chemistry it is usual to give considerable prominence to the difference between a mixture and a compound, and to prescribe obedience to the "law of combination in definite proportions" as one of the conditions which a chemical compound must satisfy. The table which is given below will show

Analysis of Oxyhaemoglobin [2].

Animal	C	H	N	S	O	Fe	P	Author
Horse	54·75	6·98	17·35	0·42	20·12	0·38	—	Abderhalden
Dog	54·57	7·22	16·38	0·57	20·43	0·34	—	Jaquet
Cat	54·60	7·25	16·52	0·62	20·66	0·35	—	Abderhalden
Guinea-pig ...	54·12	7·36	16·78	0·58	20·68	0·48	—	Hoppe-Seyler
Fowl	52·47	7·19	16·45	0·86	22·5	0·34	0·197	Jaquet
Pig	54·17	7·38	16·23	0·66	21·36	0·43	—	Otto
,, ...	54·60	7·25	17·43	0·48	19·60	0·40	—	Abderhalden

that this condition is very far from being fulfilled by haemoglobin, for the discrepancies which appear between the various analyses are too great to be accounted for by errors in the very accurate analytical method of combustion.

On the other hand the uniformity of the results yielded by accurate combustions depends upon the purity of the substance which is being analysed. And the difficulty of obtaining haemoglobin uncontaminated by other bodies, more especially by salts and

by water, is very great. We must however provisionally conclude that haemoglobin differs in different species, and perhaps even in different individuals. This difference may be explained, in part at all events, by a difference in the globin portion of the molecule, which, on account of its relatively great weight, plays a predominant part in the data of chemical analysis, whilst it is but by-play in the study of haemoglobin as a respiratory pigment. It would be enough for our purpose could we show some constancy in the iron-containing portion of the molecule and leave on one side the question of the exact composition of the protein with which it is combined.

For this reason much laborious work has been done on the determination of the "specific oxygen capacity" of haemoglobin, that is, on the number of cubic centimetres of loosely combined oxygen which correspond to every gram of iron in the compound. Suppose for instance that it appears, as the result of analysis, that 401 cubic centimetres of oxygen correspond invariably to a gram of iron. It would follow that haemoglobin obeyed the law of definite proportions so far as oxygen and iron were concerned, and also that it obeyed the law of combination in simple proportions. For, expressing this ratio of the iron to the oxygen by weight, every 56 grams of iron would correspond to 32 grams of oxygen. In other words, the oxygen and the iron would be united in the proportion of one atom of iron to two of oxygen.

The following table will however show that the history of the subject provides us with but scanty hope of reaching this ideal[3].

Observer	Animal	Number of Cases	Extreme figures	Mean
Bohr	Dog	22	328—468	375
Tobiesen	Dog and Calf	17	378—429	388
Abrahamson	Ox	32	301—391	351
,, ...	Pig	5	284—401	341
Bohr	Horse	9	379—426	411
Bornstein & Müller[4] ...	Cat	5	372—403	401
Masing & Siebeck[5]	Man, Ox, Rabbit	—	—	397
Butterfield[6]	Ox	3	389—395	397
,, ...	Man	—	—	391
,, ...	Man (diseased)	11	384—409	399

The discrepancies between the various analyses amount in some cases to one-third of the whole quantity of oxygen measured, and some little consideration must be given to their interpretation.

Till recently there have been two schools of thought with regard to the meaning of the figures given. Of these the first teaches that the sources of analytical error are so great as to make more accurate analysis impossible, whilst the second, represented chiefly by the late Professor Bohr of Copenhagen, frankly admits that the want of uniformity is so great as to render untenable the idea of haemoglobin as a simple substance. He explains the divergencies which we have noted as being due to a mixture in different proportions of four substances which he calls α, β, γ and δ haemoglobins[7], each with a different oxygen capacity from the others.

To the two schools mentioned above has now been added a third which teaches that the combination of oxygen and haemoglobin is not in the old-fashioned sense a chemical combination at all, but that it is a manifestation of the physical phenomenon known as adsorption, and that it therefore depends essentially on the surface conditions of the molecules of oxygen and haemoglobin respectively. The properties of these surfaces may presumably be altered by all sorts of variations in the collateral substances which are present in the solution of the uniting molecules. The amount and nature of the salts present for instance might be supposed to alter the charges on the molecules, and in so doing to affect the amount of oxygen with which a given quantity of haemoglobin would unite.

Within recent years, partly on account of the improvements in the analytical methods both for oxygen and iron and partly on account of the increased importance of the subject, it has become more and more desirable that some re-investigation by direct methods of this specific oxygen capacity of haemoglobin should take place. I mean by methods in which the oxygen is measured as such, and the iron as a salt of the metal, as opposed to the indirect spectro-photometric methods which have given uniform, and apparently excellent results in the hands of Butterfield[6]. This investigation has lately been undertaken by Peters[8]; for the purpose of estimating the oxygen he has used the differential method of blood analysis based upon the observation of Haldane[9] that oxygen is eliminated from haemoglobin quantitatively by potassium ferricyanide. The theoretical accuracy of the method has been confirmed by the researches of Professor Franz Müller[10] in Berlin, while its practical details have been so far simplified that some half-dozen analyses can easily be performed with as many cubic centimetres of blood in two hours. Peters therefore has been at a great advantage as compared with his predecessors, whose

individual analyses, if theoretically somewhat more accurate, extended over two or three days and entailed the use of large quantities of haemoglobin. It was impossible in their case to obtain large numbers of analyses which could be averaged and from which the errors could be eliminated to some extent by statistical treatment.

In estimating the iron by titration with titanium advantage has been taken of new methods of analysis which are much simpler and more accurate than the older permanganate titrations.

The theory of the titanium method of estimating iron is represented by the following equation:

$$TiCl_3 + FeCl_3 = TiCl_4 + FeCl_2.$$

The method has this great advantage over that of titration with potassium permanganate, that it is not vitiated by the presence of chlorides.

In practice defibrinated blood was centrifugalised, the corpuscles washed twice in isotonic salt solution, and as much as possible of the fluid was got rid of. The cream of corpuscles was laked by the addition of twice its volume of dilute ammonia (4 c.c. of strong ammonia per litre). This solution, which we shall call solution *A*, was centrifugalised again to rid it of any corpuscles which did not lake and of other débris. Portions of it were then measured out from the same burette, both for the iron estimations and the oxygen analyses. For the former 50 c.c. of the solution was evaporated in a platinum crucible and carefully "ashed." It was at this point that the nicety of the determinations really entered if accurate results were to be obtained. The ashing must take place at a temperature which is neither too hot nor too cold. It is best carried out in a "muffle furnace." To quote Peters, "If the carbon is not completely burned away iron will still remain which cannot be removed by boiling with acids; whereas on the other hand if the ash is heated to a high temperature in removing the carbon the iron becomes insoluble. In both cases the result will be a loss of iron." After the ashing is complete the iron is dissolved up in strong hydrochloric acid, and a trace of hydrogen peroxide is added to insure the complete oxidation of the iron. The excess of hydrogen peroxide is subsequently boiled off and the titration with titanous chloride is made, potassium sulphocyanide being used as an indicator.

Some idea of the scale on which Peters' experiments were conducted may be gleaned from an account of a single experiment. In addition to making two or three analyses of iron such as have just been

described, he made from the same solution, run from the same burette, sixteen oxygen estimations. These were divided into four groups: the average of each group was taken, and the mean of these four averages was taken. The greatest error which entered into Peters' blood-gas analysis was doubtless in the measurement of 3 c.c. of fluid from an ordinary 50 c.c. burette, the meniscus in the case of haemoglobin solution being none too well defined. This error, serious though it appears, is discounted by the fact that the 16 samples for analysis were run consecutively out of the burette. There may be a certain ruggedness in the individual figures, but the averages of the groups of four are very close to one another, since a positive error in one sample entails an equal negative error in the next: in the aggregate 48 c.c. used there is no appreciable error as compared with the 50 c.c. used for the iron analysis.

Peters' figures for the oxygen of a single experiment are as follows:

Oxygen in 3 c.c. of solution A in c.c.

	Group (1)	Group (2)	Group (3)	Group (4)
	·3667	·3667	·3576	·3527
	·3449	·3374	·3640	·3510
	·3482	·3439	·3638	·3614
	·3455	·3517	·3455	·3537
Total	1·4053	1·3997	1·4309	1·4188
Mean	·3513	·3499	·3577	·3547

Taking the average of these four means we get

	·3513
	·3499
	·3577
	·3547
Total	1·4136
Mean	·3534 c.c.

When it is remembered that the whole of these operations, both iron analyses and gas analyses, could be carried through successfully in a day, it will be clear what an advance Peters has made by the use of the modern technique both as regards the concordance of his figures and the certainty with which he has been able to put them forward. His figures for the volume of oxygen per gram of iron are as follows:

Ox	394, 401, 399
Sheep.....	387, 384
Pig	388
Dog	384
Average	391

It is not very easy to discern the processes by which conviction grows in the mind; probably the mere inspection of the figures given is sufficient to convince the reader that so far as the relation of the respiratory oxygen to the iron of haemoglobin is concerned, these quantities are related in the proportions of two atoms of oxygen to one of iron. To me, who had the privilege of seeing Peters work from week to week, conviction came in a slightly different way; it developed as it were like the image on a photographic plate. As one experimental difficulty after another was overcome, as one source of error after another was weeded out, as the worker himself developed in skill and in capacity, just so surely did the results which he obtained approach the theoretical figure with greater certainty till at the end when all the difficulties had been overcome and when Peters himself had attained to the rank of a first-rate exponent of the technique, I arrived at a stage of conviction in which I never doubted, when he undertook an experiment that the result would be between 385 and 405. Perhaps there could be no surer proof that all thought of the wide differences between different kinds of haemoglobin, alleged to exist by Bohr and others, had passed out of our horizon, than the fact of our almost laughable concern at the end of the work as to why the average figure was 391 and not 401. We, in the laboratory, thought perhaps that Peters did not perform sufficient experiments to obtain a true average, or that some trace of methaemoglobin was always present or, most probably, that some trifling error had crept into standardisation of the apparatus used.

The probability of the last source of error at least seemed sufficiently great to warrant the initiation of a fresh research on the subject, which was undertaken by Burn. Moreover on quite general grounds it seemed desirable to undertake something of the sort, for independence of the fallacies of a single experimental procedure is of the essence of all sound experimental work.

The problem was to find a method of calibrating the differential blood-gas apparatus[12] in such a way that the possible errors involved in the method which Peters had used would be of a different character from those involved in the new method.

The details of the differential apparatus will be found in the Appendix; the form used by Peters is shown in Fig. 1. All we need say here is that the oxygen is liberated from the haemoglobin in one of its two bottles. The pressures in these become unequal, and the difference of pressure is indicated by the movement of the fluid in the

manometer. The measurement consists in reading the difference in the level of the fluid surface on each side; the problem is to turn this difference of level read in mm. of clove oil into a measurement of quantity in cubic centimetres of oxygen which has been liberated.

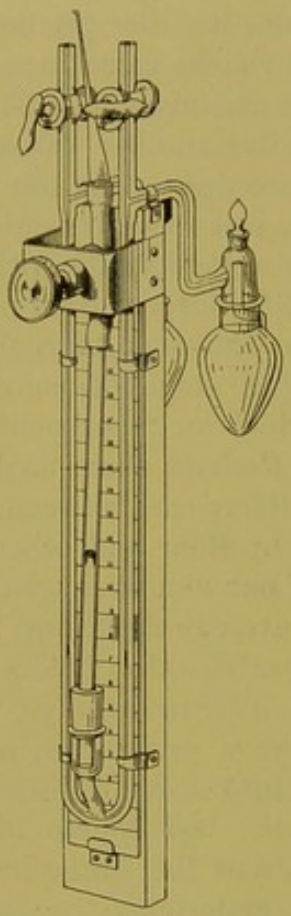


Fig. 1.—Form of differential apparatus used in Peters' and Burn's researches.

It may be shown mathematically that if x be the difference in level of the fluid surfaces, V the volume of air in each bottle, a the sectional area of the tubing, p the barometric pressure in mm. of clove oil, and q the quantity of oxygen liberated, all the quantities being expressed in mm.,

$$q = x\left(\frac{V}{p} + a\right).$$

Now the quantities V, p and a are measurable, and V and a may be measured once for all. It was by this method that Peters calculated the relation of q to x in his experiment. There is no

doubt of course as to the theoretical accuracy of the calculation, but it is evidently not impossible that when the greatest accuracy is concerned there may be weak points about its application. We are using fluids for instance which leave films on the glass and exert vapour pressures and so forth, and it may be that the trifling constant errors involved might in the aggregate amount to one or two per cent.

Burn therefore went back to quite another method of relating q to x, which was tried, though without complete success, by Roberts and myself. He first defined the conditions under which it might be relied upon, after which he obtained with it results of remarkable consistency. He liberated a known quantity of oxygen in the apparatus and read off the difference in level; thus knowing both q and x he found the constant K by which x must be multiplied to give q, and which should of course be equal to $\left(\frac{V}{p} + a\right)$.

The known quantity of oxygen was liberated from a known volume of standardised hydrogen peroxide by potassium permanganate according to the following equation :

$$K_2Mn_2O_8 + 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 3H_2O + 5O,$$
$$5O + 5H_2O_2 = 5H_2O + 5O_2.$$

So far as manipulative details are concerned the oxygen is liberated in just the same way as it is from a haemoglobin solution by ferricyanide. As the sources of manipulative error are the same in each case, the estimation of K by the H_2O_2 method should give a correct value of K for the ferricyanide method. The validity of the method depends upon the assumption that the calculated quantity of oxygen is released.

The following is the result of one series of experiments in which Burn determined K for a certain apparatus, No. 19, by the two methods.

K determined by H_2O_2				K determined by measurement of V, p, and a
3·87	3·87	3·87		3·72
3·78	3·87	3·82		
3·78	3·82	3·78	Mean 3·83	
3·82	3·81	3·89		
3·78				

The extreme results of the H_2O_2 method differ from one another by 3 °/₀.

We may now tabulate the results of three of Burn's experiments.

Series	Apparatus	No. of Exps.	Extreme results by H_2O_2 method	A Mean K by H_2O_2	B Mean K by formula $V/p+a$	A—B °/₀
I	23	13	3·55—3·68	3·62	3·53	2·6
II	19	13	3·78—3·89	3·83	3·72	2·9
III	24	7	3·50—3·61	3·56	3·48	2·2
						Mean 2·5

The chemical method of calibration therefore gives to K quite constantly a somewhat higher value, for the different apparatuses which were tried, than does the physical method. The mean difference is 2·5 °/₀.

Let us now look at Peters' results in the light of this newly acquired information. Had the constants of his apparatus (and the apparatuses alluded to in the above table were among those used by Peters) been obtained by the chemical method, his figures for the specific oxygen capacity would all have come out 2·5 °/₀ higher or thereabouts, and would therefore have been as follows:

	Specific oxygen capacity as given by Peters			Specific oxygen capacity recalculated by chemical method of calibrating apparatus		
Ox	394	401	399	404	411	409
Cat	395			405		
Sheep	387	384		397	394	
Pig	388			398		
Dog	384			394		
Average	391			401		
Theoretical figure				401		

As a third method of calibrating the apparatus has been worked out by Hofmann which gives results identical with that of Burn (see *Journal of Physiology*, vol. XLVII) it seems probable that Peters' values as corrected by Burn are more trustworthy than his uncorrected ones.

The figures arrived at by the chemical method of calibration give an average which is so near to 400·8 that there can be no doubt that the oxygen and iron are united in haemoglobin in the ratio of two atoms of the former to one of the latter.

The propositions, therefore, (*a*) that the bloods of different animals have fundamentally different oxygen capacities, and (*b*) that analyses furnish any serious reason to doubt the validity of the oxygen to iron being related as two atoms to one, have ceased to trouble my mind.

The above experiments were all made upon the blood of animals in good health, or at all events not known to be in bad health.

Now that the physiological problem may be regarded as settled the pathological ones remain, and become the more ripe for settlement. These offer quite distinct subjects from that which we have discussed, inasmuch as there may be intermediate bodies present which are being worked up into haemoglobin in the blood, or degenerate substances such as methaemoglobin. One aspect of these problems resolves itself into an investigation of whether the oxygen capacity and the colour go hand in hand in the case of anaemic patients—a most important consideration for the only real use of the haemoglobinometer is as an index of the oxygen carrying power of the blood. According to Morawitz[13] they do.

I will not enter upon a discussion of what really can only be settled by accurate analysis.

In the meantime it is clear that in doubtful cases the haemoglobinometer may be put on one side and the oxygen capacity of the blood measured directly. The following is a case in which this has been done. The oxygen capacity of the blood was observed systemically throughout the case by actual oxygen determinations, each of which was carried out upon one-tenth of a cubic centimetre—about two drops—of blood, with results which inspire complete confidence. This direct procedure obviated all assumptions as regards specific oxygen capacity of pathological blood. The case was one of so-called biliary fever in the horse investigated by Nuttall and Strickland[14] in the Quick Laboratory at Cambridge. The object was amongst other things to determine the oxygen capacity of the blood whilst it was suffering from the ravages of the blood parasite *Nuttallia equi*. This parasite makes its home in the red blood corpuscles. The stages of its development are shown in Fig. 2, the description of which I quote from Nuttall and Strickland's paper.

"*N. equi* multiplies slowly and in the following manner:

(1) The minute piriform or oat-shaped parasite enters a fresh corpuscle and (Fig. 2; 2, 3, 4, 5) grows in size, being slightly amoeboid, with a general tendency to resume a pear shape. Definitely amoeboid movements (6) are, however, only to be seen distinctly when the parasite has attained a certain size. Judging from the form of the chromatin masses, stages 7, 8, 9, 10 follow next. The rest of the cycle has been continuously observed in the living parasite: the formation and breaking-up of the cross-form, the scattering of the daughter cells within the corpuscle, and their escape from the corpuscle...."

The liberation of the parasites is associated with a great breaking down of the corpuscles. Clearly therefore the oxygen capacity of the blood cannot be judged from corpuscle counts, firstly, because of the large amount of haemoglobin in the plasma and, secondly, because there is no assumption that the fresh corpuscles formed

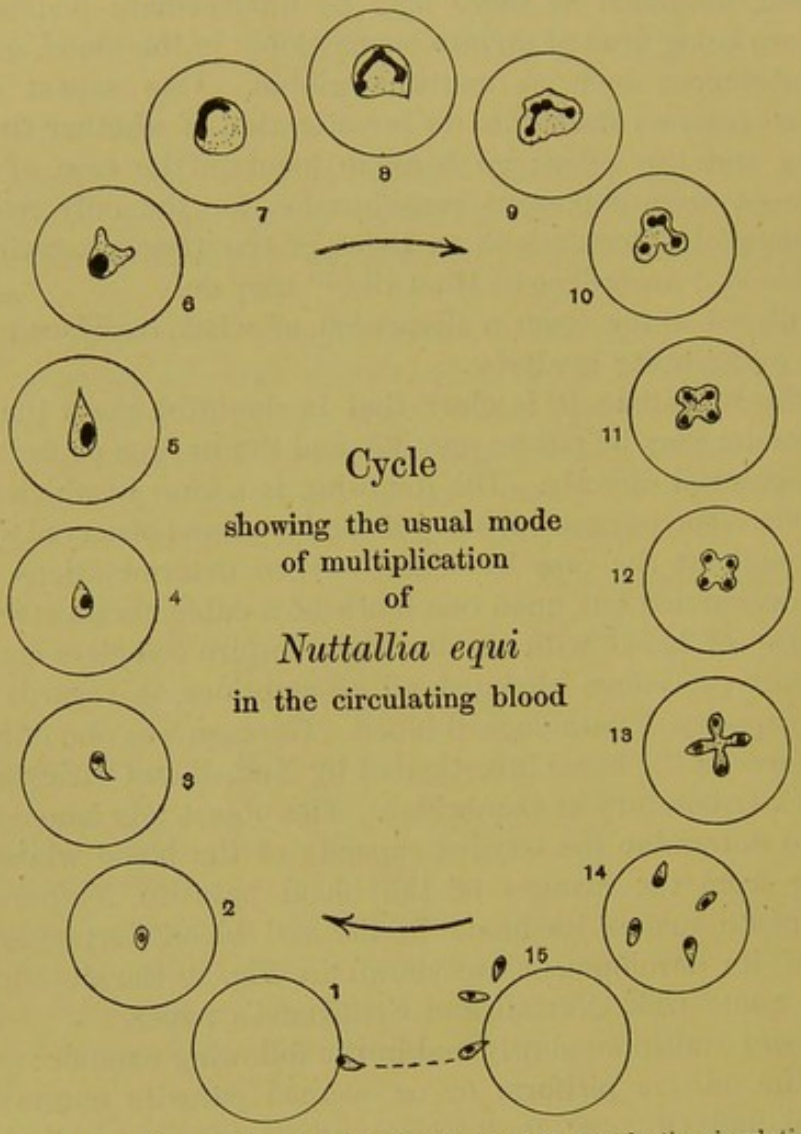


FIG. 2.—Illustrating the usual cycle of development of *N. equi* in the circulating blood.

under the stress of such a disease contain the normal quantity of haemoglobin. The oxygen capacity must be tested, if at all, either by the direct test of oxygen measurement, or by the haemoglobinometer, the use of which depends upon the assumption of the very point which we have declined above to discuss without further data.

I append the history of the case and its chart.

Nuttallia equi Horse III.

The Horse was inoculated subcutaneously with 20 c.c. of defibrinated blood taken from the jugular vein of Horse II at mid-day on 2. VIII. 1910. Horse III had previously been infected with *Piroplasma caballi* on 22. VI. 1910, but had recovered (see Protocol of *P. caballi* Horse II). *N. equi* appeared in the horse's blood on the 10th day, and it died at 5.30 p.m. on the 20th day after inoculation (21. VIII. 1910).

Day	Temp. °F. M.	Temp. °F. E.	% r.b.c. infected	(S)	(L)	(D)	(2–4)	F		R.b.c. per c.mm.	Oxygen capacity of haemoglobin	Leucocytes per c.mm.*
1	97·8	—	Inoculated.									
2	98·2	—										
3	98·2	—										
4	97·2	—										
5	98·2	—										
6	99·0	—										
7	98·2	—							.....	8,888,000	0·227	8,640
8	98·2	—							.....	8,872,000	0·230	12,900
9	97·4	—							.....	8,968,000	0·222	7,120
10	100·4	—	Parasites appeared						.....	9,155,000	0·240	7,800
10		102·0	0·2	34	65	1	·	·				
11	103·0		3·0	23	75	1	·	0·4		9,004,000	—	10,650
		104·2	2·0	43	50	4	2	·	Horse weak.			
12	104·2		—	—	—	—	—	—	Horse weak, jaundice; off	7,240,000	0·230	9,540
		106·6	11·6	23	70	3	4	1	his food.			
13	105·0		10·3	47	45	0·6	2	5	Very weak; haemoglobin-	5,390,000	—	—
		104·6	6·8	50	43	2	3	2	uria; blood appears watery, coagulation retarded.			
14	103·0		8·4	56	38	0·2	4	1	Rather better; feeding better,	5,365,000	0·141	12,000
		101·7	10·6	62	33	2	2	1	haemoglobinuria.			
15	100·6		5·2	31	65	1	3	0·4	Ditto; urine smoky.	4,812,000	0·104	7,520
		102·4	7·4	28	66	0·6	5	0·6				
16	102·4		9·2	15	80	0·6	3	0·2	Very weak, especially hind	3,840,000	0·115	9,700
		100·8	6·8	35	57	1	4	1	legs, which are stretched apart.			
17	103·0		8·0	19	75	1	3	0·2	Seems better, feeding; swell-	3,848,000	0·104	13,500
		103·0	12·8	28	67	2	3	·	ing on jaw (abscess).			
18	103·0		8·6	25	69	1	4	0·3	Weak; jaundice persists.	3,230,000	0·100	22,600
		102·2	13·2	20	73	2	5	·				
19	103·2		6·6	23	74	1	2	·	Very weak, lying down.	3,120,000	0·066	33,600
		101·0	4·8	20	75	1	4	·				
20	98·4		8·4	29	53	2	11	6	Helpless, urine smoky; found dead at 5.30 p.m.			

Mean of two readings, being the O_2 capacity of 1 c.c. of blood in c.c.

* Average number of counts = 5.

The signs denote corpuscles containing

(S) = small and medium sized piriform or oval parasites.
(L) = large rounded parasites.
(D) = dividing and cross-forms.
(2–4) = two or more parasites.
F = free forms.

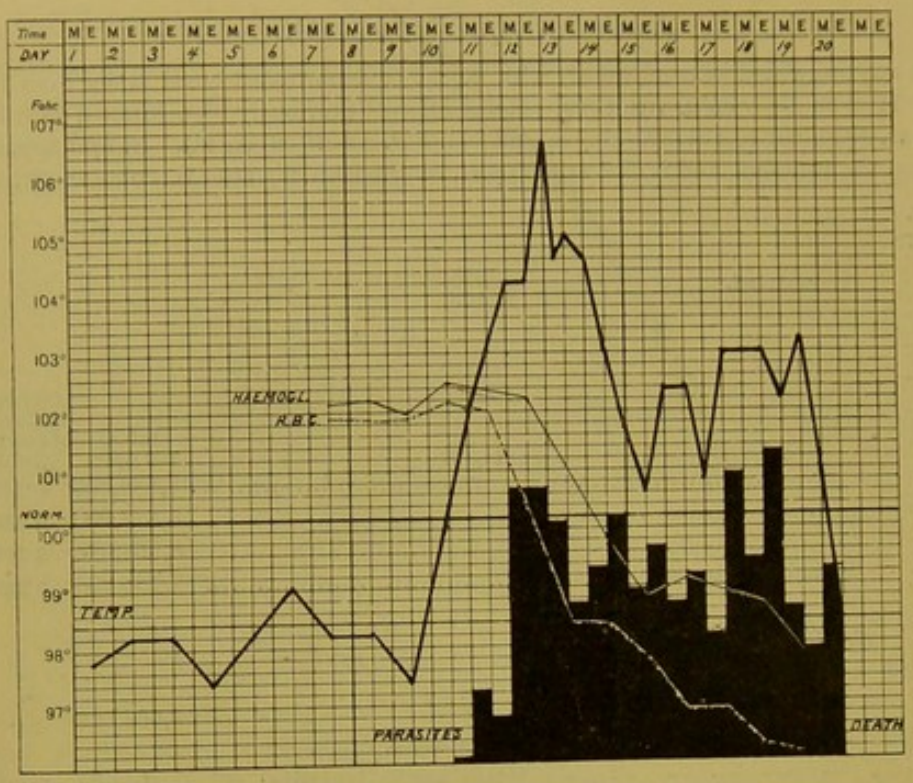


FIG. 3.—Chart of *Nuttallia equi* Horse III.

REFERENCES

(1) Chauveau and Kaufmann, *Comptes Rendus de l'Acad. Franç.* CIII, 1063.
(2) Müller, *Oppenheimer's Handbuch der Biochemie*, p. 672, 1908.
(3) For discussion of literature see Peters, *Journal of Physiol.* XLIV, p. 134, 1912.
(4) *Archiv für Anat. u. Physiol.* 1907, p. 470.
(5) *Deut. Arch. für Klin. Med.* XCIX, p. 130, 1910. Masig, *Ibid.* XCVIII, p. 123, 1909.
(6) *Zeitsch. f. physiol. Chem.* XLII, p. 143, 1909.
(7) Bohr, *Skand. Arch.* III, p. 76.
(8) Peters, *Journal of Physiol.* XLIV, p. 131, 1912.
(9) Haldane, *Ibid.* XXII, p. 298, 1898; XXV, p. 295, 1900.
(10) Müller, *Pflüger's Archiv*, CIII, p. 541, 1904.
(11) Barcroft and Burn, *Journal of Physiol.* XLV, p. 493, 1913.
(12) Barcroft and Roberts, *Ibid.* XXXIX, p. 429, 1910.
(13) Morawitz and Itami, *Deutsch. Arch. f. Klin. Med.* C, p. 191.
(14) Nuttall and Strickland, *Journal of Parasitology*, V, p. 65, 1912.

CHAPTER II

THE DISSOCIATION CURVE OF HAEMOGLOBIN

If a haemoglobin solution be shaken up with oxygen, the haemoglobin unites with a definite quantity of the gas, which is in the proportion of 32 grams of oxygen to each 56 grams of iron in the haemoglobin. However much stress we may lay upon this fact—and we cannot lay too much stress upon it—the most elementary consideration of the haemoglobin in the circulation reveals the fact that it is always united with less oxygen than the total amount possible, frequently with much less and in any case with no precise or invariable amount.

The next step therefore, if we are to regard oxyhaemoglobin as a chemical compound, is to inquire whether we can reconcile the fact that haemoglobin in the body unites now with more, now with less oxygen, with the known laws of chemical action.

The most obvious law which might illuminate this problem is the law of mass action. Our inquiry therefore resolves itself into this: granted a solution which contains (1) oxygen, (2) oxyhaemoglobin, and (3) reduced haemoglobin, does the amount of oxyhaemoglobin depend upon the concentration of oxygen in the solution?

The answer to this question can only be supplied by experiment. The experiment is not a difficult one. It is easy to obtain solutions of haemoglobin containing known concentrations of oxygen in solution, for the concentration of oxygen depends directly upon the oxygen pressure of the atmosphere with which the solution is in equilibrium. If α be the volume of oxygen which is dissolved in 1 c.c. of the solution at the temperature of the experiment and at 760 mm. pressure, then the concentration of oxygen at any other pressure p is $p\frac{\alpha}{760}$. The experiment then will consist of exposing portions of a haemoglobin solution to various atmospheres containing known

pressures of oxygen and subsequently determining the amounts of oxy- and reduced haemoglobin in each sample after an equilibrium has been established between the haemoglobin and the atmosphere.

Suppose we have five closed vessels each containing a small quantity of haemoglobin solution and also at the same time oxygen at the following pressures, namely 0, 10, 20, 40 and 100 mm. of

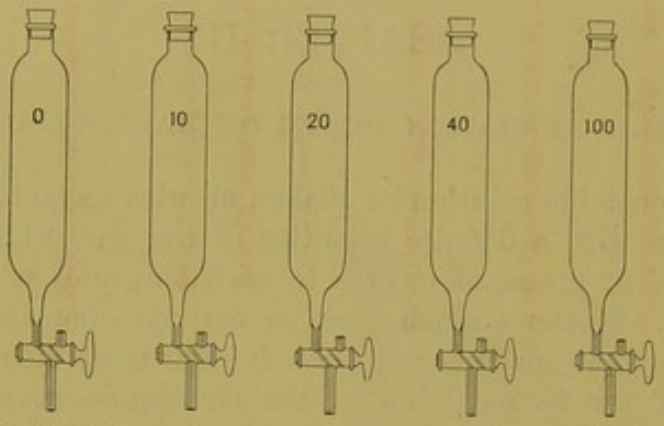


Fig. 4.—The numbers denote the pressure of oxygen in mm.

mercury. After the fluids had been shaken up thoroughly at 38° C. the concentrations of oxygen would be

(1)	(2)	(3)	(4)	(5)
$0 \times \frac{\alpha}{760}$	$10 \frac{\alpha}{760}$	$20 \frac{\alpha}{760}$	$40 \frac{\alpha}{760}$	$100 \frac{\alpha}{760}$ c.c.

That is:

0	·00029	·00058	·00116	·0029 c.c.

of oxygen in each c.c. of fluid.

Now we must find out what proportion of the haemoglobin is oxyhaemoglobin, and the following are figures such as we would obtain:

Vessel	(1)	(2)	(3)	(4)	(5)
	0 %	55 %	72 %	84 %	92 %

The best idea we can get of the relation of these numbers to one another is to place the following picture before our eyes. Suppose the haemoglobin in each case to be in a cylindrical tube and that the oxy- and reduced haemoglobin could be separated from one another, the former being red and sinking to the bottom and the latter purple and rising to the top, we should obtain five cylinders as shown in Fig. 5 corresponding to the oxygen pressures in the five tonometers.

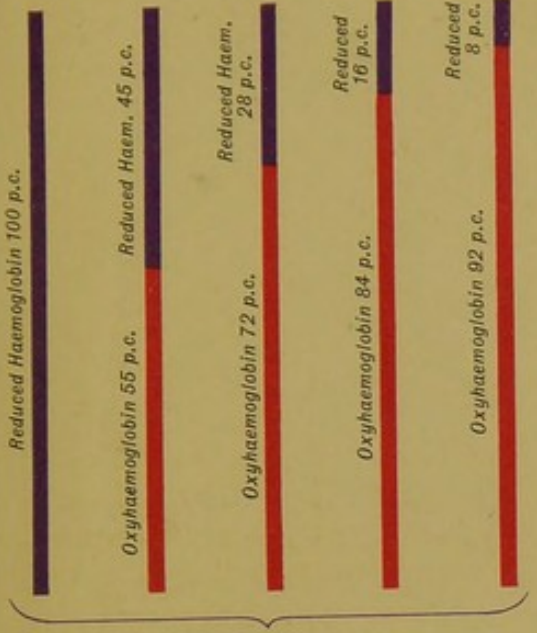


FIG. 5.

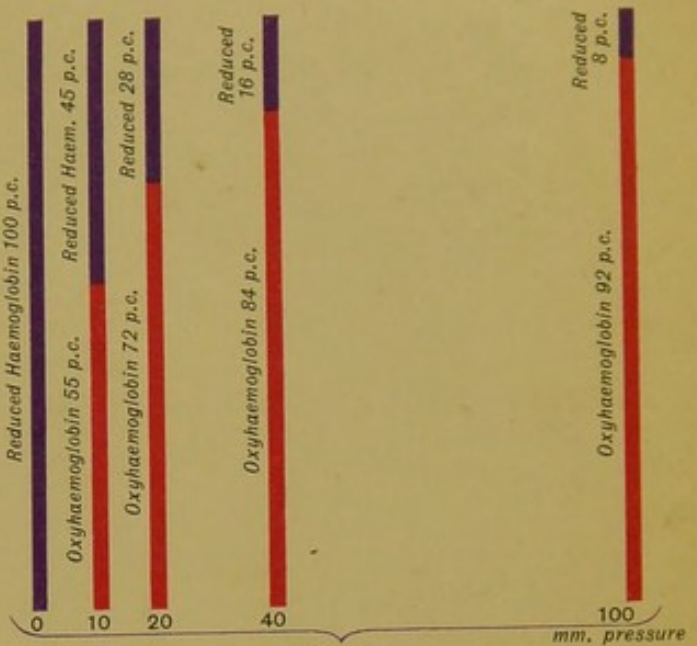


FIG. 6.

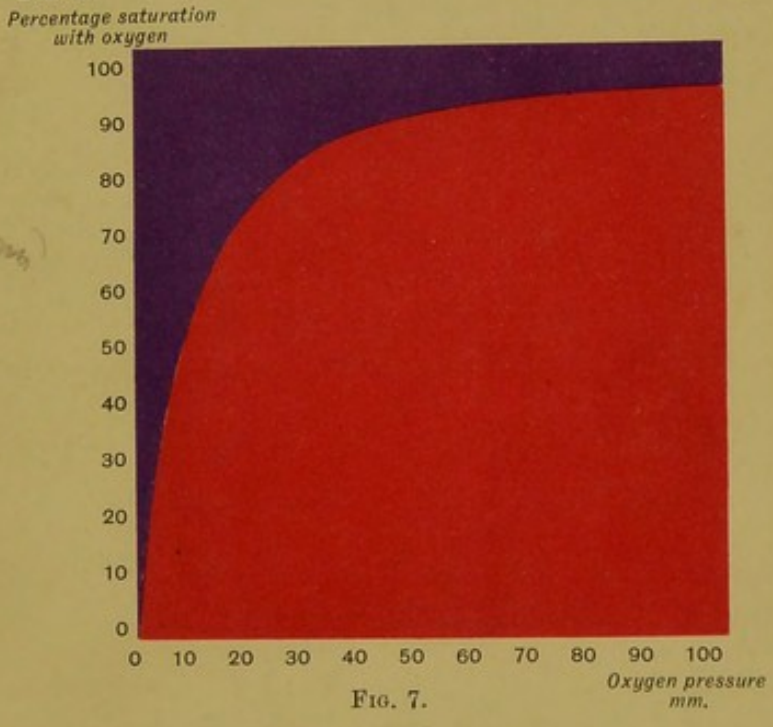


FIG. 7.

FIG. 5.—Percentage saturation of haemoglobin with oxygen at 37° C. corresponding to oxygen pressures of 0, 10, 20, 40 and 100 mm. of oxygen respectively.

FIG. 6.—The same spaced with the oxygen pressure as the abscissa.

FIG. 7.—Dissociation curve representing the equilibrium between oxygen, oxyhaemoglobin (red) and reduced haemoglobin (purple).

We are impelled to ask whether any definite relationship exists between these quantities of oxyhaemoglobin and the oxygen pressures to which they correspond. In doing so we must bear in mind that we shall shortly be face to face with a very serious position, for the very relationship which we are about to investigate is defined by the law of mass action itself, and should the relation as found not agree with that as prescribed, our theory must be abandoned and some other explanation of the properties of haemoglobin must be found. Let us introduce the element of pressure quantitatively by spacing the cylinders apart from one another at distances which are proportional to the concentrations (or pressures) of oxygen dissolved in the solution, as is done in Fig. 6.

By joining the points which divide the blue and red portions of the cylinders we obtain a curve which relates (1) the percentage of the total haemoglobin which is oxyhaemoglobin to (2) the concentration of oxygen dissolved in the fluid at all concentrations of oxygen between 0 and ·0029 c.c., and consequently to all pressures of oxygen between 0 and 100 mm. This curve representing the equilibrium between oxygen and haemoglobin is called the dissociation curve of oxyhaemoglobin. It is shown in Fig. 7.

Let us now turn from the observed properties of haemoglobin to the other side of the question, namely the requirements of the law of mass action. This law has been stated quantitatively by Guldberg and Waage in the following terms:

"The velocity of chemical change is proportional to the product of the active masses of the reacting substances." The chemical change is conceived of as taking place in both directions simultaneously, that is to say, oxyhaemoglobin is all the time being formed and being broken up, and we therefore have these two changes taking place at the same time, (1) the formation, (2) the breakdown of oxyhaemoglobin. Since these changes balance one another, the whole system being in equilibrium, the velocities of the two changes are equal. Taking first the formation of oxyhaemoglobin, the reacting substances are reduced haemoglobin and oxygen and the velocity of their reaction is proportional, says the law, to the *product* of their concentrations in the solution. If C_O be the concentration of oxygen and C_R of reduced haemoglobin, then the velocity of the reaction is proportional to, or is equal to, a constant k multiplied by the product of C_R and C_O, i.e. $k(C_R \times C_O)$. As regards the other phase of the reaction—the breakdown of oxyhaemoglobin—there

is but one active substance, namely oxyhaemoglobin; let its concentration be C_H, then the velocity of the reaction is k', another constant, multiplied by C_H. Taking the two together, we have

$$k(C_R \times C_O) = k'C_H.$$

The concentration of oxygen as we have already stated is $p \times \frac{\alpha}{760}$, where p is the oxygen pressure to which the solution is subjected and α the coefficient of solubility, therefore

$$\frac{k}{k'} \times \frac{\alpha}{760} \times p \times C_R = C_H,$$

replacing all the constants by one constant K,

$$KpC_R = C_H.$$

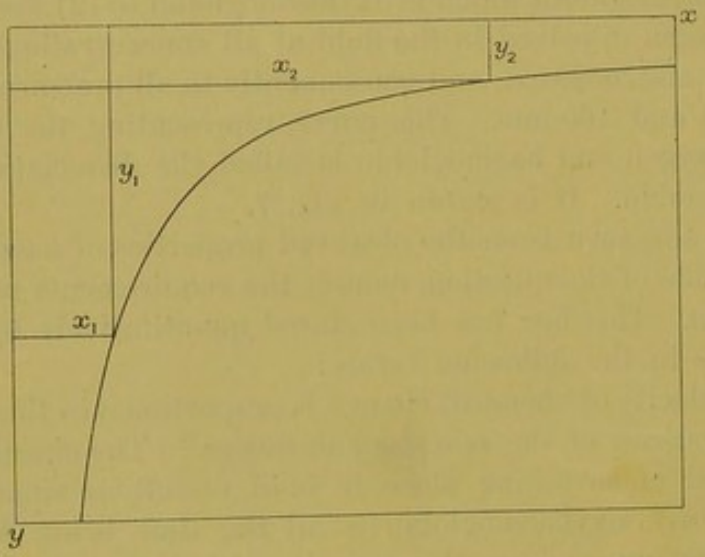


Fig. 8.—Rectangular hyperbola. The products of x and y, such as x_1y_1, x_2y_2 for all points are equal.

If the concentrations of oxy- and reduced haemoglobin are regarded as percentages of the total concentration of haemoglobin, then $C_H = 100 - C_R$,

$$KpC_R = 100 - C_R,$$

$$pC_R + \frac{C_R}{K} = \frac{100}{K},$$

$$\left(p + \frac{1}{K}\right) C_R = \frac{100}{K}.$$

If we write y for C_R and x for $\left(p + \frac{1}{K}\right)$, we obtain the relationship $xy =$ a constant, namely $\frac{100}{K}$. If we now construct a curve plotting y vertically and x horizontally, and observing the condition that for any point the product of x and y must be constant, we obtain a curve known as a rectangular hyperbola (Fig. 8).

This curve does not at first sight relate the concentration of reduced haemoglobin (C_R) to the pressure p, but to something else, namely $p + \frac{1}{K}$. It remains for us to find out what is the relation of C_R to p.

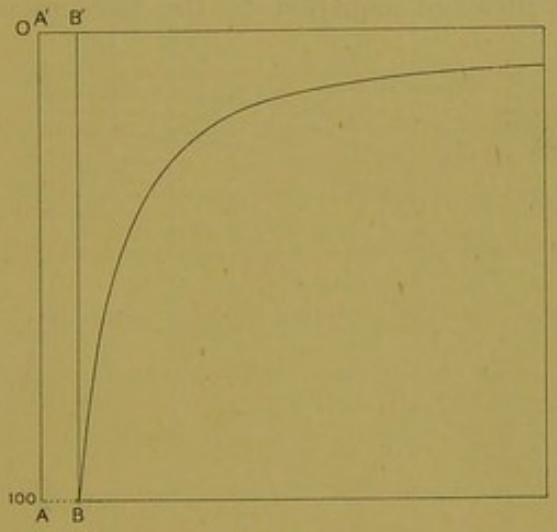


Fig. 9.

Consider the special case in which $C_R = 100$, i.e. all the haemoglobin is reduced haemoglobin,

$$\left(p + \frac{1}{K}\right) C_R = \frac{100}{K},$$

$$\left(p + \frac{1}{K}\right) 100 = \frac{100}{K},$$

$$\therefore \quad p = 0.$$

The distance AB (Fig. 9) in that case $= \frac{1}{K}$. Hence the distance from BB' of any point on the hyperbola is a measure of the pressure.

We have therefore derived the following information from the law of mass action. If the reaction is a chemical one involving single

molecules only, the relation of (1) the oxygen pressure to (2) the percentage of oxy- to total haemoglobin is capable of being represented as a rectangular hyperbola, the origin of which is at once the point at which there is no pressure of oxygen and no oxyhaemoglobin, and the curve approximates to a line representing complete saturation. If now we turn back to Fig. 7 we shall see that the curve which we have shown as representing the relation of the pressure of oxygen and the percentage of oxyhaemoglobin is identical with the curve we have just given, and therefore the law of mass action is to this extent satisfied.

Yet this satisfactory result was not reached without a struggle. The ease with which it can be demonstrated at the present time presents a very pleasant contrast to the tiresomeness of the path

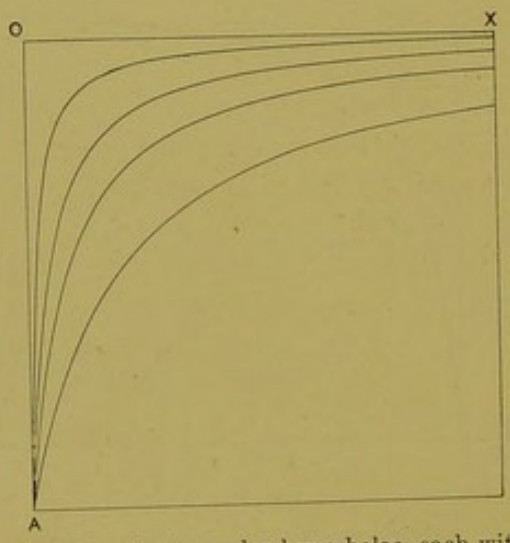


Fig. 10.—Shows a series of rectangular hyperbolae, each with *A* as its origin, and approximating to *OX*.

which had to be trod before the present point of vantage was reached.

The history of the subject forms an interesting commentary upon the psychology of research. The law of mass action was first quantitatively applied to the reaction

$$Hb + O_2 \rightleftarrows HbO_2$$

by Hüfner[1], who quite unjustifiably assumed the applicability of the law to the reaction in the form in which we have given it above and obtained a curve very similar to the one which is represented from entirely theoretical considerations.

You can have any number of rectangular hyperbolae all of which pass through the point A and approximate to OX, such as are shown in Fig. 10: these all satisfy the condition stated above

$$\left(p + \frac{1}{K}\right) C_R = \frac{100}{K}.$$

The difference between them lies in the value of K. Now Hüfner assumed the correctness of the equation and set out to find the value of K. This can be done from one point. He used a number of samples of haemoglobin prepared in different ways, determined a point for each, found the value of K, averaged these values and produced a curve. But a nemesis awaited Hüfner. His speculations

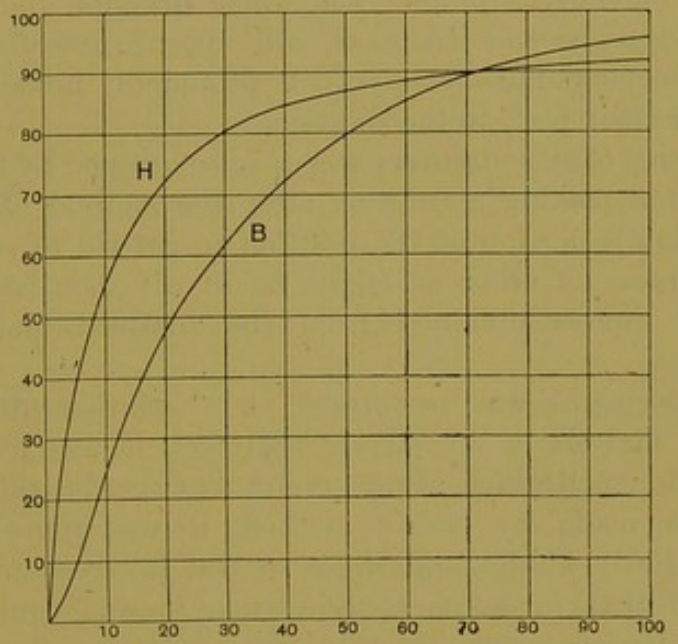


Fig. 11.—Dissociation curves of haemoglobin. H according to Hüfner. B according to Bohr.

fell in the hands of a physiologist of a diametrically opposite school. Bohr[2] had inherited a tradition from the great laboratory of Ludwig which, though it may carry its holders to excessive lengths, at least forms a useful corrective to unjustifiable generalisations. Bohr's motto was that every experiment had a value, nothing which was obtained as the result of a test in the laboratory was set aside on the ground of its inherent unlikelihood, of its failure to fit general principles. Bohr therefore determined to map out the curve relating the pressure of oxygen to the relative quantities of oxy- and reduced

haemoglobin point by point, irrespective of laws, and to find out experimentally what it actually was like.

Fig. 11 shows that the actual curve determined point by point differs fundamentally from Hüfner's hyperbola. The two cross, therefore Bohr's cannot be a hyperbola, and further it has a double contour and therefore is not a curve of the same order at all.

At the present time it is scarcely necessary to dwell on the theory which Bohr propounded to explain his curve. In two words it was that when oxygen broke from oxyhaemoglobin, the haemoglobin itself split into globin, and something rather like haematin which he called haemochrome. Another explanation of this curve will be alluded to in another place. The whole question took on a new aspect when it became clear, as will appear hereafter, that the affinity of haemoglobin for oxygen is profoundly influenced by the nature of electrolytes [3] in the solution.

The bearing of this discovery was at once grasped by Ff. Roberts [4] who suggested making a solution after the method of Bohr* and then dialysing a portion of the solution to get rid of such residual salts and traces of ether as might be in it; then comparing the dissociation curves obtained from the dialysed and undialysed portions.

The experiment was performed on a solution obtained after three days' dialysis in an aseptic dialyser; it was free from the characteristic smell which always clung to our preparation of dog's haemoglobin made by Bohr's method, it was quite neutral in reaction; Hardy kindly undertook to test its freedom from salts, and showed that in saline concentration it was equivalent to a ·004 N solution of sodium chloride. The points on the dissociation curves of the two solutions were then determined, and are shown in Fig. 12. The round points are those of the dialysed solution, the square ones are those of the undialysed solution. It is at once apparent that the latter are in very close agreement with the curve published by Bohr (denoted by II in the diagram), whilst the former fall so nearly on the rectangular hyperbola (denoted by I) as to make it in the highest degree probable that could the salts be entirely eliminated the coincidence would be complete. Whilst in our opinion the theory advanced by Bohr to explain his own curve may be set aside,

* I.e. precipitating the haemoglobin crystals with ether from centrifugalised corpuscles, washing them, redissolving them and shaking out the ether.

another theory also advanced to explain the same curve demands consideration. It is the theory that oxygen is united to the haemoglobin, not chemically in the ordinary sense of the word but by the physical process known as adsorption, that is to say that small masses of haemoglobin, each composed of a number of molecules, exist in or

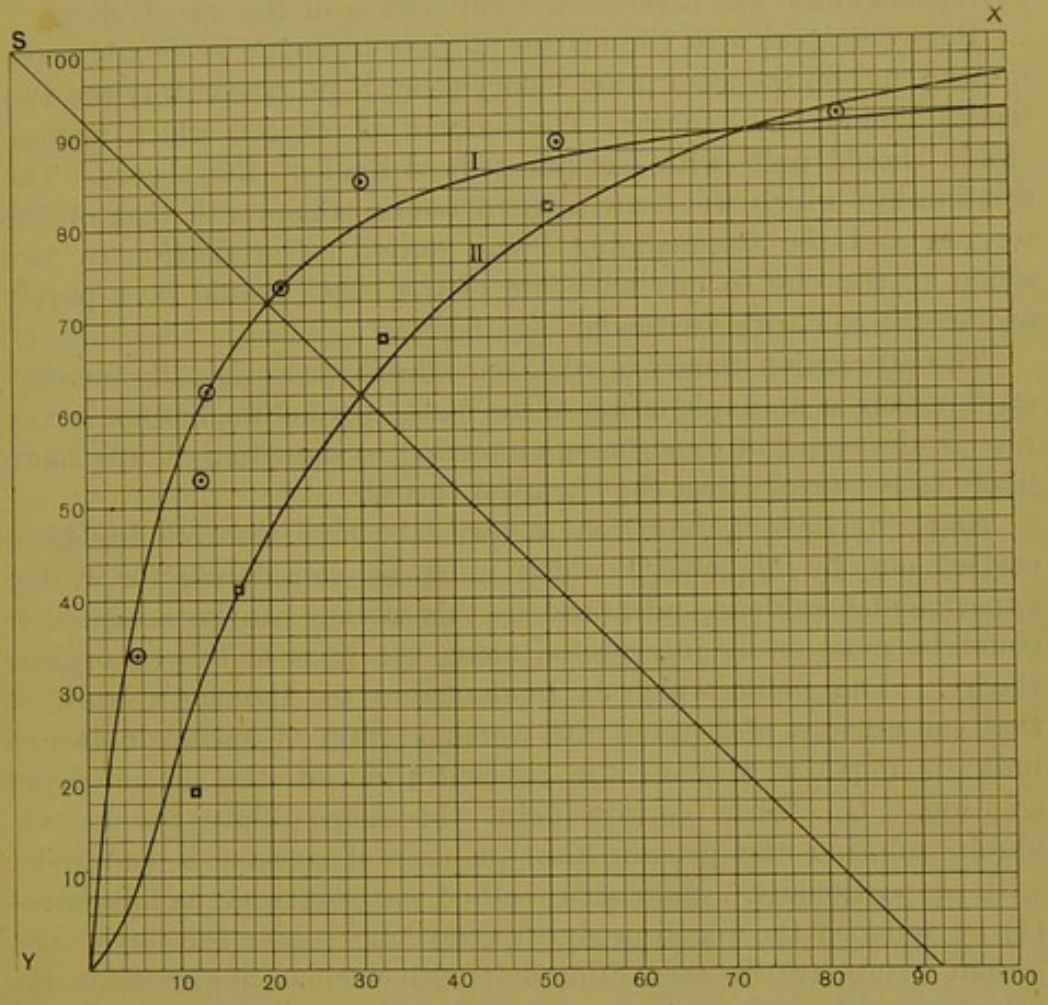


Fig. 12.—Ordinates = percentage saturation of haemoglobin with oxygen. Abscissae = tension of oxygen in mm. of mercury. Curve I = rectangular hyperbola. $XY = 800$. Curve II = Bohr's dissociation curve of haemoglobin.

⊙ Points determined from dialysed solution.

□ Points determined from undialysed solution.

out of the solution and that the surface charges on them attract molecules of oxygen which adhere to the surface of the haemoglobin. The phenomena of surface effects are destined to play so great a part in the future of physiology that this theory, which is due to Wolfgang Ostwald[5], is well worth the consideration of those interested in the

subject. Moreover it presents another very attractive feature. The difference between oxyhaemoglobin and the body believed to be isomeric with it, methaemoglobin, has always been somewhat of a mystery. It would therefore be a relief to regard methaemoglobin[6] as a chemical compound of oxygen and haemoglobin, whilst oxyhaemoglobin was a physical combination of the two bodies.

Before dealing with the matter at greater length we should make it clear that on the adsorption theory there is no reason why the two bodies should be isomeric. The amount of oxygen which haemoglobin should unite with has not a fixed limit on this theory. The relationship which we have pointed out in Chapter I, that of the oxygen and the haemoglobin being united in proportion to two atoms of the one to one molecule of the other, would be a purely accidental one with particular significance.

The adsorption theory of oxyhaemoglobin was originally put forward merely as an explanation of the facts then known, but not in any way as the result of experiments intended to show that it was more likely to be correct than the chemical theory.

Since its advent, however, a very laborious research has been carried out by Manchot[7] which must be considered, for if the experimental evidence advanced by this worker can be accepted it could be most easily interpreted along the lines of this adsorption theory. The point which is fundamental to Manchot's work is that the amount of oxygen with which 1 gram of haemoglobin can unite is dependent upon the concentration of the haemoglobin solution. Up to a certain point the more dilute the haemoglobin solution the greater the quantity of oxygen it can unite with proportionally. Manchot's method was somewhat as follows:— (1) he reduced the blood used, till it no longer gave the oxyhaemoglobin bands, (2) he then diluted it with various fluids, shook the solutions with oxygen and measured the amount absorbed. The experimental procedure failed to satisfy us on two counts: (1) we do not regard the spectroscope as a satisfactory index of the initial reduction, (2) no attempt was made to test whether the haemoglobin at the end would yield, to a vacuum or to ferricyanide, the amounts of oxygen alleged to have been taken up by adsorption. Burn[8] has been at great pains to verify Manchot's results, having due regard to the conditions just stated. He reduced the blood with the pump till no more gas came off. He then determined the amount of gas which 1 c.c. of blood would absorb when shaken with air in the differential

apparatus, and the amount which it would afterwards give out when treated with potassium ferricyanide. When due allowance had been made for the fact that "pumped-out blood" absorbs the carbonic acid of the air as well as the oxygen, and for other trifling physical factors, Burn obtained the following figures :—

Degree of Dilution of Blood	Oxygen capacity by absorption method	Oxygen capacity by ferricyanide method
Undiluted	17·6 %	17·4 %
5 times	17·6 %	17·5 %
7 times	17·8 %	17·1 %

Each figure represents the average of four determinations: clearly dilution has no effect on the oxygen capacity.

In passing it is interesting to note that Burn was able to simulate some of Manchot's results, when he used only the absorption method.

A caution must here be given against the attractiveness of analogies between certain inorganic oxidations and that of haemoglobin. There is perhaps no greater danger at the present time, to one who would research in this particular subject, than the temptation of trusting to analogies instead of to analysis.

Before leaving the point of analogies we must warn the reader against one which is frequently put forward, namely, that of the union between calcium oxide and carbonic acid. In this case if the pressure and the amount of carbonic acid united to the calcium oxide be plotted after the manner described above we get quite a different type of curve. At a certain critical pressure the carbonic acid begins to come off and at that pressure it all comes off. The essential difference between the two cases lies in the fact that, in the case of the haemoglobin reaction the reacting substances are all in solution, while in the case of the calcium carbonate the reaction is between a solid and a gas and therefore involves a change of phase. The number of phases being one greater, the number of degrees of freedom is one less.

REFERENCES

(1) Hüfner, *Arch. f. Anat. und Physiol.* v, p. 187, 1901.
(2) Bohr, *Zentrlb. f. Physiol.* XVII, p. 682.
(3) Barcroft and Camis, *Journal of Physiol.* XXXIX, p. 118, 1909.
(4) Barcroft and Roberts, *Ibid.* p. 149.
(5) Wolfgang Ostwald, *Biochem. Ctrlb.* p. 386, 1908.
(6) Hasselbalch, *Biochem. Zeitschr.* XIX, p. 435, 1909.
(7) Manchot, *Liebig's Annal. d. Chem.* p. 369, 1909; *Biochem. Ztschr.* XLIII, p. 438, 1912.
(8) Burn, *Journal of Physiol.* XLV, p. 482, 1913.

CHAPTER III

THE EFFECT OF TEMPERATURE ON THE AFFINITY OF HAEMOGLOBIN FOR OXYGEN

In perusing the last chapter one point will not have escaped the reader. I may state it as follows. Let it be granted that the dissociation curve of dialysed haemoglobin takes the form of a rectangular hyperbola, passing through the point A and approximating

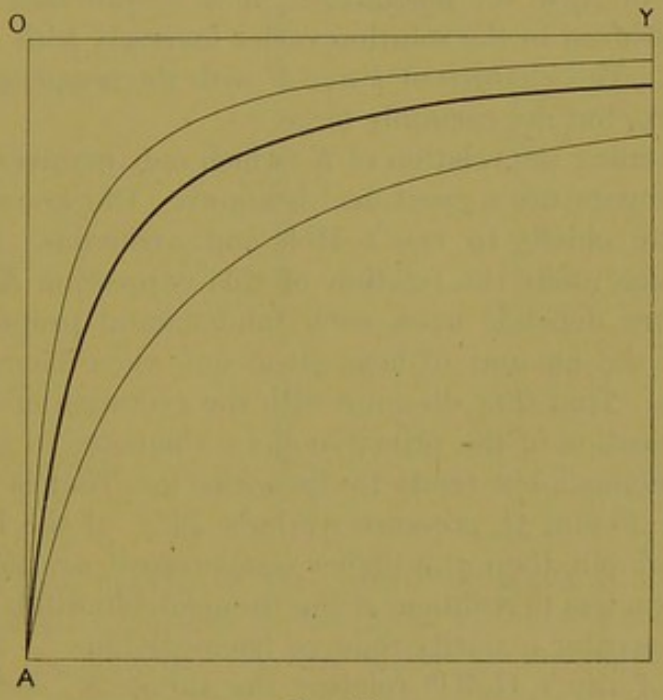


Fig. 13.

to the line by OY. It is possible to draw any number of such curves. Why should the curve of haemoglobin be this particular one, which is represented by the thick line in Fig. 13, rather than any other, such as those represented by the thin lines?

Let us go back upon the argument which we have already used.

The equation which gave us this hyperbola was

$$\left(p+\frac{1}{K}\right)C_R=\frac{100}{K}.$$

For any value of p there can be only one value of C_R so long as K, which we have already described as a constant, remains invariable.

To go back one stage further K itself involved three other expressions which were also treated as constant—α, the amount of oxygen which is dissolved in 1 c.c. of water at 760 mm., and k and k', the velocity constants of the forward and backward phases of the reaction

$$Hb+O_2 \rightleftarrows HbO_2.$$

Now in treating all these expressions as constants, as we have already done, we have made the assumption that the temperature is constant, for in reality they all vary with the temperature. Take the simplest of them for instance, α; it is known that the amount of oxygen dissolved in the solution varies inversely with the absolute temperature. The changes of k and k' with the temperature are not exactly known, but are certainly large.

But concerning the relation of K (which only involves the ratio of k to k') to temperature a great deal is known. Our knowledge of the subject is due chiefly to van 't Hoff and Arrhenius. So far from being a chance affair the relation of this expression K to changes of temperature depends upon such fundamental properties of the substance as the amount of heat given out when haemoglobin and oxygen unite. That they do unite with the evolution of heat is clear from an application of the principle of Le Chatelier to the fact that to heat oxyhaemoglobin tends to dissociate it. That is to say, if at 38° C. and at 20 mm. O_2 pressure we have 71 °/₀ of the haemoglobin as oxyhaemoglobin, then at a higher temperature, say 49° C., and the same pressure a less percentage of the haemoglobin will be oxyhaemoglobin and a greater quantity reduced haemoglobin.

The law of van 't Hoff[1] relating the values K_1 and K_2 for any temperatures T_1 and T_2 is

$$K_2=K_1e^{\frac{-q}{2}\cdot\frac{T_2-T_1}{T_2T_1}},$$

e being the base of the Napierian system of logarithms, and q the heat evolved when one gram-molecule of haemoglobin unites with one gram-molecule of oxygen.

As soon as we have grasped this a whole world of facts is before us.

Firstly then we must have a different hyperbola for every different temperature. The one we have obtained is not a general expression of the relationship of oxygen to haemoglobin, it is merely the relationship at the particular temperature at which we chanced to do our experiments, namely 38° C. Therefore the law of mass action has become doubly exacting. Not only must the dissociation curve be a certain shape, but it must be that shape at all temperatures, and moreover the curves at all temperatures must be related according

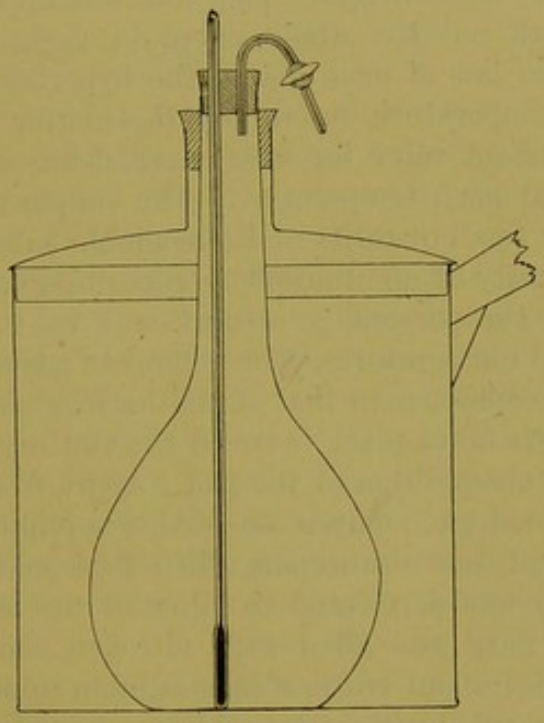


Fig. 14.

to a certain law. Have we the means by which to test the truth of the chemical conception in the light of these exacting requirements? Of the quantities in the above formula we have already a value of K_1 corresponding to the temperature T_1, viz. 38° C. One could determine the value of K_2 for some other temperature T_2, and then if only $-q$ were known one would have all the data for testing whether K_2 as calculated was the same as that which was obtained by experiment. But the truth is that we do not know q, or rather we did not when the problem first attracted the attention of Hill [2]; it was therefore impossible to attack it in just this way. But though it was impossible to make this test, it was possible to do something

less—namely this. Assuming the truth of the equation we have all the data for the determination of q. Now if our assumption is correct we may determine q not merely from the values of K at these two temperatures, but from the values of K at numerous other temperatures; and the test lies in the fact that the values of q so determined should all be identical, they being independent determinations of a definite physical constant—the heat of formation of one gram-molecule of oxyhaemoglobin. Apart from the law of mass action such an identity would be mere coincidence as unlikely as if half a dozen church spires of different design taken at random proved to be the same height. This test was applied; it was not necessary to work out the whole curve in each case. Assuming as a result of the law of mass action the hyperbolic nature of the curve at each temperature, or, to put the matter in another way, that K has a constant value for each curve, it was only necessary to take one point at each temperature. The simple method of doing this was to put a small quantity of haemoglobin solution into a flask with a large quantity of an atmosphere containing but little oxygen, and to determine the percentage saturation of the haemoglobin with oxygen at several temperatures. The volume of gas must of necessity be so large in comparison to that of the haemoglobin solution that any reaction which takes place between the two may be regarded as not altering the composition of the gas. Apart from the necessary apparatus for blood gas analysis no further equipment was needed for this experiment than a saucepan, a litre flask and a thermometer. The saucepan lid was perforated to allow of the neck of the flask protruding; the flask was filled with nitrogen containing a small quantity of oxygen; about 10 c.c. of haemoglobin solution were put in. The saucepan was filled with water which surrounded the flask, and the lid was fixed immovably on the saucepan. At any temperature it was a simple matter to establish an equilibrium between the haemoglobin solution with its small volume and large surface and the gas in the flask. When the equilibrium was once established the apparatus was inverted. The solution ran down into the neck, where it presented a very small surface to the atmosphere above it, and therefore did not alter sensibly in percentage saturation whilst a sample was being abstracted for analysis. Such an experiment carried out at five different temperatures gave values as follows for the percentage saturation:

Temperature	16°	24°	32°	38°	49°
Percentage saturation ...	92	71	37	18	6

Are these figures such as to give a constant value for q? The answer is as follows. If q were 28,700 the following would be the percentage saturation:

Temperature	16°	24°	32°	38°	49°
Percentage saturation ...	90	71	41	22	6

There is no greater divergence here than can be accounted for by the error of the method used. A similar experiment, at a much higher oxygen pressure, with a haemoglobin solution made in a different way but also dialysed, yielded the following results:

Temperature		16°	25°	32°	38°
Percentage saturation	Observed ...	96	89	77	52
	Calculated...	97	89	74	54

The value for q used in this calculation was 27,700. It seems clear then, that as far as our present experimental methods will carry us, q has a constant value of approximately 28,000 gram-calories over a range of temperature of 16° to 49°.

There is never the same feeling of exhilaration about testing known phenomena by known laws as there is about breaking absolutely fresh ground. Though the conception which we were testing had passed triumphantly through a successful ordeal, the research which we are describing took on a new and extremely interesting aspect at this point, for now that the *bona fide* nature of q seemed to be established it was clear that we had hit upon a new method of attacking the molecular weight, and this aroused our enthusiasm the more as the molecular weight determinations of haemoglobin were in a very chaotic condition. It is true that, shortly before his death, Hüfner[3], in collaboration with Gansser, had made a determination by a measurement of the osmotic pressure of haemoglobin. The haemoglobin was placed in an osmometer which was permeable to salts; the final osmotic pressure observed corresponded to a molecular weight of about 16,000. In the performance of this experiment Hüfner had assumed that the salts distributed themselves equally on each side of the membrane. This assumption seems to be unjustified, though the extent to which unequal distribution of the salts would vitiate such a result has not been accurately ascertained. Moreover others had attacked the same problem. Weymouth Reid[4] and Roaf[5] had made similar experiments. The former arrived at a value of 48,000, the latter at the conclusion that the value varied within very wide limits according to the nature of the solvent used.

The reader may say at this point that we have ourselves proved that the molecule of haemoglobin contains but one atom of iron and is therefore the simplest possible. That assumption underlies the hyperbolic form of the curve which is based upon the equation

$$Hb + O_2 \rightleftarrows HbO_2.$$

For inasmuch as there are two atoms of oxygen to one of iron, if the haemoglobin molecule contained two atoms of iron the equation of the reaction would not be that just given but

$$(Hb)_2 + 2O_2 = Hb_2O_4,$$

using Hb to denote the simplest possible formula for haemoglobin.

Let us therefore inquire more particularly where we stand in this matter. Let us see what we have assumed and what we have proved. We have shown in Fig. 12 that the points obtained experimentally from a dialysed haemoglobin solution are consistent with the view that the dissociation curve is a hyperbola and, not as Bohr supposed, inconsistent with that view; moreover it must be remembered that though the fluid in that experiment had a low electric conductivity it was not absolutely salt free. We have shown then that the facts are consistent with the equation

$$Hb + O_2 \rightleftarrows HbO_2.$$

But have we shown at that point that they are inconsistent with the equation

$$Hb_2 + 2O_2 = Hb_2O_4$$

or

$$Hb_3 + 3O_2 = Hb_3O_6?$$

As a matter of fact the points as determined would agree nearly as well with a curve which was drawn on the basis of the second of these equations though not on one which was based on the third. Let it be clear however that we are not giving up the position which we established in Chapter II, namely the probability of the oxygen being chemically united to the haemoglobin.

I am indebted to Hill for calculating the curve (Fig. 15, Curve II) which would be yielded by the equation

$$Hb_2 + 2O_2 = Hb_2O_4.$$

The points determined when compared with this curve appear to be not altogether out of conformity with it and suggest that the points are really between the two curves. And this is very likely to be the case. For on our supposition it is clear that the actual

hyperbola could only be reached if all the salts were got rid of, that is to say that the hyperbola is a theoretical limit which cannot actually be attained, and similarly the unimolecular is a theoretical limit of the aggregation of the molecules. In actual fact it is not unlikely that the great majority of the haemoglobin molecules are single molecules and that these are mixed with a certain number of others. Therefore there is the possibility of considering what curve would be given if the average number was $n = 1{\cdot}5$. This curve agrees with the determined points just about as well as the hyperbola and it is therefore not unreasonable to suppose that the actual solution,

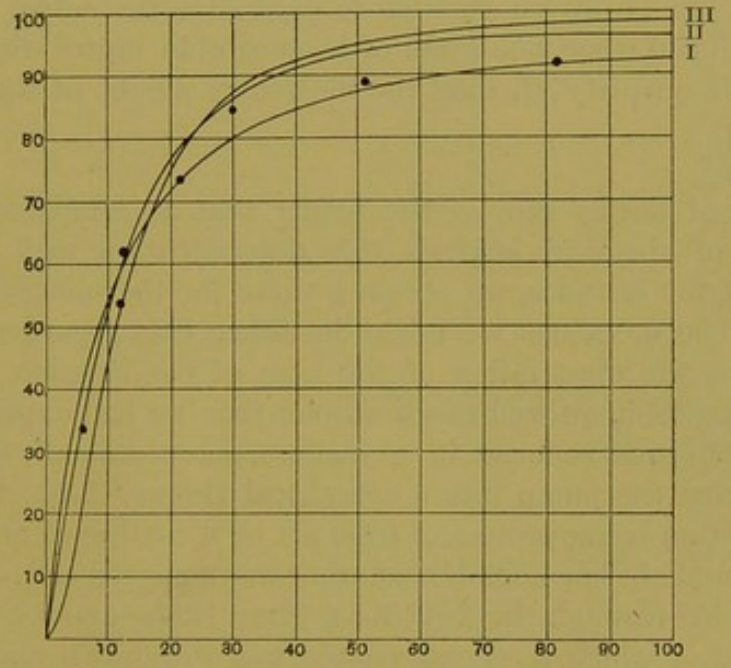


FIG. 15.—Curves I, II and III represent dissociation curves in which the mean number of molecules in each aggregate would be 1, 1·5 and 2 respectively. The points are those experimentally determined.

which was equivalent to a ·004 normal salt solution—and it is the first traces of salt which would exercise the most profound influence in causing the molecules to aggregate—contained some aggregated molecules.

Such an hypothesis would at once hold out a hope of bringing our own results into line with those of Roaf[5] whose general theme is that the osmotic pressure of the protein, and therefore its molecular weight, varies with the nature of the solvent in which the haemoglobin is dissolved. As we shall have to discuss this matter more particularly in the next chapter we shall leave it at this point.

In the present chapter we have established a value for the heat of molecular combination of oxyhaemoglobin: this involves the idea that K is constant at any temperature but not the assumption that haemoglobin is necessarily Hb_1 rather than Hb_2 or Hb_3, i.e. that its molecular weight is approximately 16,700 rather than 33,400 or 50,100. We are therefore in this position; osmotic pressure experiments have yielded all of these values as well as many others, our own results hitherto lean to the first, do not exclude the second but seem to exclude the third. Can we use the heat of formation of a gram-molecule of oxyhaemoglobin, Hb_nO_{2n}, to decide the question? We can. For it proved possible to determine the actual amount of heat developed when one gram of haemoglobin unites with oxygen: calling this quantity H, then the molecular weight of the molecule

$$(Hb)_n = \frac{q}{H}.$$

We need hardly remind the reader that as compared with the accuracy of chemical analysis this determination may be rather rough. If, for instance, we obtain a value for the molecular weight of say 13,000 or 20,000 we might in either case regard n as unity. In practice the observation of the heat of combination of oxygen with haemoglobin proved much simpler than we had expected. The haemoglobin was reduced in a vacuum pump and allowed to run directly from the pump into a cylindrical Dewar's flask, the surface of the solution being protected from air by a coating of neutral olive oil. A sample of haemoglobin solution was analysed and oxygen was then bubbled through the fluid for a given time—about 5 minutes—at the end of which a second sample was withdrawn and analysed. The rise of temperature was observed with a simple Beckmann's thermometer. The necessary corrections were made for the amount of heat which the solution acquired if standing by itself, and so forth, and when this had been done we arrived at the rise of temperature in the haemoglobin solution as the result of the oxidation. We have therefore two measurements: (1) the amount of heat produced by the oxidation and (2) the amount of oxygen absorbed. On the assumption, the correctness of which has been shown in Chapter I, that each gram of haemoglobin unites with 1·34 c.c. of oxygen we may calculate the number of grams of haemoglobin which have been oxidised; we therefore have all the data which we require. The result of our observations was that H, the quantity of heat evolved when 1 gram of haemoglobin unites with oxygen, is

1·85 calories*. We have indicated above that the molecular weight of haemoglobin $(Hb)_n$ is to be found by dividing the quantity q by H. The result of which is approximately 15,200—a figure very near to the theoretical figure 16,669.

We have then by the above reasoning shown that the molecular weight of $(Hb)_n = 16{,}669$, therefore Hb must either be that figure or some submultiple of it. Thus if n was 2, then Hb would be 8335,

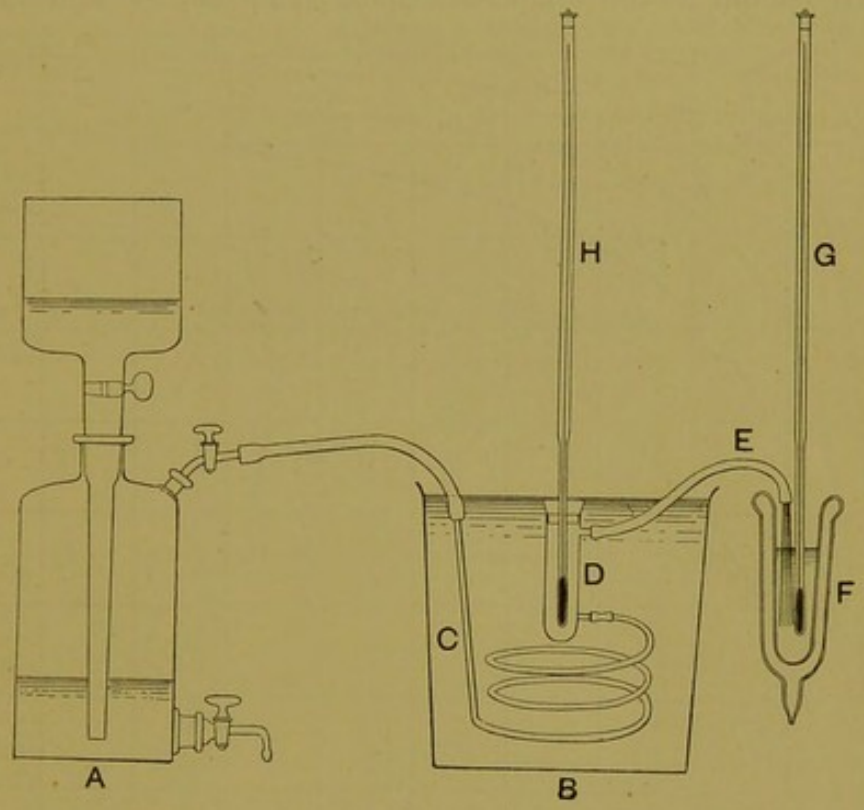


Fig. 16.—*A*, oxygen holder. *B*, warm bath for heating oxygen up to temperature of haemoglobin. *C*, coil of metal tubing. *D*, chamber for measuring temperature of oxygen. *E*, rubber pressure tubing. *F*, Dewar's flask containing haemoglobin. *G* and *H*, Beckmann's thermometers.

and so forth. Clearly this last alternative is an impossible one, for it would involve the splitting of the atom of iron. The figure 16,669 containing 56 parts of iron is the smallest figure for the molecular weight which would fit the facts of chemical analysis. The analyst would admit a multiple of 16,669 but not a submultiple. We have therefore no alternative but to regard n as unity, a result which

* This result is the mean of three experiments which gave values of 1·82, 1·98, 1·75, respectively.

agrees with all we have as yet considered with regard to haemoglobin.

We are now in a position, from the values of K which we have determined above, and from the knowledge that dialysed haemoglobin exists in single molecules, to calculate the percentage saturation of haemoglobin with oxygen at any oxygen pressure and at any temperature. Below we give a diagram of the dissociation curves of oxyhaemoglobin at the temperatures 16°, 25°, 32°, 38° and 49° C.

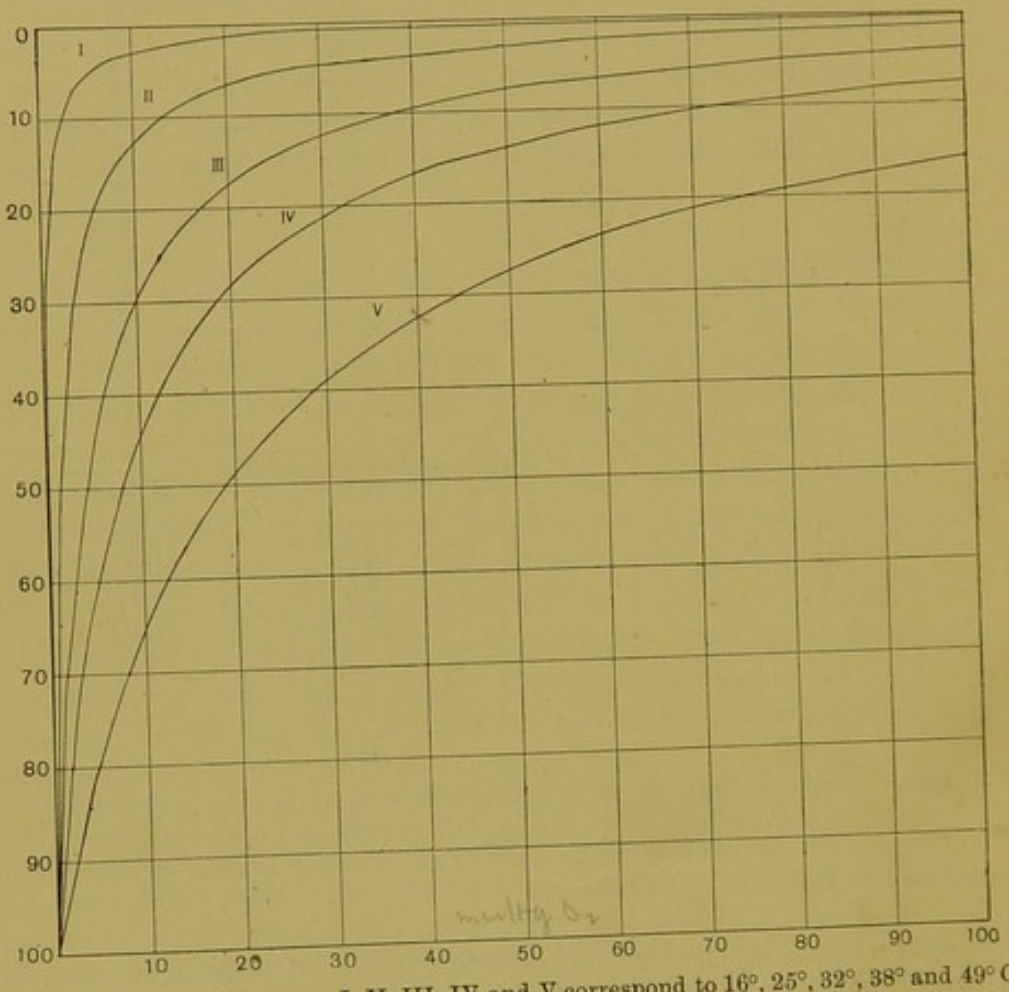


Fig. 17.—Dissociation curves I, II, III, IV and V correspond to 16°, 25°, 32°, 38° and 49° C. respectively. Oxygen pressure plotted horizontally, percentage of reduced haemoglobin vertically downwards.

In order to arrive at some sort of knowledge, as to how fast the forward and backward reactions of the oxidation and reduction of haemoglobin go on, and as to how far these rates are affected by temperature, some further experiments were done by Barcroft and

Hill. Nitrogen was bubbled through a suitably protected solution of haemoglobin at a constant rate but at two different temperatures. The rate at which the haemoglobin was oxidised was noted in each case. The experiment was first performed as follows. We started at a temperature of 18° C. with the blood fully saturated. The course of the experiment may be followed by reference to Figs. 18 and 19; the former shows the apparatus used, the latter the results obtained. As regards the apparatus, the haemoglobin solution was placed in the cylinder *A*. Nitrogen was passed at a constant rate in

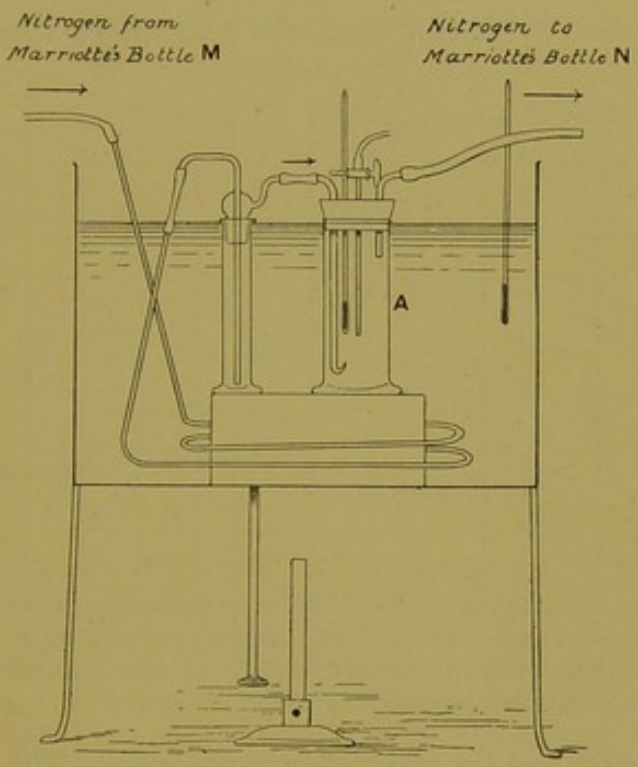


Fig. 18.—Apparatus for the determination of the rate of oxidation of haemoglobin.

the direction of the arrows, first through a wash-bottle containing an alkaline solution of pyrogallol, then through the haemoglobin solution; the whole apparatus was immersed in a water-bath and in that was kept at the required temperature. Samples of haemoglobin, which were small relative to the whole volume of haemoglobin used, could be abstracted from *A* at any time through a tube let in through the cork for the determination of the percentage saturation with oxygen. At any time in the experiment the inlet and outlet tubes for the nitrogen could be clamped and the whole apparatus

warmed up rapidly by replacing the water in the bath by water at a higher temperature. The flow of nitrogen then was resumed at the altered temperature.

Nitrogen was allowed to run for 35 minutes, by which time the haemoglobin was slightly reduced. An analysis showed that 6 °/₀ of the haemoglobin was reduced haemoglobin and 94 °/₀ oxyhaemoglobin. The point at which we have arrived is represented as *D* in Fig. 19. There is possibly an error of about 2 °/₀ in this measurement either way, i.e. the percentage saturation may have been 92 °/₀ or 96 °/₀ (*d* or *d'*). The nitrogen was now stopped, the temperature of the bath was raised to 38° C. (the time which the process took is omitted

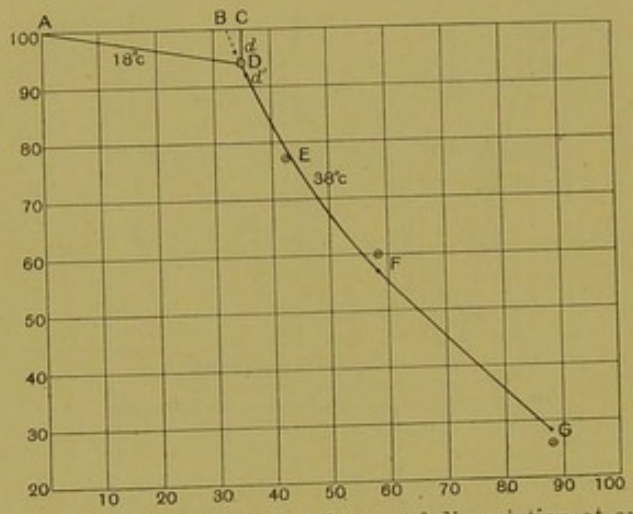


Fig. 19.—Curve representing the calculated degree of dissociation at any time of haemoglobin which was reduced by a stream of nitrogen bubbled at a uniform rate. Percentage saturation plotted vertically, time in minutes horizontally. The points represent actual determinations, *A*—*D* at 18° C., *D*—*G* at 38° C.

from the diagram) and the nitrogen was restarted. The haemoglobin now reduced very rapidly. After this three other determinations were made as follows:

Point on curve	*D*	*E*	*F*	*G*
Time (minutes) measured from *D*	0	7·5	23	53
Percentage saturation of haemoglobin	94 °/₀	77 °/₀	60 °/₀	26 °/₀

Thus whilst at 18° C. 35 minutes had been required for the reduction of the haemoglobin from 100 °/₀ to 94 °/₀ saturation, at 38° C. it only required 7·5 minutes to reduce it from 94 °/₀ to 77 °/₀. Perhaps the best comparison of the times necessary to produce a given reduction can be obtained by extrapolating the curve *DEFG* backwards to *B*, in which case *AC* represents the time necessary to

produce the reduction from 100 to 94 °/₀ at 18° C., whilst *BC* represents the time necessary for the same reduction at 38° C.; the former is 35 minutes the latter about 2·5 or at most 3 minutes. It is usual to compare the rates at which chemical changes take place, not over a range of 20° C. as in this case, but over a range of ten degrees. If the times necessary to produce a given change at temperatures 20° C. apart be in the ratio 35 : 3, the corresponding ratio for a difference of 10° C. will be the square root of the ratio of 35 : 3, namely 3·4 : 1, i.e. the coefficient is increased between 3 and 4-fold by a rise of temperature of 10° C.

A few lines back I spoke of extrapolating the curve *DEFG*. Let me say a word or two in justification of this phrase. I do not mean

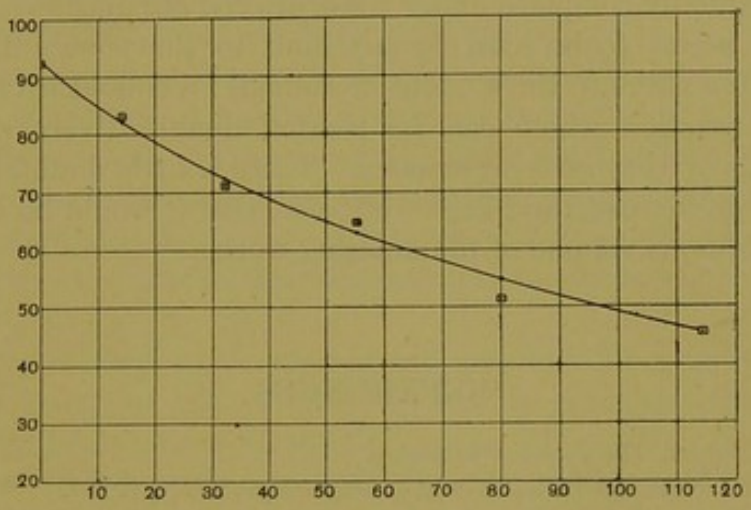


Fig. 20.—Curve representing the calculated degree of dissociation at any time in a haemoglobin solution through which nitrogen bubbled at a uniform rate. Percentage saturation plotted vertically, time in minutes horizontally. Points represent actual determinations.

merely producing the curve backwards by its general appearance, though for the present purpose no practical error would be involved by this procedure. I wish to make it clear that the line *DEFG* is a definite curve with a definite equation. The reduction of haemoglobin by nitrogen, if it takes place as a chemical breakdown, must obey laws which prescribe a definite form to the curve relating the percentage saturation to the time. The equation relating these two quantities has been worked out by Hill, and *DEFG* as drawn in the figure is such a curve. The coincidence of the points as determined experimentally with the curve is sufficiently good. The test would however have been more crucial had the points been more numerous.

Hill and I therefore performed another experiment from this point of view, i.e. to see whether the actual points determined followed a curve prescribed by the laws of mass action. We varied the conditions by making the haemoglobin in a different way, making the rate of reduction much slower and determining six points instead of four. The result worked out as follows :

Temperature 38° C.

Points	*A*	*B*	*C*	*D*	*E*	*F*
Time from beginning of Exp.	0	14·5	32	55	80	114 mins.
Percentage saturation observed	92·5	83	71	64	55	46
Ditto calculated	92·5	82	72·5	63	51·5	46

The correspondence of the determined points with the theoretical values is equally complete under these altered circumstances.

There seems to be scarcely any limit to the possibilities which haemoglobin offers to the student of colloids: a colloid with a simple chemical reaction which can be treated quantitatively with little difficulty is of very rare occurrence. There is little doubt, if haemoglobin could be made and kept with ease, that it would be a medium for an enormous volume of illuminating work.

REFERENCES

(1) Nernst, *Theoretical Chemistry.*
(2) Barcroft and Hill, *Journal of Physiology*, XXXIX, p. 411.
(3) Hüfner and Gansser, *Archiv f. Anat. und Physiol.* p. 209, 1907.
(4) Weymouth Reid, *Journal of Physiology*, XXXIII, p. 12, 1905–6.
(5) Roaf, *Proc. Physiol. Soc.* p. 1, 1909.

CHAPTER IV

THE EFFECT OF ELECTROLYTES ON THE AFFINITY OF HAEMOGLOBIN FOR OXYGEN

Up to the present we have made no attempt to treat of the work, which has recently been carried out on the affinity of haemoglobin for oxygen, on any sort of chronological basis, and now, having arrived at the fourth chapter we have also arrived at the point at which much of the work discussed in the preceding pages really began, and from which it proceeded always from the point of greater complication to the point of greater simplicity. It was the constant effort to unravel a knot which gave us the threads from which ultimately to weave a fabric.

What we wished to do may sound simple. In practice it has proved to be quite the reverse. It was to calculate the oxygen pressure in the capillary circulation.

We were therefore face to face with the problem of whether it was possible from the percentage saturation with oxygen of a given sample of blood to calculate from the dissociation curve of blood the oxygen pressure to which the blood was exposed. This was our first practical introduction to the dissociation curve. When we consulted the literature of the subject we found that each paper brought our ideas into a state of greater complexity. The authors who had written most recently on the subject were Zuntz [1], Loewy [2], Bohr [3], and Franz Müller [4]. Zuntz and Loewy had made the important contribution that apparently no two samples of blood, whether from man or beast, could be relied upon to have the same dissociation curve. Their view had been supported by Bornstein and Müller who worked on cat's blood, whilst Bohr and his colleagues had stated that this was in large part due to the fact that the dissociation curve is very sensitive to the presence of carbonic acid; their observations were discounted or neglected by many authors. Müller, however, had also

shown that, even taking this fact into consideration, the blood of the dog had a smaller affinity for oxygen than had that of the horse.

Camis[5] and I determined to perform some experiments to test the accuracy of the above observations: this did not seem difficult in view of the fact that we had at hand the differential method of blood gas analysis—a method which offered the prospect of making in a few days analyses that would previously have taken many weeks.

Like those of the proverbial golfer who strikes the ball with unerring accuracy on the occasion of his first visit to the links and then only, so our very early efforts seemed to augur speedy success—in our case the construction of a uniform dissociation curve for the blood of various animals. When we came to the blood of man however we could never make the dissociation curve agree with that of the cat or the rabbit. We went back to it time after time; the result was the same, human blood took up less oxygen at low tension than did that of the cat or rabbit. It was then clear that we had found our way into the morass in which our predecessors had already floundered so hopelessly, and our newer and more certain methods instead of saving us from their embarrassments had only made the uncertainty of our position more certain. There was no overlapping of the curves, no confusion of the points—human blood had a smaller affinity for oxygen than cat's blood.

We then entered upon six months of research which became weekly more and more depressing. Occasional flickers of light appeared—they turned out to be but will-o'-the-wisps—each of which beckoned us to a more inevitable disappointment than the last. It is easy to discuss the merits of apparatus, to gauge the help given by this or that method, yet as I look back upon this period of gloom, I discern more and more clearly as time goes on that the determining factors which made this research fruitful did not come from methods or from apparatus, but from the unfailing zeal and good will of my colleague Camis. His goodness of heart rose superior to the trials caused not only by his own disappointments but by mine.

We thought to simplify the issue by ceasing to work with blood and substituting haemoglobin. The simplest way of making this substitution was to lake the blood. In order to do this efficiently we added an equal part of dilute ammonia solution such as we used in our gas analysis apparatus, which solution was made by adding 4 c.c. of strong ammonia to a litre of distilled water.

When we left off working with blood and commenced working with solutions of haemoglobin, the discrepancies were quite as great as, if not greater than, they had been with blood, and intellectually the same difficulties presented a much greater embarrassment because they were much more paradoxical. With regard to blood, it might be said with some reason that the haemoglobin was modified by the fact of its being enclosed in the red blood corpuscles. Indeed, many suggestions might be put forward as to how this could happen. The surface charge of the corpuscle, if it has one, might increase or decrease the affinity of a corpuscle as a whole for oxygen. The surface membrane of the corpuscle might in different cases present

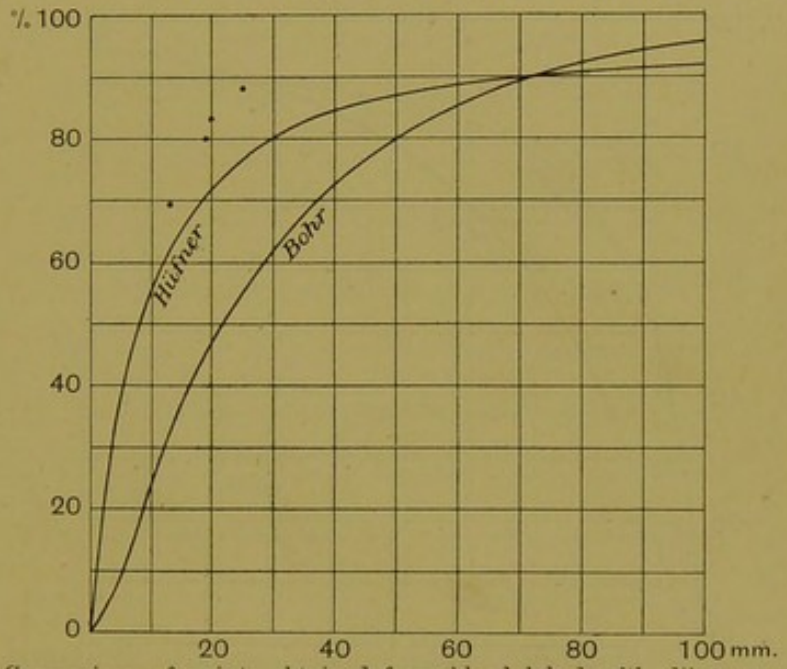


Fig. 21.—Comparison of points obtained from blood laked with dilute ammonia with dissociation curves of Bohr and Hüfner. Oxygen pressure horizontal. Percentage saturation vertical.

different obstacles to the diffusion of the oxygen within its walls. The existence of points or particles in a fluid is known to have a considerable influence on the accumulation of gas in their vicinity. All these unknown factors might be advanced with regard to the corpuscle.

When, however, we laked the blood, and so obtained a solution of haemoglobin, free from corpuscles, it was not possible to apply any of these considerations, and therefore the fact that no two solutions of haemoglobin presented identical affinities for oxygen was much more difficult of explanation.

Moreover the curves obtained did not agree with those either of Bohr or of Hüfner and they certainly were not in any rectangular hyperbola.

We thought it best to get rid of the proteids of the serum which might in some way adsorb oxygen. So we dissolved the haemoglobin in Ringer's solution. The only trace of uniformity in our result seemed to be that it differed from all our other results even as they had done from one another.

The curve which we obtained was not of any particular mathematical form which we could discern. It even differed from that of a suspension of washed corpuscles in Ringer's solution.

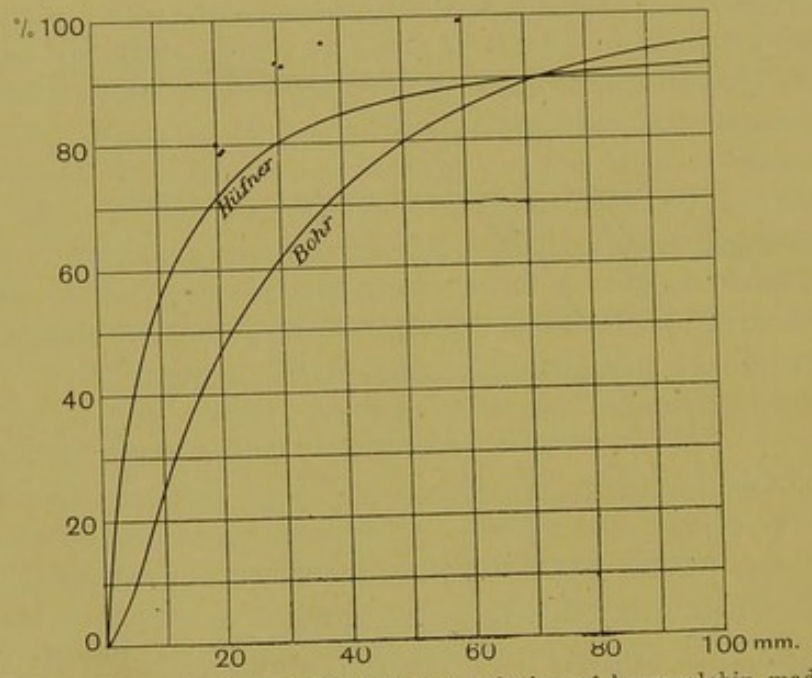


Fig. 22.—Comparison of points obtained from a solution of haemoglobin made alkaline with ammonium carbonate, with the dissociation curves of Bohr and Hüfner. Oxygen pressure horizontal. Percentage saturation vertical.

Lastly we made solutions of haemoglobin in different ways.

Our great effort was to obtain the maximum degree of concentration of the haemoglobin. The importance of this point had been rendered evident by the earlier work of Hüfner, whose solutions of haemoglobin had been so dilute that the oxygen physically dissolved in the solution bore a considerable ratio to the total oxygen present. To this fact we then attributed the discrepancies to be found in Hüfner's results. On looking back now we can see that it merely served to obscure discrepancies of a much more deep-seated nature, of

which Hüfner himself appears to have been oblivious, but which were, at the time of which we were speaking, only too evident in our own experiments. It seems certain that Hüfner's solutions of haemoglobin, which he regarded as identical, must have differed from one another in their affinities for oxygen as much, if not more, than our own.

To get the maximum amount of haemoglobin into solution we found it advisable to add a little ammonium carbonate ; but we were always assailed with the same result, namely, that no two solutions gave identical dissociation curves.

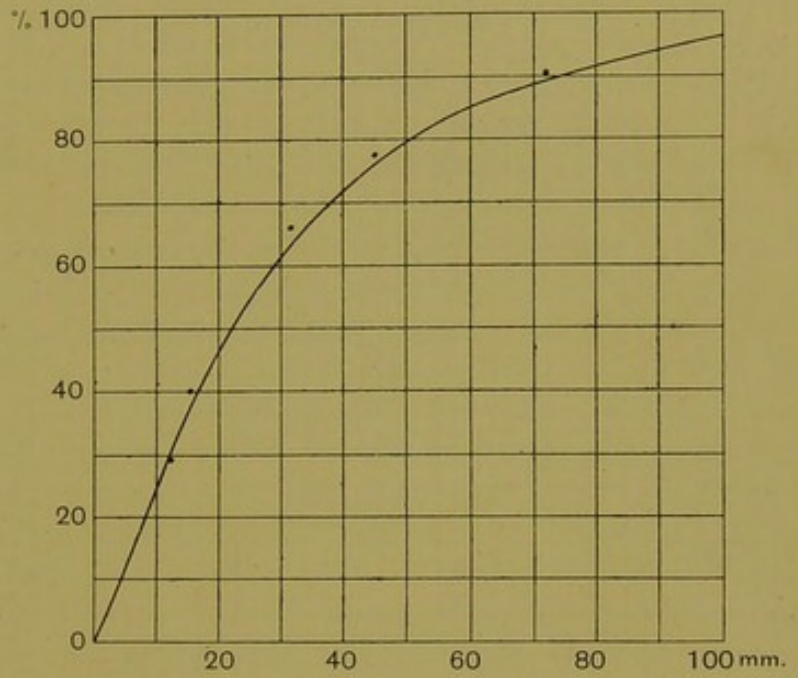


Fig. 23.—Comparison of points on haemoglobin solution prepared by Bohr's method, with Bohr's dissociation curve. Oxygen pressure horizontal. Percentage saturation vertical.

One afternoon Camis, Hill and myself, over the laboratory tea, fell to discussing a subject which had frequently been discussed before, namely Bohr's haemochrome theory. We had long since given up any attempt to harmonise our curves with those of others, but the question in our mind was whether with different constants the most recent curve which we had obtained could be made to agree with the general mathematical expression of the theory. Camis read out the positions of the points.

Tension of oxygen	12·5	15·5	31	45	72
Percentage saturation ...	29	40	66	77·5	90·5

Hill dotted them in in pencil on Bohr's curve and showed me the figure; it was as shown in Fig. 23.

The solution like most of those made previously had been made by Bohr's method, quoted in the last chapter; in this particular case however we had omitted, whether by intention or forgetfulness I cannot now remember, to add any ammonium carbonate.

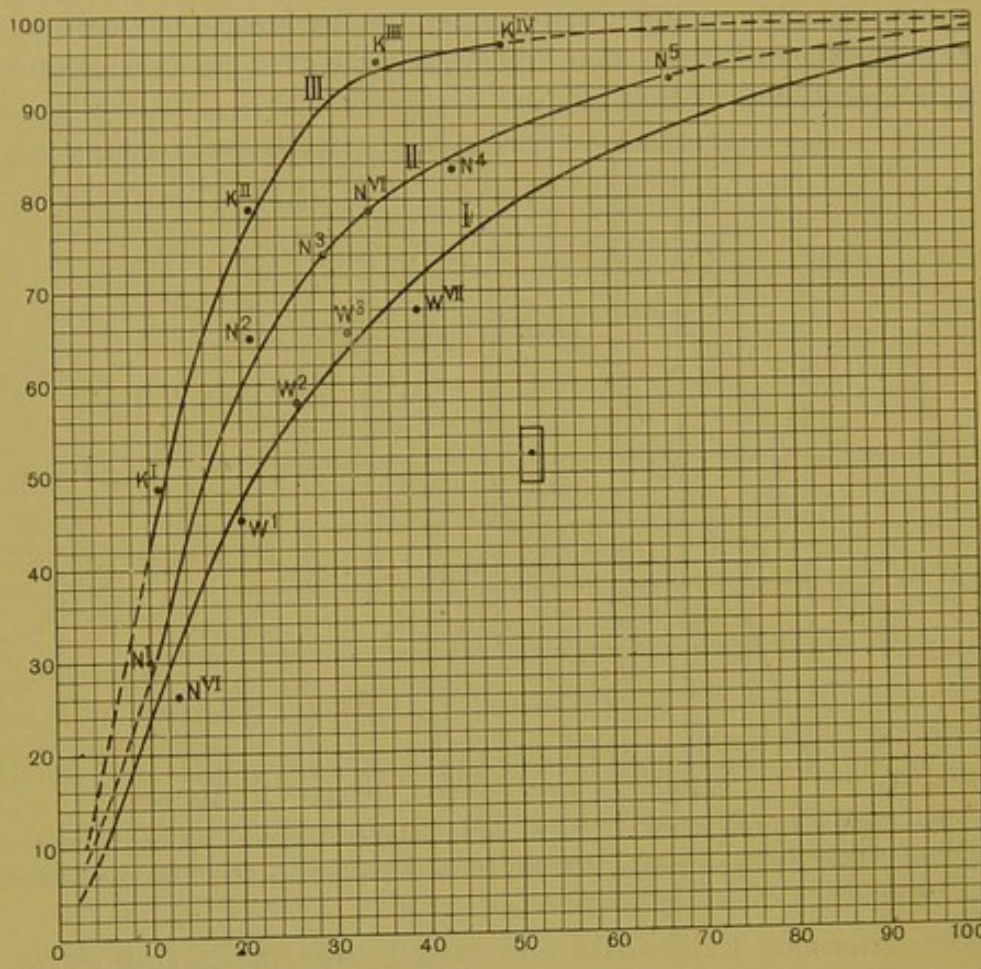


Fig. 24.—Ordinate = percentage saturation of haemoglobin. Abscissa = tension of oxygen in mm. of mercury.

I. Dissociation curve of haemoglobin dissolved in water.
II. ,, ,, ,, ,, ·7 % NaCl.
III. ,, ,, ,, ,, ·9 % KCl.

Rectangle surrounding point = magnitude of experimental error. Temperature 37—38° C.

We could only attribute the divergence of our previous curves from that of Bohr to the ammonium carbonate which had been added somewhat casually. We therefore dispensed with this and

then we were able time after time to obtain solutions which were identical with that figured by Bohr for haemoglobin.

To test the effect of salts in a more systematic way we made solutions of haemoglobin, divided them into various portions, added various salts to the portions and compared their dissociation curves with that of the original haemoglobin as prepared by Bohr's method.

In Fig. 24 are given the dissociation curves of oxyhaemoglobin in the solutions of electrolytes which are of greatest physiological importance, namely sodium chloride and potassium chloride isotonic with one another. We shall reserve the consideration of some others till later. From this point constructive work on the dissociation curve of blood commenced. In the red blood corpuscle haemoglobin is dissolved in a solution of salts which differs very widely in different members of the animal kingdom. But it is evident that the first step towards building up the curve of blood from that of haemoglobin is to investigate a haemoglobin solution dissolved in the actual salts which are present in the red blood corpuscles. But here a fresh complication arose, for the salts differ in the corpuscles of each different species. We performed the following experiment: a solution of haemoglobin was divided into two portions, (*a*) and (*b*). To the portion (*a*) were added the salts of the human red corpuscles as determined by the old, though still classical, analysis of Schmidt[6]. To the portion (*b*) were added the salts of the dog's red blood corpuscles as determined by the recent, and excellent, analysis of Abderhalden[7]. The dissociation curves of these two solutions were determined in the presence of approximately 40 mm. pressure of carbonic acid, and gave the results depicted in Figs. 25 and 26.

It was at least evident that the two curves were quite different, that the difference could by no possibility be explained by experimental errors, and that one had arrived at at least one tangible reason why the blood of one animal should differ from that of another; but even then it seemed that we were far from a complete solution of the whole problem before us. It was not unlikely that any number of other reasons might conspire to add or detract from the differences we had observed, and therefore our next move was to make as faithful a comparison as possible between the curves which we had just observed, and the actual curves which could be obtained from human and canine bloods respectively when exposed to the same conditions as the haemoglobin solutions (*a*) and (*b*). We hoped

that by finding out what discrepancies still existed, to shed some light on the factors which remained for investigation. Great then was our excitement and delight when we found that point after point on the curve of human blood lay also on the curve of the haemoglobin

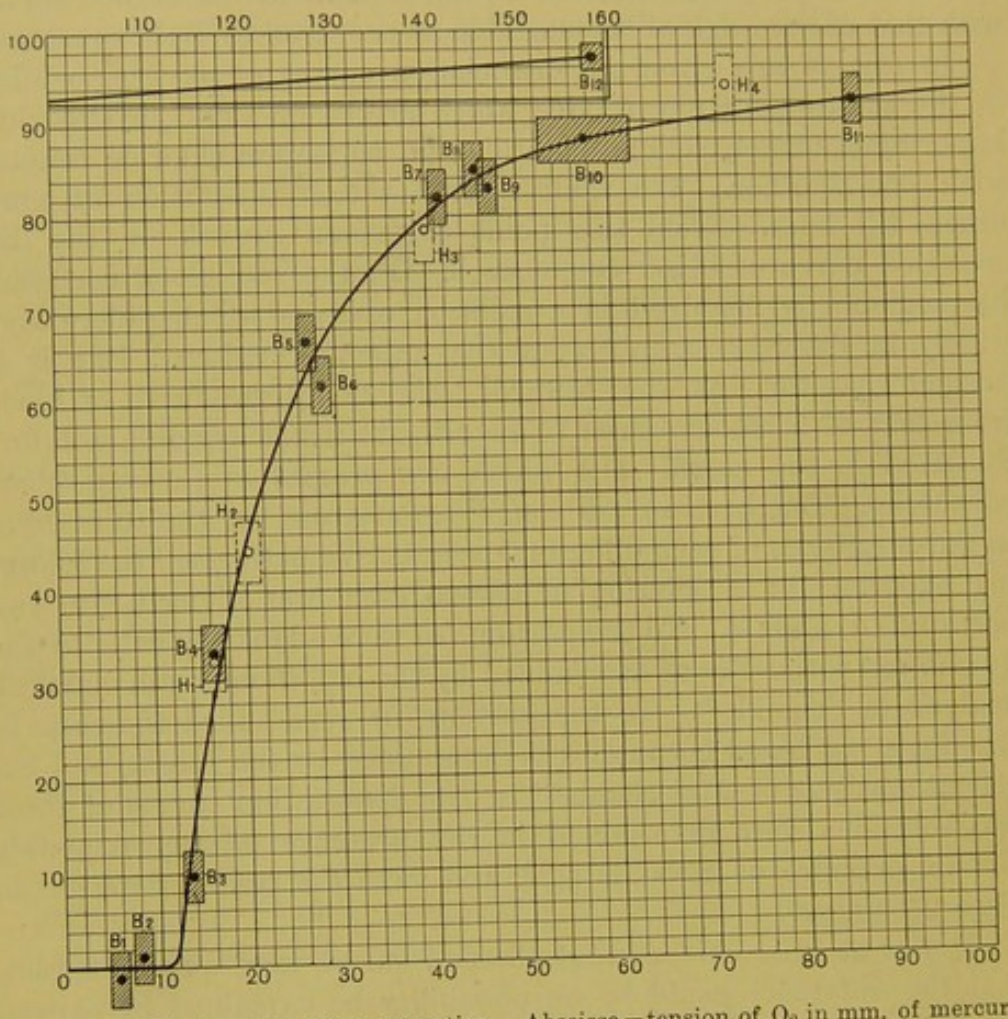


FIG. 25.—Ordinate = percentage saturation. Abscissa = tension of O_2 in mm. of mercury. Dissociation curve of human blood at 40 mm. tension of CO_2. • = determination for human blood. o = ditto for haemoglobin solution with salts of human red blood corpuscles. The area drawn round the point is the experimental error in each case. Temperature 37—38° C. More recent observations have shown that the portion of the curve below 15 mm. pressure, while traversing the shaded areas, approaches the base line more gradually.

solution (*a*), whilst the corresponding points for dog's blood adhered with equal fidelity to the curve obtained from solution (*b*). The points in question are also given in the figure, and the reader will observe that only in one point on each curve does there seem to be

a slight tendency for the actual points obtained from blood to diverge a little from the curve obtained from the haemoglobin solution. That is just where the curve bends round at the top. To this we must add that more recently the curve of the same human subject, Dr Camis himself, has been redetermined at Pisa by more exact methods than were

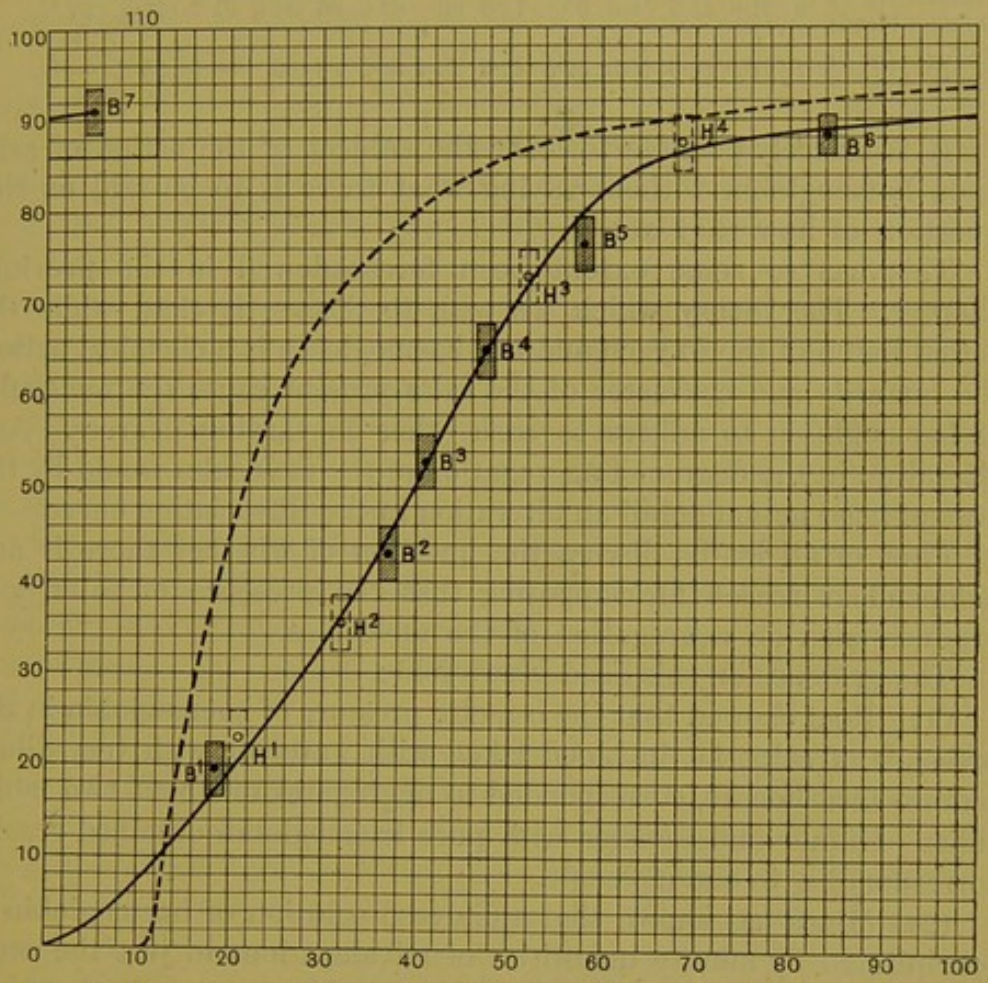


FIG. 26.—Ordinate = percentage saturation of haemoglobin. Abscissa = tension of O_2 in mm. of mercury. Dissociation curve of dog's blood at 40 mm. tension CO_2. Dotted line = dissociation curve of human blood (see Fig. 25). • = determination for dog's blood. o = ditto for haemoglobin solution with salts of dog's red blood corpuscle. The area drawn round the point is the experimental error in each case. Temperature 37—38° C.

then at our disposal. These methods enabled us to work accurately at pressures of oxygen under 10 mm. The Pisa curve seems to differ from that given above to a trivial though appreciable extent. The difference, however, is quite negligible as compared with the differences which exist between his blood and that of a dog, and it is

highly probable that it depends upon the method employed. The following are the data of the curves obtained for man and the dog:

Blood at approximately 40 *mm. tension of* CO_2 *and* 37—39° *C.*

Abscissa	10	15	20	25	30	35	40	45	50	60	80	100	160 mm. O_2
Ordinate for human blood	0	21·5	45·5	59·5	69	75·5	80	83·5	86	88·5	91·5	93·5	97 %
Ordinate for dog's blood	7	11·5	18·5	20·5	32·5	41·5	50·5	60·5	69	80	88·5	90	— %

It is scarcely possible to leave the subject of the action of salts upon haemoglobin without saying what there is to be said on this question of how they act.

This has to some extent been foreshadowed in the previous chapter. The theory which we wish to discuss is that, while the actual union of the oxygen and the haemoglobin is a chemical action, the quantity and character of the salts present cause the molecules of haemoglobin to adhere to each other in varying degrees. Any alteration in the degree of aggregation of the molecules would by the laws of mass action entail an alteration in the dissociation curve.

First of all let us discuss the question of whether there is any independent reason for supposing that the presence of salts does produce such an aggregation of the molecules.

The evidence on this head is two-fold:

(1) Roaf showed that the osmotic pressure of haemoglobin in the presence of salts, i.e. in blood laked with water or with very dilute sodium bicarbonate, corresponded to aggregates of about two molecules. Hüfner and Gansser with pure haemoglobin obtained results corresponding to one molecule.

(2) Mines has shown that a dialysed solution of haemoglobin is precipitated by minute quantities of trivalent ions in just the same way as it is by acids, and precipitation is only one stage further than aggregation.

The colloidal nature of haemoglobin.*

Although it is certain that the union between haemoglobin and oxygen is a true chemical combination and not a mere surface adsorption, the colloidal condition of haemoglobin remains of great importance as a factor influencing the equilibrium between haemoglobin and oxygen at relatively low oxygen tensions. For although the oxygen capacity of haemoglobin must be independent of the state

* I am indebted to Mr Mines for the following note.

of dispersity of the substance, the extent to which oxyhaemoglobin will dissociate in the presence of low tensions of oxygen may conceivably be largely influenced by this factor.

Solutions of haemoglobin however finely dispersed will remain colloidal, for the haemoglobin molecule is of such dimensions that it presents surface and itself constitutes a colloidal particle.

As regards the production of actual visible agglutination or precipitation of haemoglobin by electrolytes, haemoglobin may be said to present the characters of a negatively charged emulsoid or lyophil colloid. It has been shown that a number of negative suspensoid colloids have their electric charge affected, and thus are precipitated, quite as readily by complex trivalent kations (such as $Co(NH_3)_6^{\cdots}$) as by simple trivalent kations (such as $La^{\cdots}$, $Nd^{\cdots}$, &c.), while emulsoids, many of which have their charge affected readily by simple trivalent kations, are almost uninfluenced by complex trivalent kations. The latter is the case with oxyhaemoglobin. A carefully dialysed solution of the pigment is precipitated by a moderate concentration of a salt of lanthanum or cerium (cerous) while no visible effect is produced by the addition of luteo-cobalt chloride even in large concentration.

Oxyhaemoglobin also shares a property possessed by some suspensoids and some emulsoids, namely, extreme sensitiveness towards the hydrogen ion. A low concentration of acid suffices to precipitate oxyhaemoglobin.

It is to be expected that a change in the hydrogen ion concentration in the direction of increased acidity, insufficient to cause visible agglutination, will yet cause some aggregation of the molecules of haemoglobin. The fact that gross aggregation is readily obtained by acid lends support to the idea that the effects produced upon the dissociation curves of oxyhaemoglobin by still smaller amounts of acid (Bohr, Barcroft and Orbeli) are due to a minor degree of aggregation of the molecules.

If this hypothesis is a true one, it is to be expected that of the simple kations, exclusive of the hydrogen ion, those of different valency will produce very different effects. Thus a certain small concentration of a salt yielding simple trivalent ions (for instance lanthanum nitrate) will produce an effect on the dissociation curve of oxyhaemoglobin which will be equalled only by a concentration of a magnesium salt some hundreds or perhaps thousands of times as great, while of such salts as NaCl a relatively enormous concentration will be required. Further we should expect to find phosphates and

citrates very active in opposing the changes produced by polyvalent kations.

We are now face to face with the influence of "reaction" on the dissociation curve of blood.

REFERENCES

(1) Zuntz and Loewy, *Archiv f. Anat. u. Physiol.* p. 166, 1904.
(2) Loewy, *Ibid.* p. 246.
(3) Bohr, Hasselbalch and Krogh, *Skand. Arch. f. Physiol.* XVI, p. 412.
(4) Bornstein and Müller, *Archiv f. Anat. u. Physiol.* p. 478, 1907.
(5) Barcroft and Camis, *Journal of Physiol.* XXXIX, p. 118.
(6) Schmidt, *Halliburton's Text-book of Physiol. and Pathol.* p. 265.
(7) Abderhalden, *Lehrbuch d. Physiol. Chem. Zweite Aufl.* pp. 732 *et seq.*

CHAPTER V

THE EFFECT OF ACID ON THE DISSOCIATION CURVE OF BLOOD

BEFORE entering into a discussion as to whether the influence of "reaction" on the affinity of blood for oxygen is different in nature or only in degree from that of salts, I will state the facts which must form the basis of any such discussion.

Doubtless the affinity of haemoglobin for oxygen is very sensitive to small changes in acidity or alkalinity. This statement unfortunately does not rest on experimental determinations of the influence of minute quantities of acid or alkali on the dissociation curve of an otherwise pure solution of haemoglobin, for no such determinations exist. In the case of acid it remains to be seen whether such an experiment can be carried out without the precipitation of the haemoglobin. What information there is on the subject, then, is derived from experiments on blood and not on haemoglobin.

In two words, the effect of increased acidity of the solution is to lessen the effective concentration of the oxygen in the solution which contains the haemoglobin. To give a concrete instance, that of my own blood. The presence of 40 mm. pressure of carbonic acid halves the effective concentration of oxygen. In order therefore to produce a given degree of saturation in the presence of 40 mm. CO_2 pressure, the concentration of oxygen must be about twice the value which would have sufficed in the absence of the carbonic acid.

The concentration of free carbonic acid in the solution, necessary for the production of a measurable change in the affinity of haemoglobin for oxygen, is very small, and amounts to something of the order of one part in one hundred million. This fact is directly deducible from the data furnished by Bohr, Hasselbalch and Krogh [1],

who were the discoverers of the influence of carbonic acid on the dissociation curve.

Carbonic acid however is not specific in its effect. Lactic acid acts in precisely the same way[2]; so also do mixtures of lactic and carbonic acids.

Having made the rough statement that the effect of acidity is to reduce the effective concentration of oxygen, I must now state more precisely (1) what I mean by "reaction," and (2) what I mean by reduction of the effective concentration of oxygen.

It seems that the influence of acids depends on the change in hydrogen ion concentration which they cause in the blood: and this appears to be different from the change of hydrogen ion concentration which they would cause in water or salt solution.

For instance, mineral acids in equal molecular concentration are generally much more powerful than acids such as lactic, acetic or carbonic. They cause a much greater concentration of hydrogen ions. Yet when they are added to serum the change in the hydrogen ion concentration which they produce is nearly the same. This change has been measured by the method of titration with an indicator which changes colour gradually over a considerable range of hydrogen ion concentrations.

Added to normal salt solution the following strengths of acid would produce equal changes in the hydrogen ion concentration:

Lactic		Hydrochloric		Formic	Acetic
M/200	=	M/300		—	M/80
M/110	=	M/200	=	M/130	M/50
M/50	=	M/170		—	—
M/10	=	M/130		—	—

But the relative quantities of these acids which produce equal changes in hydrogen ion concentration in serum are

Lactic		Hydrochloric	Acetic	CO_2	Hydrogen ion conc.
M/200	=	M/200	—	—	$10^{-6\cdot8}$
M/100	=	M/120	—	—	$10^{-6\cdot1}$
M/100		weaker than	M/100	—	—
·2 °/₀	=	exposure to atmosphere of		100 °/₀	$10^{-4\cdot6}$

These results were obtained by titration with the indicator referred to above.

Now so far as the affinity of the haemoglobin for oxygen is concerned, the same change is wrought by increasing the concentration

of lactic acid to ·2 °/₀ as by exposing the blood to 100 °/₀ CO_2, a change which in each case increases the hydrogen ion concentration of the plasma from $10^{-7\cdot5}$ to $10^{-4\cdot5}$.

Similarly, blood made up with lactic acid to M/120 has about the same affinity for oxygen as blood made up to M/130 with hydrochloric acid, and so forth.

The affinities of the samples of blood for oxygen were not compared in these cases by means of the dissociation curve, but by another method, that of bubbling a uniform stream of nitrogen through the blood and testing the rate at which it became reduced, i.e. the time taken to attain a given degree of reduction. Allusion has already been made to this procedure. The method was adapted somewhat for the purpose by Mathison[3], who performed the experiments with which I am now dealing. It is described in Chapter XI. Here I am discussing the results.

This method no doubt expresses dynamically the facts concerning the relation of haemoglobin to oxygen, just as they are expressed statically by the dissociation curve. It is very desirable however that actual determinations should be carried out in which the hydrogen ion estimations are measured with the gas chain battery, and the affinity of the haemoglobin for oxygen by means of the dissociation curve. This is a very difficult matter, but the work which has recently been published from Hasselbalch's[4] laboratory offers a bright promise in this respect.

In the meantime, let me give as examples the data of the two cases I have mentioned. From them it will be seen (1) that the addition of acids greatly accelerates the reduction of the blood; (2) that the concentrations of the acids necessary to produce approximately equal effects are those in which they produce equal increments in the hydrogen ion concentration.

From Fig. 27 it will be seen that the times taken, by blood containing ·2 °/₀ lactic acid, to be reduced by nitrogen, and by blood without lactic acid to be reduced by 100 °/₀ CO_2, are indistinguishable, and are about one-tenth as great as the times necessary for the same reduction without the acids; i.e. the blood is reduced from 100 °/₀ saturation to 80 °/₀ saturation, in about 2 minutes with the acids present, and about 22 minutes with the acids absent.

From Fig. 28 it will be seen that normal blood is reduced under the conditions of the experiment (in which the bubbling was more rapid and the temperature higher than in the preceding one) from

100 °/₀ to 35 °/₀ in 15 minutes, while when it contained M/130 HCl and M/120 lactic acid the same reduction was effected in about 6·5 and 7·5 minutes respectively.

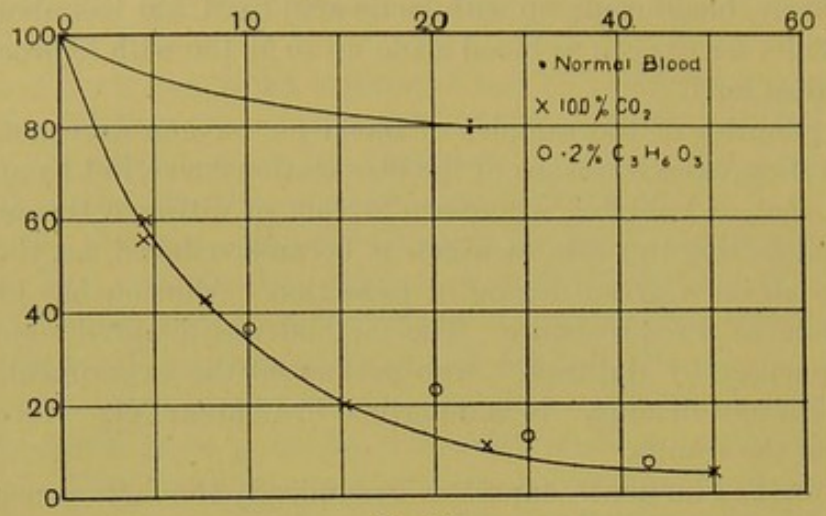


Fig. 27.

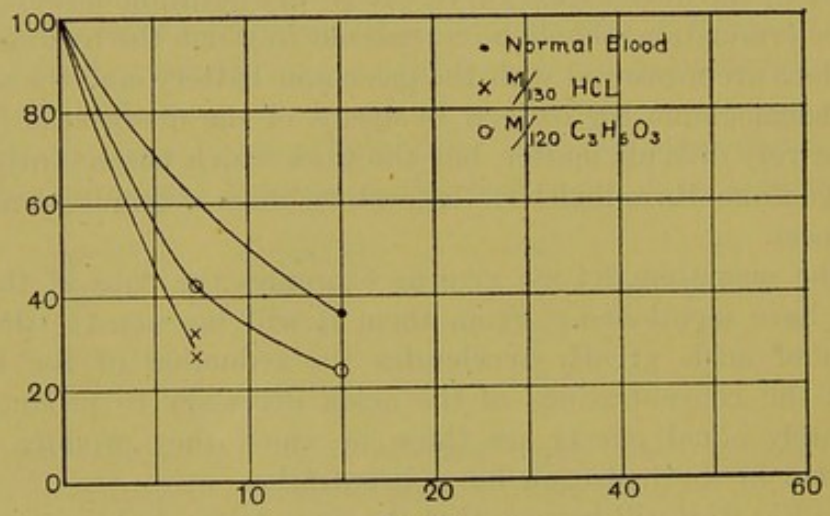


Fig. 28.

Figs. 27 and 28.—Ratio of reduction of blood with a uniform stream of oxygen free gas. Percentage saturation vertically. Time in mins. horizontal. Fig. 27.—Comparison on normal blood, with blood containing lactic acid and with blood reduced by carbonic acid instead of nitrogen. Fig. 28.—Shows the approximate equality of M/130 HCl and M/120 lactic acid in their effects.

Having made it clear that the sense in which I use the word "reaction" is the strict one, namely the hydrogen ion concentration, I will leave this point and pass to the consideration of the effective concentration of oxygen.

The simplest meaning which would attach to the words "increased hydrogen ion concentration reduces the effective concentration of oxygen" would be as follows: In order to obtain a given percentage saturation of the blood with oxygen in the presence of the various concentrations of acid, the pressure of oxygen with which the blood would be in equilibrium would require to be higher in proportion to the increase in the H-ion concentration: this proportion also should be the same all along the curve.

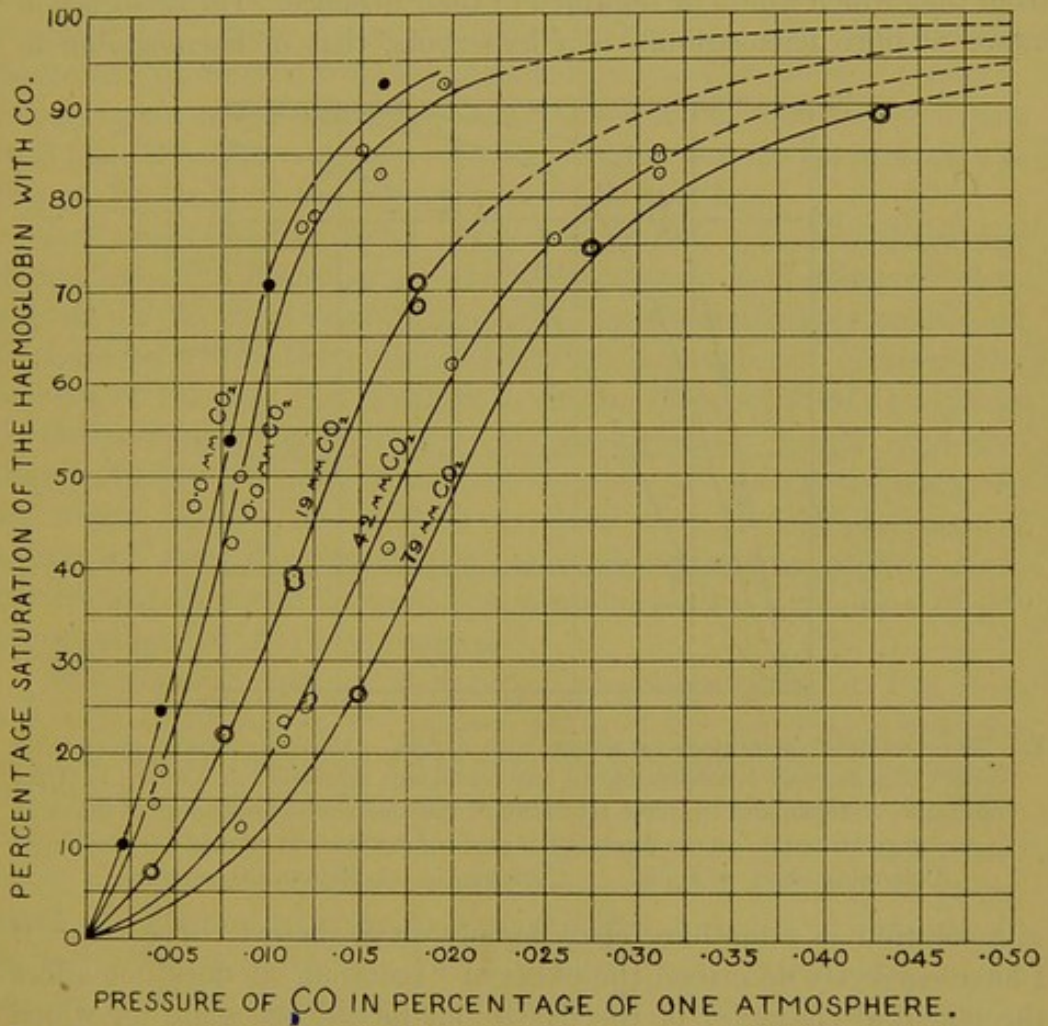


FIG. 29.—Dissociation curves of CO-haemoglobin in absence of oxygen, at 38° C. and with various pressures of CO_2.

o Blood of Douglas. • Blood of Haldane.

For instance, my blood is 45 % saturated at 10 mm. oxygen pressure in the absence of CO_2, in the presence of 40 mm. CO_2 it is 45 % saturated at 24 mm. O_2: now if the relation which we have

defined be true the oxygen pressures on the two curves (0 CO_2 and 40 mm. CO_2) for any given percentage saturation should be in the ratio of 10 to 24. In other words the effect of the CO_2 is to make 24 mm. oxygen pressure behave like 10 mm., and x mm. pressure behave like $x \times \frac{10}{24}$.

Here we find ourselves facing the question of whether the effect of "reaction" is of a fundamentally different character from that of salts. The effect of salts was to produce a curve essentially different from that which would be obtained in their absence. The dissociation curve of pure haemoglobin is a hyperbola, that of haemoglobin in

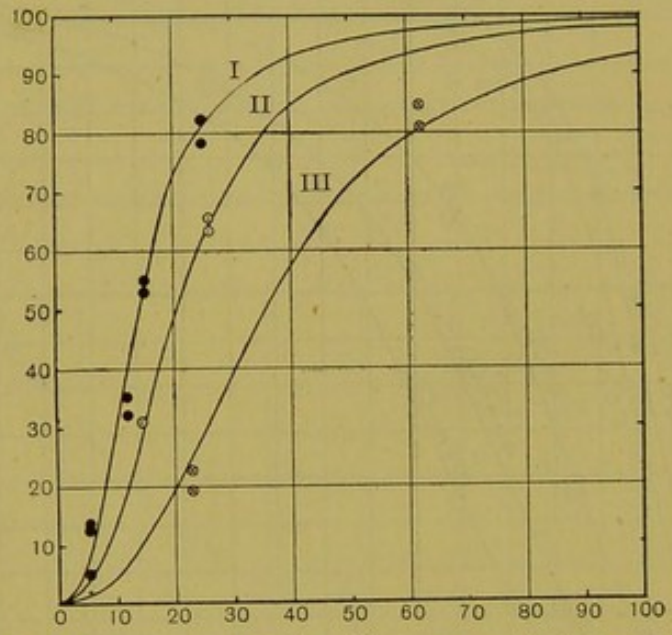


FIG. 30.—Ordinate = percentage saturation with oxygen. Abscissa = oxygen pressure in mm. The abscissa corresponding to any percentage saturation on Curve I is 11/20, on Curve II 16/20, and on Curve III 27/20, of the abscissa corresponding to the same percentage saturation on the dissociation curve of my blood at 40 mm. CO_2 pressure.
Determinations: • 3 mm. ⊙ 20 mm. ⊗ 90 mm. CO_2 pressure.

salt solution is something else, namely an S-shaped curve. But if I have correctly described the effect of "reaction," it does not affect the essential character of the curve, but merely the scale on which it is drawn. This conception is fundamentally different from that with which I finished Chapter IV. There was no other suggestion in it than that the effects of acids and salts might turn out to be the same.

The question is one which must be put to the test of experiment. The effect of acid, as I have described it, was suggested to Douglas

and Haldane by the curves which they obtained for the dissociation of carboxyhaemoglobin into reduced haemoglobin and CO, in the presence of different CO_2 pressures. Clearly they approximate to this ideal.

It seemed desirable to redetermine the corresponding points for oxyhaemoglobin for the purpose of ascertaining whether there were any difference between the curves at various CO_2 pressures other than the scale on which they were drawn. To this matter Poulton and I addressed ourselves. We made determinations of points on the dissociation curve in the presence of 3, 20 and 90 mm. CO_2 respectively. These points are shown in Fig. 30. The curves in this figure are all derived from my dissociation curve at 40 mm. pressure. For any percentage saturation the pressure on the line corresponding to 3 mm. CO_2 line is $\frac{11}{20}$, that on the 20 mm. line is $\frac{16}{20}$, and on the 90 mm. line is $\frac{37}{20}$ of the pressure on my 40 mm. line.

Not until Poulton and I arrived at 90 mm. CO_2 was there any suggestion of a discrepancy. The discrepancy that occurred then may be due to experimental error, for the determinations could just be included within the extreme limits of such errors. Nevertheless in view of the fact that there is a similar disposition of the points on the corresponding curve for CO haemoglobin (Fig. 29), and also that a similar tendency is evident in some of the curves obtained on Monte Rosa, I am inclined to think that at CO_2 pressures of about 100 mm. the dissociation curve does become slightly more curved than it would be were it a mere horizontal expansion of the curves obtained at lower pressures *.

This slight divergence at a very high concentration of CO_2 does not seem to invalidate the general truth of the conception that the main action of increased hydrogen ion concentration is to reduce the effective concentration of oxygen in precisely the way we have described. One of the most unbending of physical laws (Boyle's law) bends when pushed to extremes. The laws of ionic dissociation do the same, and it could scarcely be expected that our present case should be more rigid than these. The important point is that over ranges of CO_2 pressure which are not extreme the curves appear to differ merely in the scale on which they are drawn. This, as I have indicated, suggests that in the presence of considerable quantities of salt small changes in hydrogen ion concentration do not appreciably alter the degree of aggregation of the molecules.

Now that I have stated the main facts about the relation of salts

* See Appendix II.

and acids to haemoglobin it may be profitable to give a brief sketch of an attempt which has recently been made to find a physical explanation of these facts.

Any such explanation starts from the proven fact that the dissociation curve is a rectangular hyperbola, representing a simple reaction

$$Hb + O_2 \rightleftarrows HbO_2.$$

This reaction involves single molecules only, and has a definite equilibrium constant.

The equation for this curve may be written

$$\frac{y}{100} = \frac{Kx}{1 + Kx},$$

where y = the percentage saturation of the haemoglobin with oxygen, x = oxygen pressure, and K is the equilibrium constant of the curve.

This curve, as I have said, assumes that the molecules of haemoglobin are single ones. Hill [7] amplified this equation with the object of ascertaining the shapes of the dissociation curves which would be obtained on the assumption that the molecules of haemoglobin fall into aggregates as the result of the addition of salts. At the end of Chapter IV I pointed out that there was independent reason to suppose that this was the case.

If on the average there are n molecules in each aggregate, the equation becomes

$$\frac{y}{100} = \frac{Kx^n}{1 + Kx^n}.$$

The solution is conceived of as containing haemoglobin in the various degrees of molecular aggregation, the reactions of which would take place in the following way:

$$Hb + O_2 \rightleftarrows HbO_2,$$
$$Hb_2 + 2O_2 \rightleftarrows Hb_2O_4,$$
$$Hb_3 + 3O_2 \rightleftarrows Hb_3O_6, \text{ &c., &c.}$$

The equation however has certain limitations of which the only one to be considered, so long as we are confining our attention to oxyhaemoglobin, is that there is no considerable quantity of intermediate oxides such as Hb_2O_2 in the solution [8]. The theory does not preclude the formation of such substances. It is highly probable that Hb_2O_2 for instance is a stage on the way to Hb_2O_4. But to make the equation hold, the suboxides can only exist as stages in the reaction; in short the assumption is that if two molecules of Hb_2O_2 met, they

would resolve themselves into Hb_2 and Hb_2O_4 as being the more stable arrangement.

And now does the theory fit the facts?

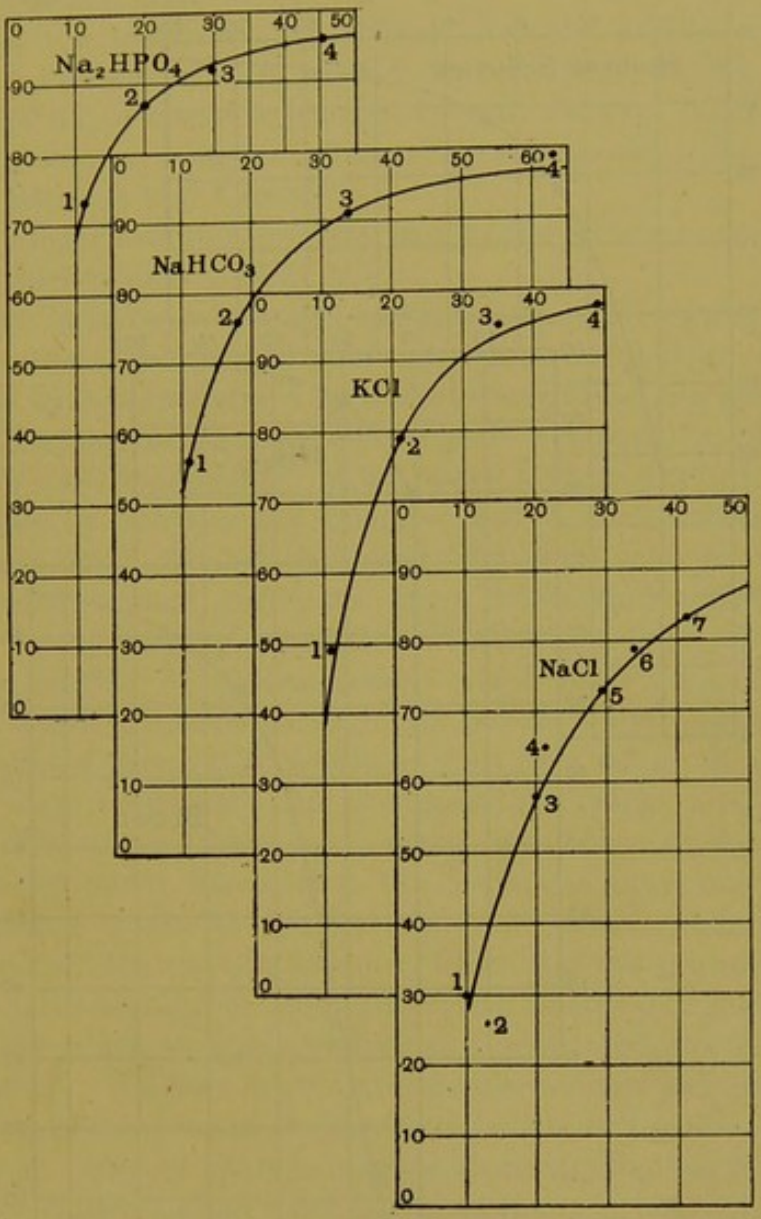


FIG. 31.—Dissociation curves of haemoglobin solutions in Na_2HPO_4, $NaHCO_3$, KCl and NaCl. • Points actually determined. Curves drawn from formula $y/100 = \frac{Kx^n}{1+Kx^n}$. Ordinate = percentage saturation with oxygen. Abscissa = oxygen pressure in mm.

First let me consider the simplest cases. Below is a table of the values of K and n which correspond to the various solutions of haemoglobin which have been investigated.

Solvent	Na_2HPO_4	$NaHCO_3$	KCl	NaCl	$(NH_4)_2CO_3$	Distilled water dialysed
K	·0453	·0155	·00204	·0062	·006257	·125
n	1·670	1·835	2·49	1·778	3·19	1

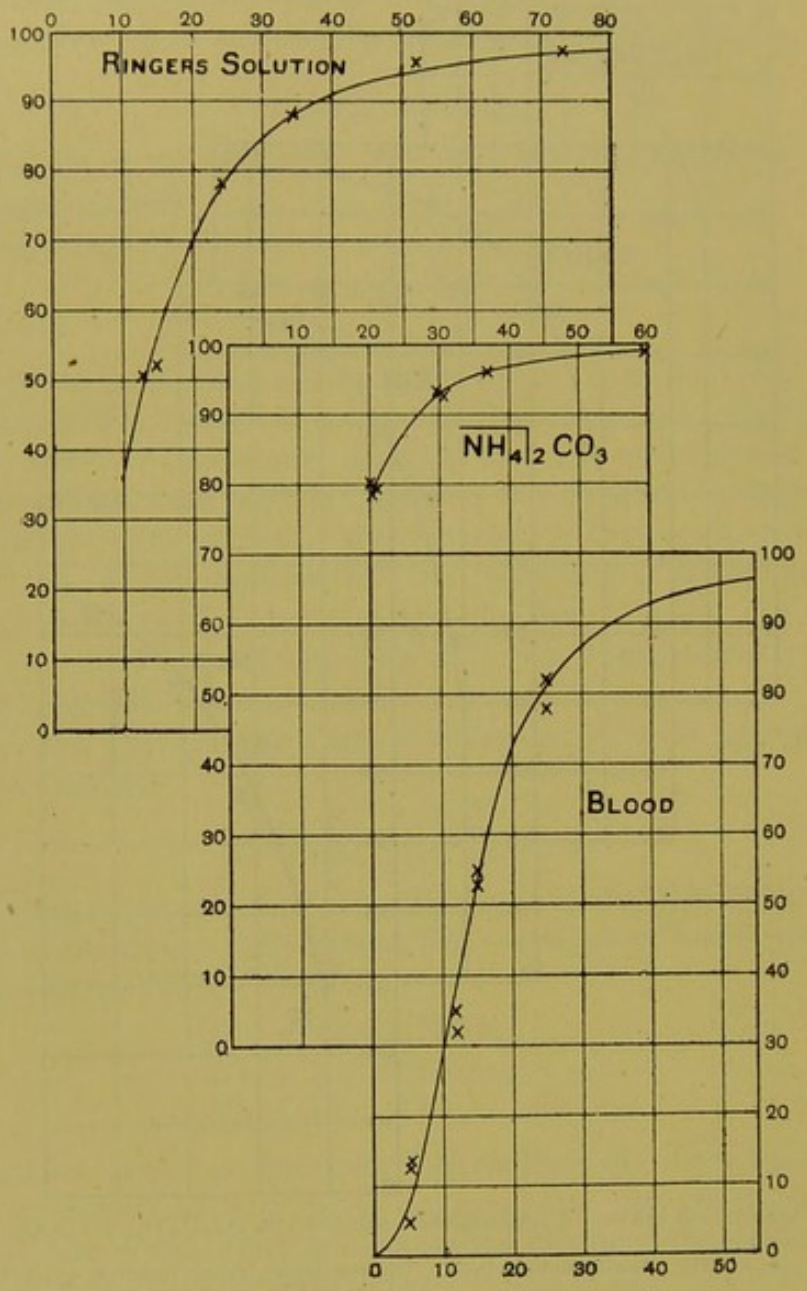


FIG. 32.—Dissociation curves of haemoglobin solutions in Ringer's fluid and $(NH_4)_2CO_3$, and of blood. × Points actually determined. Curves drawn from formula $y/100 = \frac{Kx^n}{1+Kx^n}$. Ordinate = percentage saturation with oxygen. Abscissa = oxygen pressure in mm.

Whether or no the curves drawn from these data fit the points as determined may best be seen by actually drawing the curves, and placing the points in relation to them.

This is done in Figs. 31 and 32.

To pass to a more complicated case—that of a solution of haemoglobin in a mixture of salts, namely Ringer's fluid: taking $n = 2{\cdot}111$ and $K = {\cdot}00427$, a curve is produced which satisfies the properties of haemoglobin in Ringer's solution, as well as does the freehand curve of Barcroft and Camis[9].

From Ringer's solution we may pass to the still more complicated problem which is presented by blood.

Here we have the advantage, in some cases at all events, of working with curves upon which much more time and labour have been spent than have been claimed by the solutions of haemoglobin.

Some forty or more determinations have been made on the normal blood of Mr C. G. Douglas[10] of St John's College, Oxford, some by myself, some by Haldane and Douglas; and as our methods differed somewhat in detail, the fact that we arrived at the same result proves that Douglas' normal curve has been determined with as much accuracy as our present methods will admit of. Yet in spite of the great number of determinations which have been made, the curve as drawn freehand through the points is extremely close to that calculated from the equation, with the values $K = {\cdot}000212$, $n = 2{\cdot}5$. This curve and the corresponding points are given in Fig. 33. The curve differs very much from those of the solution given just previously, inasmuch as the S-shape is much more evident. This difference is due to the fact that the curve is determined in the presence of 40 mm. CO_2 pressure, which was the pressure of that gas in Douglas' alveolar air and presumably in his body generally.

Each test which we apply, and which is met satisfactorily, leads us on to another. We were drawn into the discussion which has formed the subjects of this present chapter and the last by the discovery that the bloods of different animals had not identical affinities for oxygen. The theory we are testing must therefore not only hold true for the blood of a particular person, but it must also hold good for the bloods of different species.

It has been tested in the case of man, sheep, dog and cat, and seems to be true for them all.

But the differences which have been found between different species apply to a less extent to different individuals, at all events to

different individuals of the human race. Probably my own blood has been studied in greater detail than that of any person except Douglas. It is quite certain that my blood differs from his. It is also certain that with a slightly different value for K a curve for my

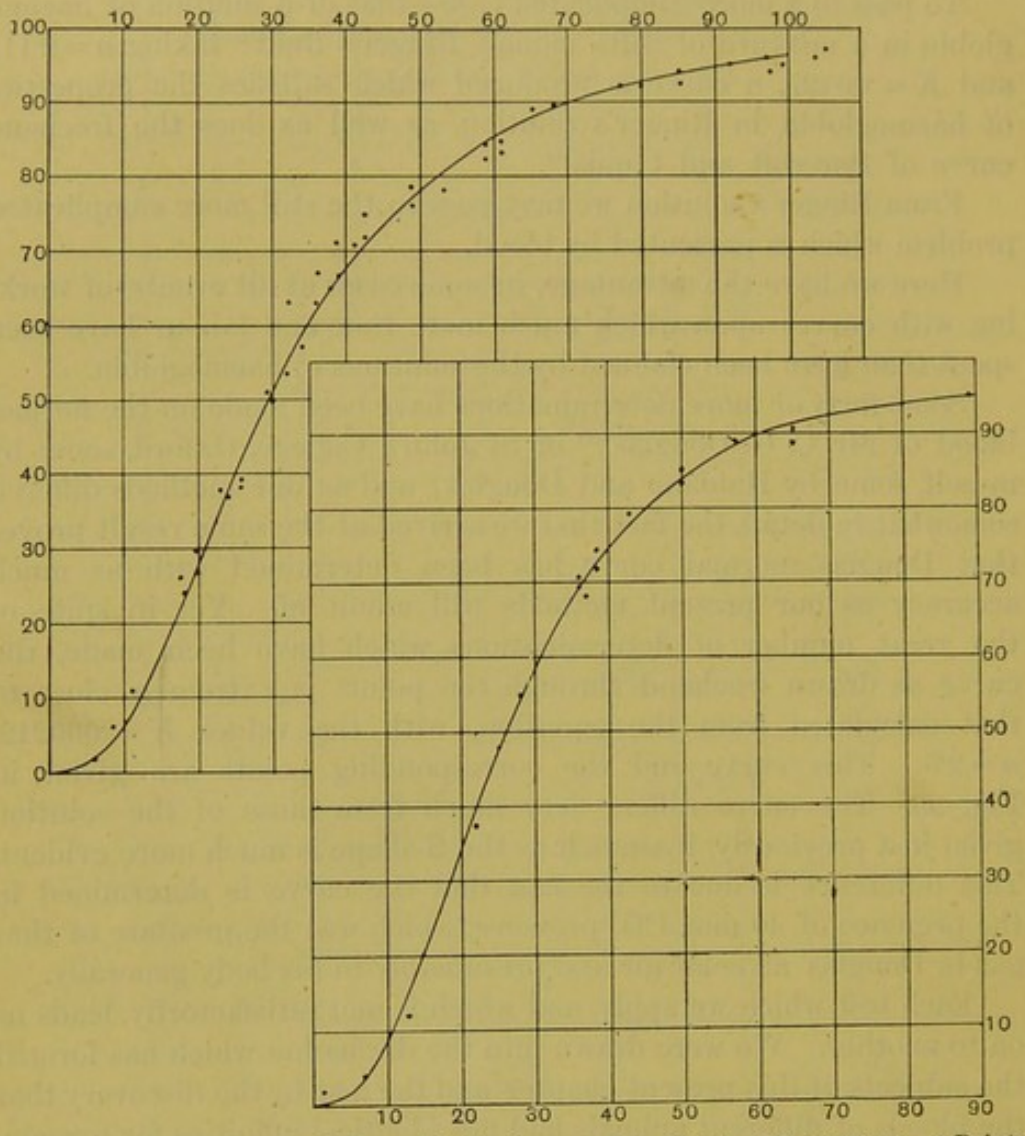


Fig. 33.—Douglas' blood and Barcroft's blood exposed to 40 mm. CO_2 pressure. • Points actually determined. Curves drawn from formula $y/100 = \frac{Kx^n}{1 + Kx^n}$. Ordinate = percentage saturation. Abscissa = oxygen pressure in mm.

blood can be calculated from Hill's equation, which fits the points which have been determined (Fig. 33).

The normal dissociation curves for Douglas' blood and of my own have been determined in the presence of CO_2 as I have said

above. The amount of CO_2 present has been in each case that present in the alveolar air. We arrive at a very interesting result, however, by studying the curves of the same blood with different quantities of carbonic acid. In a couple of words it is this: the equations for all such curves seem to* differ from one another in the value of K only, n remaining constant for the series. This is but another way of saying that the series consists of a single curve drawn to different horizontal scales and can be derived from the statement that the effect of the CO_2 is to lessen the effective concentration of oxygen.

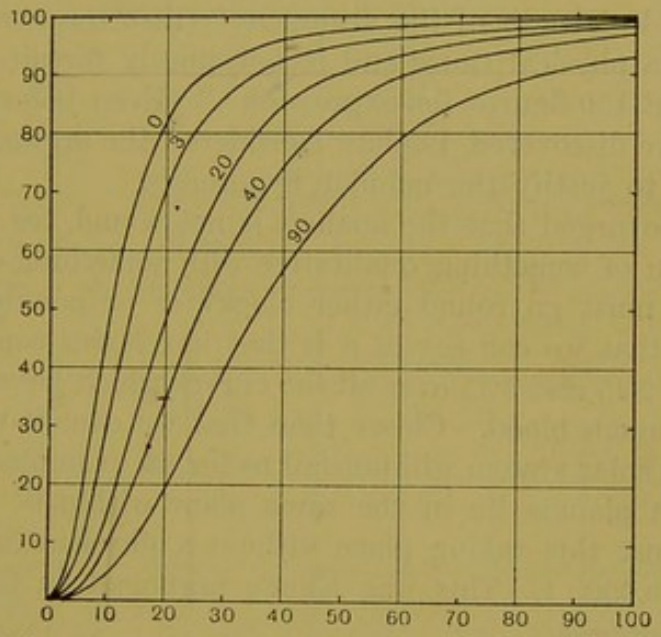


Fig. 34.—Dissociation curves of Barcroft's blood. Exposed to 0, 3, 20, 40 and 90 mm. CO_2. Ordinate = percentage saturation. Abscissa = oxygen pressure.

In Fig. 34 n is 2·5 and the values of K for the various CO_2 pressures concerned are

CO_2 pressure in mm.	0	3	20	40	90
K	·00258	·00130	·000505	·000292	·000135

The fact that n remains constant throughout the whole series of curves places the theory under discussion upon a wholly different stratum of probability.

Up to this point we have considered K and n as being merely mathematical constants though it is true they were arrived at by a physical process of reasoning; all that we have claimed for them is that by suitably changing them we can reproduce the curves obtained by analysis. Now however we are face to face with the fact that one

* See Appendix II.

of these remains constant over a large series of curves. It is very improbable that such a constancy is merely fortuitous.

The classical example of this principle was first put forward by Laplace with reference to the direction of the orbits of the members of the solar system. In its up-to-date form it is as follows: "...the tale of the asteroids has now approached five hundred and out of this huge number of independent planetary bodies there is not a single dissentient in the direction of its motions. Without any exception however they all perform their revolutions in the same direction as the sun rotates at the centre. When this great host is considered the numerical strength of the argument" (that the arrangement is referable to a physical cause and is not purely fortuitous) "would require about 150 figures for expression*." Even before any of the asteroids were discovered, Laplace considered the argument a strong enough one to justify the nebular hypothesis.

It may be urged that the analogy is not sound, for it looks like a comparison of something qualitative with something quantitative. The planet must go round either clockwise or counter-clockwise, whereas all that we can say of n is that it remains constant within the limits of 2·45 and 2·55 over all the curves which have been determined for human blood. Closer than that we cannot determine it. Even so, the solar system will not fail us for an example. The orbits of the seven planets lie in the same plane within 9° of arc. The chance against this taking place without a physical explanation is about 10,000,000 : 1. This was Kant's argument in favour of the nebular hypothesis.

We can calculate the value of n—on any one of the curves of which we have been speaking—to within about four or five per cent., and within these limits it is the same for all. I leave it to some mathematician to say what the chances may be of n being the same within four per cent. in a dozen curves, when, if it were a perfectly fortuitous mathematical expression, it might be anything between zero and infinity in any given case; but I have probably said enough to convince the reader that since n remains so constant it is probably the expression of some definite physical fact.

It is clear that any theory which applies to the formation of oxyhaemoglobin must also apply to CO-haemoglobin. Therefore the equation may be put to the further test of applying it to the parallel data with regard to the dissociation of carboxyhaemoglobin in the

* Quoted from the *Earth's Beginning* by Sir Robert S. Ball, 1909, p. 316.

presence of various pressures of carbon dioxide. It is not a little startling to find that the curves given in Fig. 34 for oxyhaemoglobin are almost superposable upon those in Fig. 29 given for CO-haemoglobin by Douglas, Haldane, J. S. and Haldane, J. B.[11] *. In the figure the curves or some at least of them are drawn in freehand.

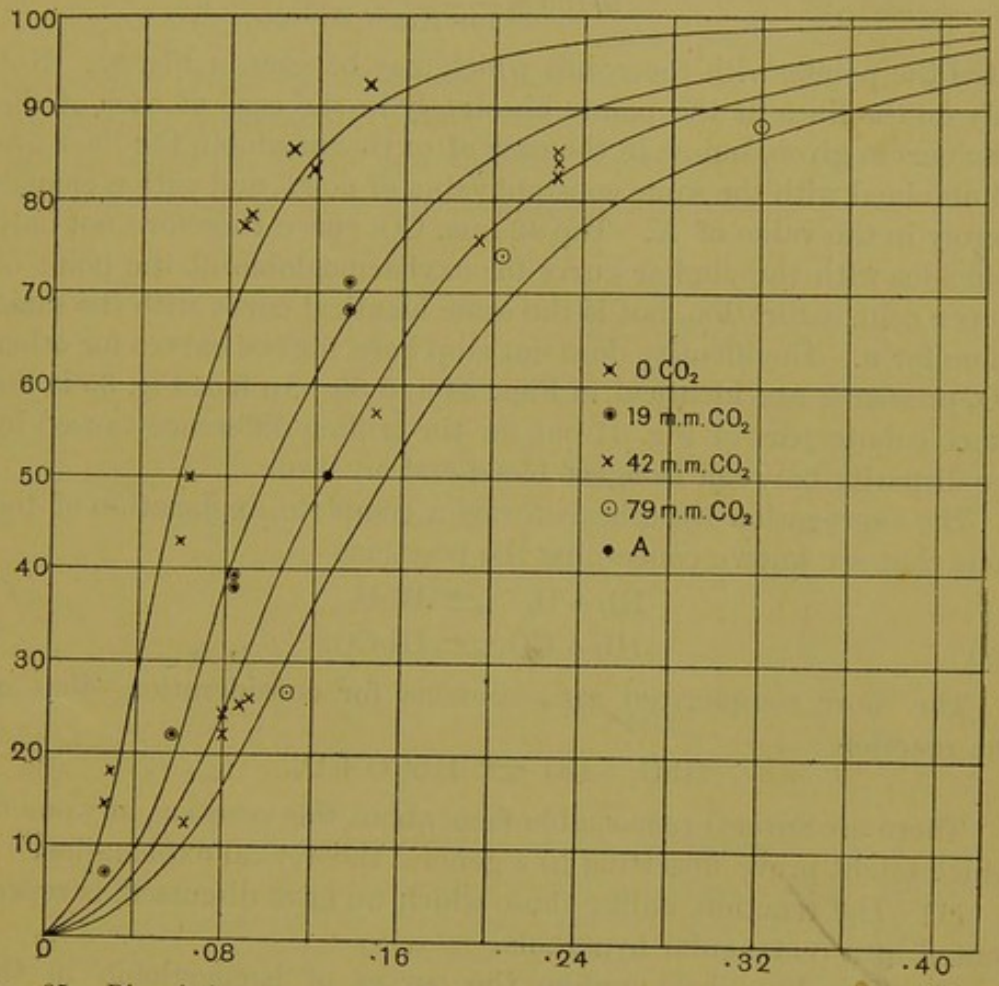


Fig. 35.—Dissociation curves of CO-haemoglobin. Curves drawn from formula $y/100 = \frac{Kx^n}{1+Kx^n}$. Ordinate = percentage saturation with CO. Abscissa = CO pressure in mm. Points indicated are the determinations of Haldane and Douglas.

The points determined by these observers have been transcribed from their figure as faithfully as possible and are shown in Fig. 35. The figure is drawn to a somewhat different scale from theirs for the purpose of comparing the oxygen dissociation curves with the

* The aggregation theory has been modified by these authors in certain respects. My reasons for preparing the original form of the theory are given in the *Biochemical Journal*, VII, p. 481.

CO-haemoglobin curves. It is so chosen that the middle point of the CO curve (Fig. 35, *A*) at 42 mm. CO_2 pressure coincides on paper with the middle point for the corresponding curve of oxyhaemoglobin.

I then tested whether curves drawn from Hill's equation

$$y/100 = \frac{Kx^n}{1 + Kx^n}$$

fitted the points, with the result which may be seen in Fig. 35. Not only do the lines fit the points absolutely in the case of each of the four curves given, but as in the case of oxyhaemoglobin the lines are all obtained with the same constant value of n, 2·5, and with a change merely in the value of K. The 42 mm. CO_2 curve therefore not only coincides with the similar curve for oxyhaemoglobin at the point of 50 per cent. saturation, but is the same identical curve with the same value for n. The identity does not stop here, for the curves for other CO_2 pressures are identical in Figs. 34 and 35. In fact Fig. 35 is the exact counterpart of Fig. 34 but for the trifling difference caused by the disparity between Douglas' blood and my own.

The aggregation hypothesis forms a complete explanation of the facts that are known concerning the reactions

$$Hb + O_2 \rightleftarrows HbO_2$$

and

$$Hb + CO \rightleftarrows HbCO.$$

The more complicated case remains for consideration—that of the reaction

$$HbO_2 + CO \rightleftarrows HbCO + O_2.$$

There are several remarkable facts about this reaction, any one of which might prove upsetting to a general theoretical explanation.

(1) The reaction, unlike those which we have discussed, is represented by a rectangular hyperbola.

(2) This hyperbola, unlike the curves of haemoglobin in the presence of oxygen, or of CO separately, is almost unaffected by acids and by salts.

(3) While the curve of the reaction

$$HbO_2 + CO \rightleftarrows HbCO + O_2$$

is a rectangular hyperbola in the presence of an ample supply of O_2 and CO, it ceases to be so when there is insufficient CO and O_2 present to saturate the Hb, i.e. when there is a considerable quantity of reduced haemoglobin present. Under these circumstances the entire form of the curve changes in the way which is shown in Fig. 36[11].

Perhaps the most telling feature of the aggregation theory is its adequacy to meet these unexpected and peculiar requirements.

At first sight it wholly failed to do so. If two assumptions be made, the matter, as Hill has shown[8], becomes quite clear. Of these one has already been made: it is the instability of unsaturated compounds; the other is that the ratio $\frac{\text{affinity for CO}}{\text{affinity for } O_2}$ is even greater in the case of the half-saturated than in the case of the completely unsaturated compounds. With regard to the first assumption it may be said that (1) no unsaturated oxide has ever been isolated

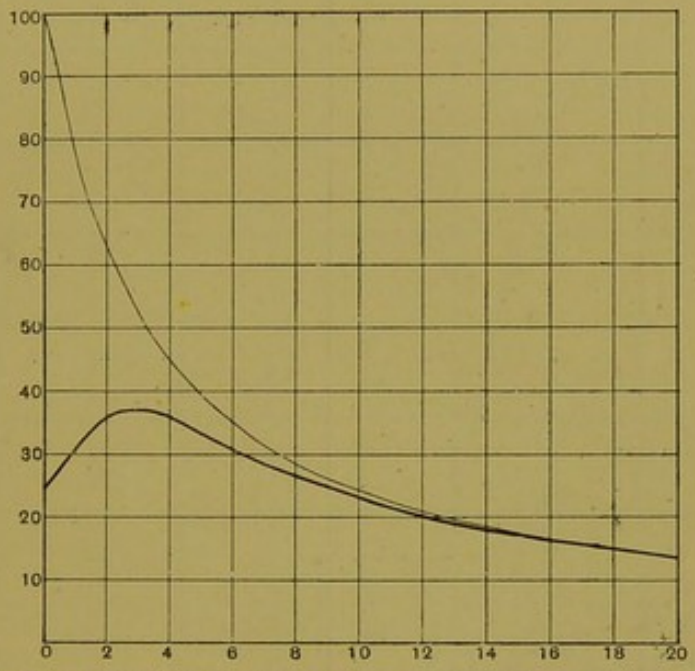


Fig. 36.—Heavy line represents dissociation curve representing partition of haemoglobin between O_2 and CO at 38° C. CO pressure = 0·00854 % of an atmosphere throughout. Light line represents the corresponding hyperbola.

(2) there is no transitional spectrum between that of oxy- and of reduced haemoglobin.

It remains only to state the numerical relation between K and the carbonic acid pressure. It will not surprise the reader to hear that this relation is similar in the cases of CO-haemoglobin and oxyhaemoglobin. The two fall on the same curve, the difference lying only in the scale on which the curve is plotted. The temptation to speculate on the form of this curve is considerable, but till a greater number of points are forthcoming the temptation must be repressed. It must suffice to say that it is not a parabola—the curve which

would occur if the influence of the CO_2 were simply to cause a greater or less degree of adsorption of the haemoglobin. It is of interest to record that the influence of CO_2 is as evident at low temperatures as at high ones. In this respect there is a sharp contrast between the effect of CO_2 and also of the acids engendered by oxygen want, and the effect of oxygen want itself (a vacuum), on the reaction

$$Hb + O_2 \rightleftarrows HbO_2.$$

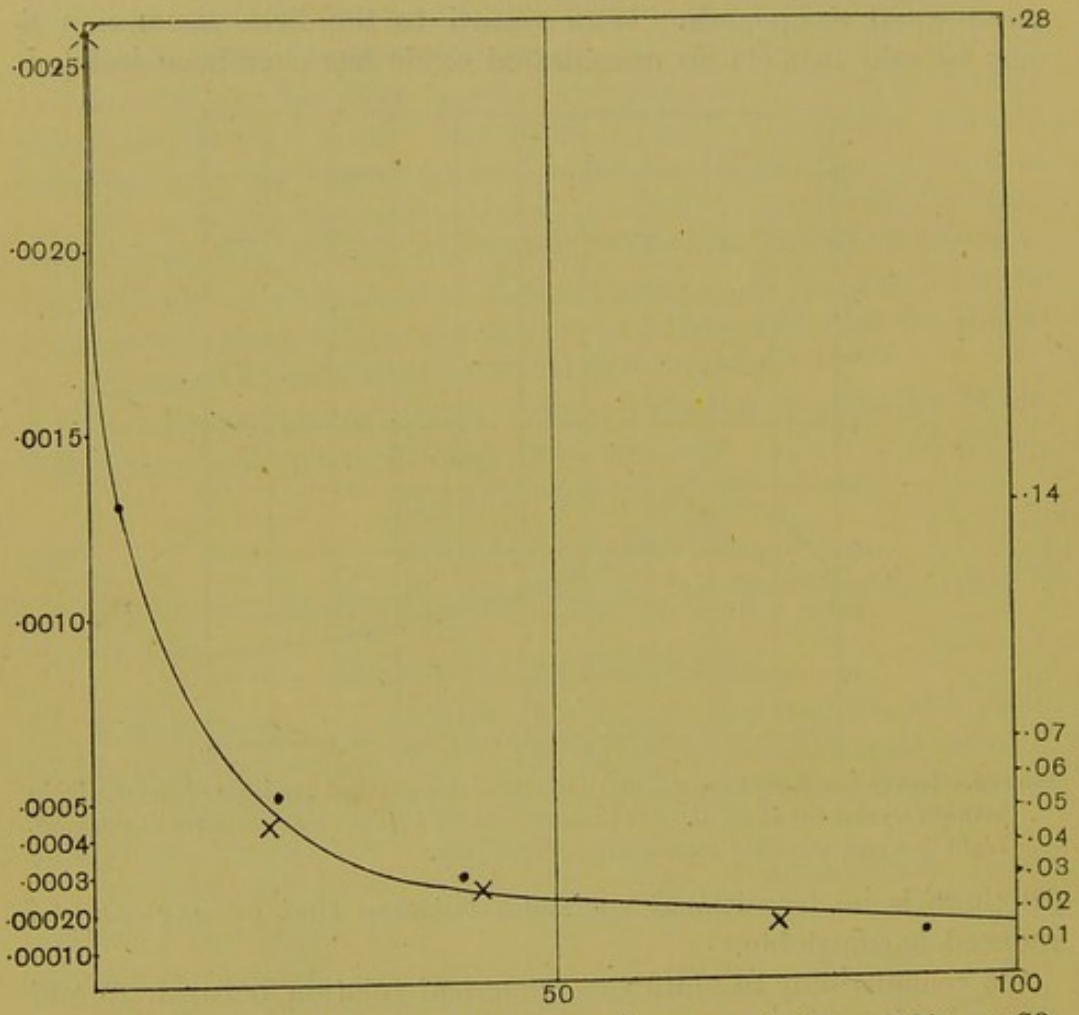


Fig. 37.—Ordinate = K. Abscissa = CO_2 pressure in mm. • Oxyhaemoglobin. × CO-haemoglobin. (Left hand = oxyhaemoglobin, right hand = CO-haemoglobin.)

The reduction of the haemoglobin by the acids goes on both at 37° C. and at room temperature with equal activity, but the reduction of haemoglobin by a vacuum is so slow at low temperatures that one is tempted to conceive of the presence of acids rather than of absence

of oxygen as being the factor which determines the reduction of blood in cold-blooded animals.

In the opening sentences of this book I alluded to the speculations which I sometimes allow myself. I drew a contrast between morphology and biochemistry in their relation to natural selection. The fundamental basis of natural selection is variation, but it would

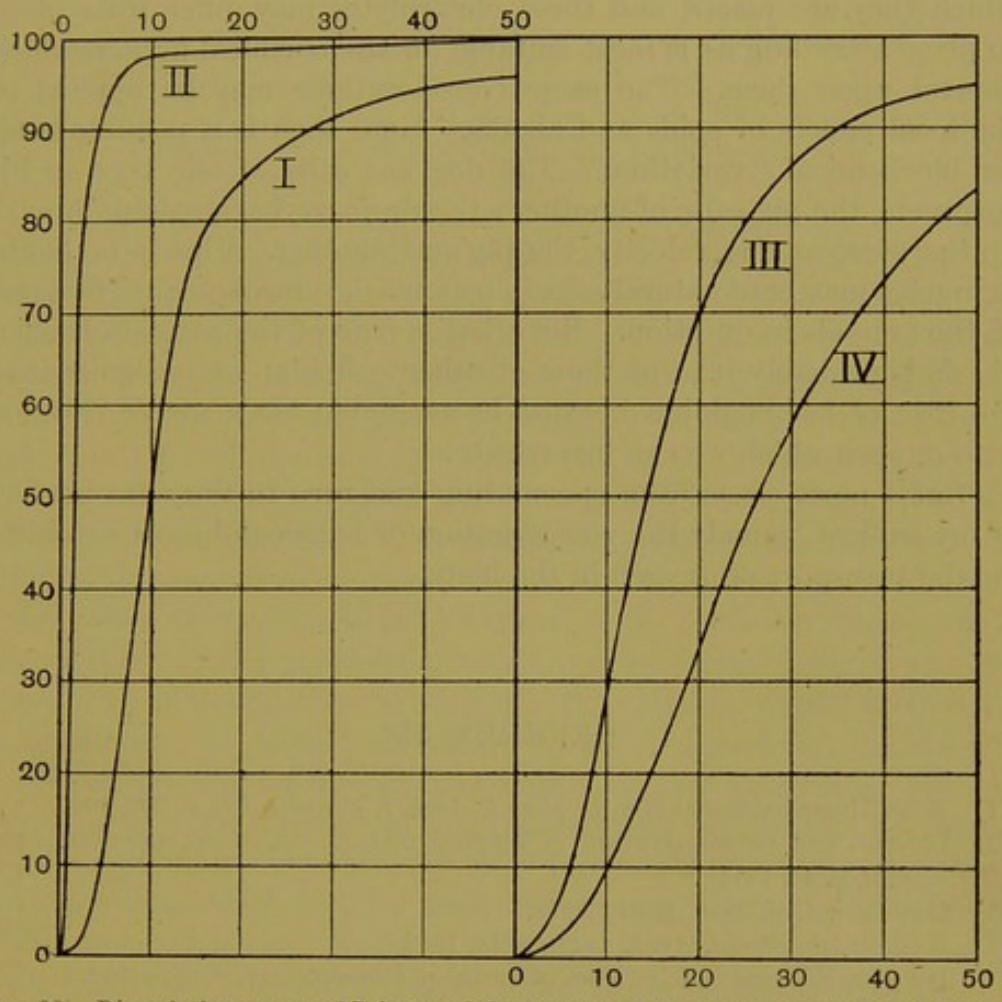


Fig. 38.—Dissociation curves of I, haemoglobin in ·9 % KCl in presence of 25 mm. CO_2 at 15° C.; II, ditto in absence of CO_2; III, blood at 37° C. exposed to 3 mm. CO_2; IV, ditto exposed to 20 mm. CO_2.

seem that the chemical properties are fixed. They are immutable properties of the substance. The reader will not have read the preceding chapters without discovering for himself that a way out of this apparent *impasse* has been discovered. At all events in the case of the reaction $Hb + O_2 \rightleftarrows HbO_2$ this is so.

The velocity of this reaction is dependent to some extent upon

temperature, that is so much to the good, but the temperature of the body is roughly speaking the same throughout. It has to suit itself to a thousand reactions. But in virtue of the fact that the haematin is allied to a protein the rate of reaction is influenced by the degree of aggregation of the protein elements, and this may "vary" with the concentration and nature of the electrolytes in which they are placed and these electrolytes may differ from place to place according as is most suitable for the chemical actions which depend upon them. The same considerations may be applied to the local effects of acids and alkalis. Here then is a possible basis for biochemical "variation." The dog has salts of one type in his corpuscle, the pig salts of another; the dog's oxyhaemoglobin breaks up therefore at one velocity, the pig's at another. This is no doubt as much a matter of natural selection as are the morphological features of the animals in question. But what is true of the reaction haemoglobin is probably true of those of other colloids; we recognise it in the case of haemoglobin because haemoglobin has a simple reaction with oxygen which we can investigate.

But I must cease from speculating and turn to the next portion of my subject, namely the consideration of haemoglobin as a vehicle for the transport of oxygen in the body.

REFERENCES

(1) Bohr, Hasselbalch and Krogh, *Skand. Arch. f. Physiol.* XVI, p. 390, 1907.
(2) Barcroft and Orbeli, *Journal of Physiol.* XLI, p. 353, 1910; Barcroft, *Ibid.* XLII, p. 44, 1911.
(3) Mathison, *Ibid.* XLIII, p. 347, 1911.
(4) Hasselbalch, *Skand. Arch.* XXVII, p. 13, 1912.
(5) Douglas, Haldane and Haldane, *Journal of Physiol.* XLIV, p. 237, 1912.
(6) Barcroft and Poulton, *Ibid.* XLVI.; *Proc. Physiol. Soc.* Feb. 1913.
(7) Hill, *Journal of Physiol.* XL.; *Proc. Physiol. Soc.* p. iv, 1910.
(8) Hill, *Biochemical Journal*, VII, p. 471, 1913; Barcroft, *Ibid.* p. 481.
(9) Barcroft and Camis, *Journal of Physiol.* XXXIX, p. 118, 1909.
(10) Barcroft, *Ibid.* XLII, p. 44, 1911.
(11) Douglas, Haldane and Haldane, *Ibid.* XLVI, p. 275, 1912.
(12) Barcroft and King, *Ibid.* XXXIX, p. 375, 1909.

PART II

THE PASSAGE OF OXYGEN TO AND FROM THE BLOOD

CHAPTER VI

THE CALL FOR OXYGEN BY THE TISSUES

THE classical work of Pflüger[1] on the combustion of living material settled for all time, it seems to me, the logical order in which the constituent processes of respiration should be treated.

The issue before Pflüger may be stated in a few words. Is the quantity of oxygen taken up by the cell conditioned primarily by the needs of the cell, or by the supply of oxygen? The answer was clear, the cell takes what it needs and leaves the rest. Respiration therefore should be considered in the following sequence. Firstly the call for oxygen, secondly the mechanism by which the call elicits a response, the immediate response consisting in the carriage of oxygen to the tissues by the blood and its transference from the blood to the cell. Thirdly in the background you have the further mechanism by which the blood acquires its oxygen. It is not the habit of writers on respiration to adopt this order, quite the contrary, but their reason for placing pulmonary respiration in the foreground of the picture is a purely practical one—pulmonary respiration is more evident, both to the eye and to the understanding. I imagine they will not quarrel with me if I make an attempt to treat the matter in what appears to be its logical sequence and make some estimate of the call which the blood has to meet, before entering into a discussion of how the call is to be met.

The present chapter will deal with the following theme: "There is no instance in which it can be proved that an organ increases its activity, under physiological conditions, without also increasing its demand for oxygen."

The importance of the principle that increased activity of an organ entails a call for oxygen has been self-evident ever since the days of Ludwig. The reason why the work has not been carried out on an extensive scale till recently is because the old methods were quite inadequate.

Skeletal muscle. Nevertheless I cannot pass over the bold attempt made by Chauveau and Kaufmann[2]. My reasons for drawing attention to their work on the levator labii superioris and the masseter muscles of the horse depend less upon the results which they obtained (some of which do not altogether inspire confidence), than on the grasp which they had of the problem. Their work was conceived along physiological lines; their idea was to determine the gaseous exchange of the muscles with the least possible abnormality in the conditions of the animal. They took no elaborate precautions against clotting of the blood; they simply had recourse to the horse as an animal whose blood did not readily clot. They wanted a considerable quantity of blood, for their samples for analysis had to be of the order of 100 c.c. each. They therefore chose a smallish muscle and one belonging to an animal so large that the bleeding entailed was not felt by the animal. They record their surgical operation as being so simple that the animal did not cease chewing its oats while they were at work: thus they reaped the double advantage that the muscles which they were studying had (*a*) a normal stimulus and (*b*) a metabolism which was unhampered by anaesthetics whether in rest or activity. There is one final point in which one could wish that other workers had been able to follow Chauveau and Kaufmann—they made an attempt to measure the degree of activity which was induced in terms of absolute units of energy. For this purpose they made measurements both (*a*) of the work done, by attaching a weight to the muscle, and also (*b*) of the heat given out by the muscle during its contractions. Nothing in short could have been more complete than the scheme of their research. It has been a loss to science that the actual number of experiments performed was small and that the complete scheme was not carried through in any one experiment.

The following is an example of the figures which they obtained:

Extent of Gaseous Exchange in the Levator Labii Superioris of the horse in c.c. per gram of muscle per minute.

	Rest		Activity	
	Oxygen absorbed	CO_2 given out	Oxygen absorbed	CO_2 given out
1	0·0032	0·0019	0·054	0·063
2	0·0079	0·0058	0·014	0·018
3	0·0028	0·0026	0·010	0·013

The above figures leave no room for doubt that the quantity of oxygen used increases during activity.

Recent work on Skeletal Muscle. Quite recently the inquiry has been pushed a good deal further by Verzàr[3], who investigated the time relations of the oxidation as compared with the muscular activity. His technique was very different from that of Chauveau and Kaufmann; armed with the modern methods by which it is possible to work on minimal quantities of blood, he made a muscle nerve preparation of the cat's gastrocnemius muscle. The general relations of the dissection will be seen in the accompanying figure.

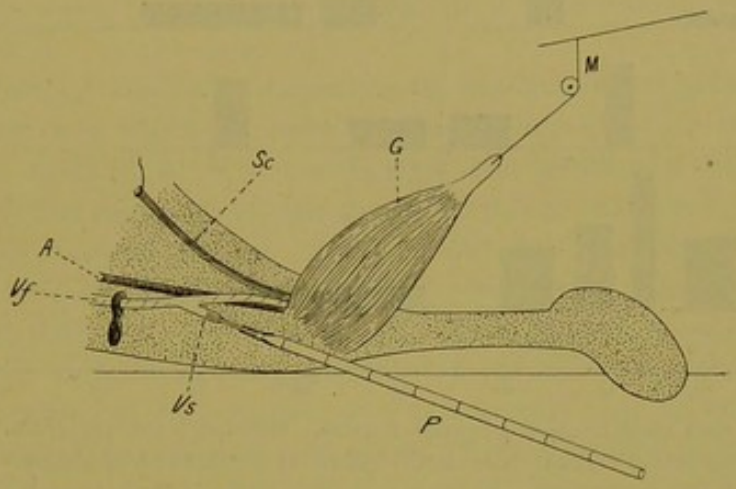


FIG. 39.—*G* = gastrocnemius muscle. *Sc* = sciatic nerve. *A* = artery. *Vf* = femoral vein. *Vs* = saphenous vein. *P* = pipette. *M* = myograph.

The veins which contributed to the femoral vein below the saphenous were all tied with the exception of that which came from the gastrocnemius muscle. A cannula was introduced into the saphenous vein, and when a sample of blood was required the clip was removed from the saphenous vein and placed on the femoral vein above the junction of the two vessels. The blood was collected into a graduated 1 c.c. pipette so that the time taken for 1 c.c. to flow through the muscle might be measured. The blood was prevented from clotting by the intravenous injection of hirudin. The muscle was thrown into contraction by the application of an electrical stimulus to the sciatic nerve. The muscle lifted a weight, doing about 70 grm. cm. of work at the beginning of each tetanus.

The following diagrams with the figures on which they are based show quite distinctly the time relations of the tetanus and the call for oxygen. The latter takes place:

(1) During the contraction. The adequacy of the oxygen supply depends upon the rate of blood flow in the muscle and this in turn depends upon the pressure in the blood vessels.

(2) After the contraction. The response to the call is in every case at its maximal value after the tetanus passes off, which shows that the call for oxygen continues for some time after the actual work is performed which the oxidation is designed to meet.

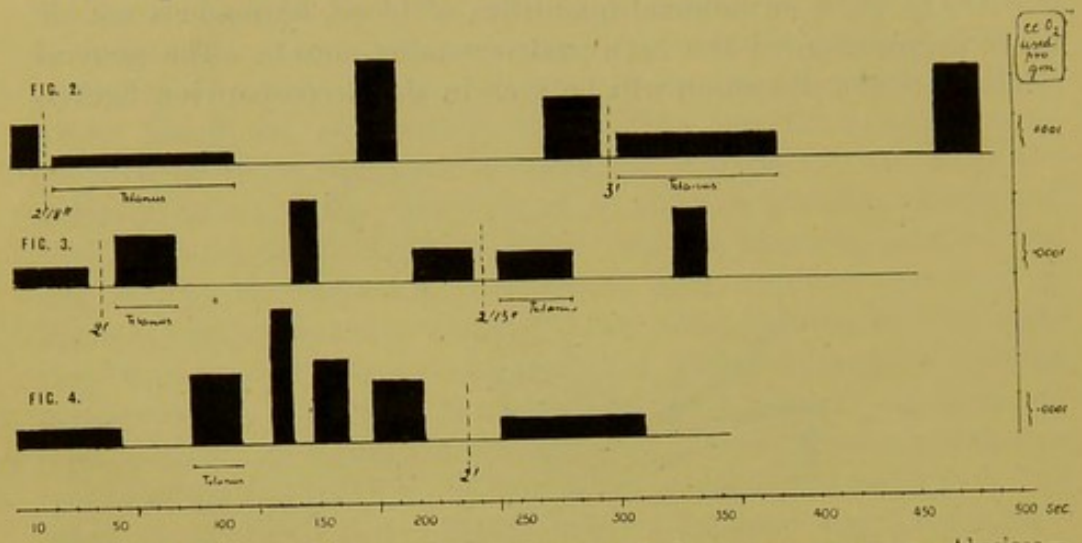


FIG. 40.—Oxygen used by gastrocnemius muscle. Ordinate = c.c. per gram. Abscissa = seconds. The dotted vertical lines signify points at which the kymograph was stopped.

Exp.		C.c. O_2 necessary to saturate 1 c.c. blood		Percentage saturation of oxygen		O_2 pressure in blood mm. Hg		Rate of blood-flow c.c. per min.	C.c. O_2 used by muscle		Total oxygen capacity of 1 c.c. blood (c.c. O_2)	Wt. of muscle gm.
		Venous	Arterial	Venous	Arterial	Venous	Arterial		per min.	per gm. min.		
16	Normal	·057	·013	67	93	48	100	4·30	·190	·00670	·175	28·6
	Tetanus	·096		45		37		·67	·056	·00196		
	61″ later	·163		7		11		3·00	·450	·01580		
	73″ ,,	·136	·007	22	96	27	100	2·14	·276	·00960		
	Tetanus	·158		10		14		·75	·114	·00397		
	80″ later	·156		11		15		2·61	·390	·01360		
17	Normal	·046	·006	62	95	45	100	1·54	·062	·00320	·121	19·7
	Tetanus	·084 (?)		31		32		2·00	·144	·00770		
	57″ later	·061		50		39		4·45	·245	·01250		
	45·5″ ,,	·056	·007	54	94	41	100	2·07	·102	·00520		
	Tetanus	·061		50		39		1·62	·088	·00450		
	50″ later	·069		43		36		3·52	·215	·01090		
18	Normal	·055	·012	69	93	49	100	1·16	·050	·00303	·179	16·5
	Tetanus	·085		53		41		2·40	·178	·01080		
	15″ later	·068		62		45		6·00	·336	·02030		
	11″ ,,	·071		60		44		3·52	·208	·01260		
	11″ ,,	·076	·011	58	94	43		2·40	·154	·00935		
	146″ ,,	·072	·007	60	96	44	100	·90	·059	·00358		

Verzàr's results have reformed our view as to the terms in which we should express the increased oxidation which results from increased activity. In treating of Chauveau and Kaufmann's work we followed their method of stating their result when we said that the "coefficient of oxidation" was increased say thirty-fold. This is clearly an inadequate statement of the case. At a certain point in the train of events the oxidation was increased to that extent. Really we should ask, How much extra oxygen was used by the muscle as the result of such and such a piece of work? In order to answer this question we must, it is true, consider the events taking place *during* the work, but just as necessary is it to consider those taking place *after* it.

The third of the three experiments quoted above gives the most satisfactory data which we have at the present time for relating the functional activity of the muscle and the oxidation taking place within it. The duration of this experiment was long enough to allow the oxygen consumption to return almost to its original level. We can therefore, by calculating the whole increase in the oxygen used during the period of the experiment, obtain a minimal value for that required—a value which is probably not very far from the true one. Of the experiment in question one can say (1) that as the result of the stimulus given, which lasted about 25 seconds, the muscle showed increased oxygen intake for 220 seconds at least, and (2) that in this time it used up ·753 c.c. of oxygen as against ·260 which it would have used up at its normal rate; therefore the stimulus which was given was responsible for at least ·5 c.c. of oxygen used by the muscle.

The fact that the increased oxygen consumption of the muscle survived the increased functional activity must be viewed in conjunction with the recent work of Hill [4], who investigated the relation in time of the functional activity of amphibian striated muscle and the heat-evolution of the same.

Hill's method was as follows: he compared the curve of deflection of a galvanometer registering thermo-electrically the rise of temperature of a live muscle when stimulated, with the curve of deflection given by the rise of temperature due to electrical warming of the same muscle after death. He found that the curve of deflection (coming back to the base line in 4 or 5 minutes by reason of heat-loss) was the same for a muscle in nitrogen as for a muscle warmed after death by a very short tetanising current: there is therefore in nitrogen no heat-production except in the few moments immediately following an excitation. In O_2 however the curve of

deflection of the live muscle continually diverged from the control curve due to electrical warming for a short period. The only possible explanation of this is that heat is being produced by the muscle in O_2 for long periods after the contraction is over. For examples of this see Figs. 41 and 42. The fall of the deflection is due to heat-loss and, where there is no delayed heat-production, is a simple exponential curve. From the control curve of electrical warming it is possible to calculate the coefficient of heat-loss in the case of any particular thermopile used, and from this to ascertain, in the case of the live muscle in O_2, the true curve of heat-production.

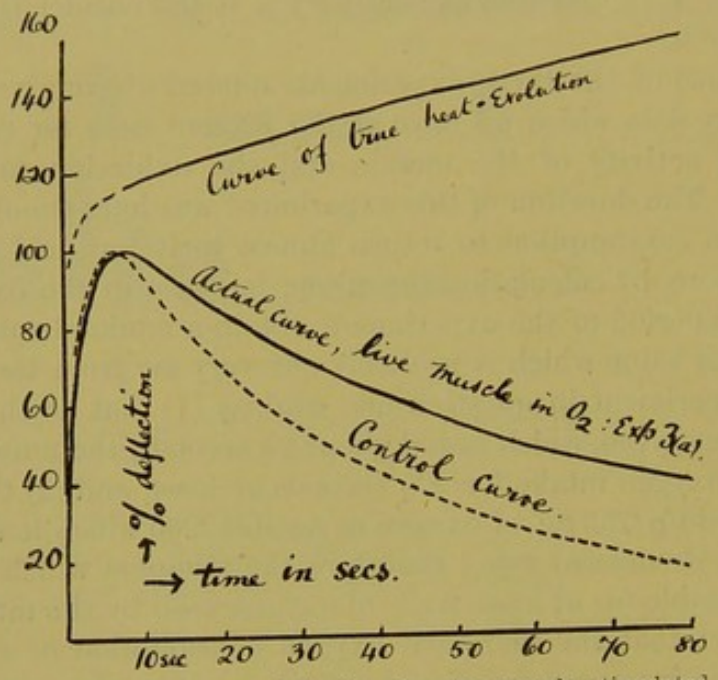


Fig. 41.—Curves of galvanometer deflection for live muscle stimulated, and for dead warmed (control). Also calculated curve of true heat-production of live muscle, i.e. curve corrected for heat-loss.

It was found that approximately as much more heat is produced in the 4 or 5 minutes following a single shock in O_2 as was produced in the first few moments after excitation occurred. There is therefore, but only in the presence of O_2, a very large recovery heat-production lasting for some minutes after the contraction is over, which recovery heat-production one can scarce but associate with the oxidative removal of fatigue products (lactic acid, Fletcher and Hopkins [12]).

It was found moreover that any process, as e.g. a previous tetanus, which uses up the O_2 existing already in the muscle delays or abolishes the recovery heat-production. Hill concluded that oxygen is used,

and the delayed heat-production occurs in recovery processes: and that these processes cannot occur in the absence of oxygen. He argued moreover that his results were in favour, neither of the hypothesis of intra-molecular oxygen, nor of the idea that oxygen is used as required in the simple processes of energy liberation. He suggested in fact that his results were in favour of the old view that oxygen is used largely in the processes whereby the molecular

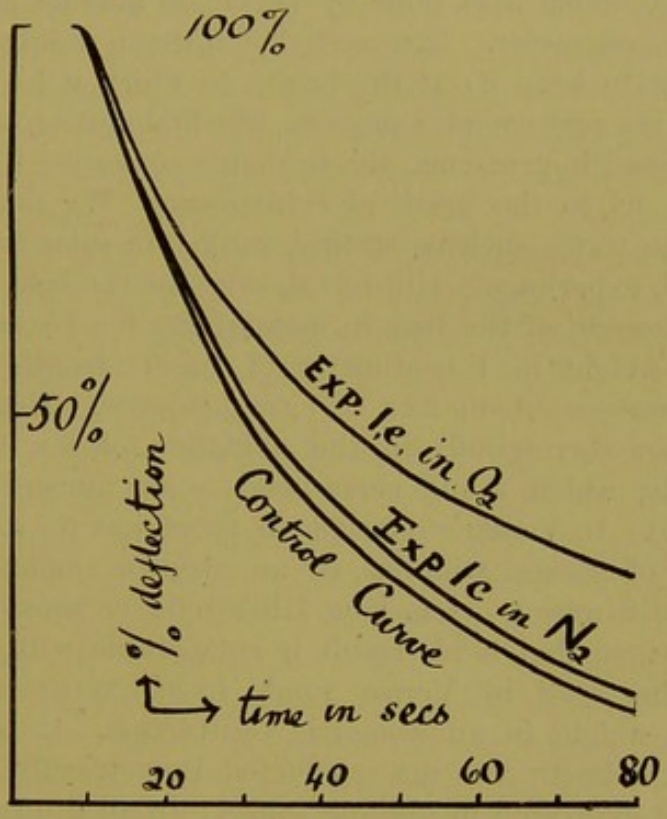


Fig. 42.—Galvanometer deflection for rise of temperature of muscle excited in nitrogen, and later in oxygen, and finally warmed when dead (control). Note that the curve of the deflection for the live muscle in nitrogen very nearly coincides with the control curve, and that the curve for the live muscle placed in oxygen after nitrogen is considerably displaced to the right.

machine—like a steam-engine charging an accumulator—builds up bodies containing considerable amounts of potential energy which (like the accumulator) can be discharged whenever desired on subjecting the tissues to appropriate stimuli: that oxygen is used in maintaining the activity, the state of potential energy, of the organism, and is therefore largely used after activity has occurred in preparation for the next period of activity*.

The construction which is to be put on these experiments is that

* I am indebted to Hill for the above statement of his results.

the chemical activity, whether expressed as heat or as the call for oxygen, is not merely something which accompanies the contraction of the muscle, but the contraction sets going a chain of chemical events which are necessary for the restitution of the muscle into its former state; that the increased functional activity is responsible for the increased oxidation is certain.

In the light of what has since been published one is inclined to wish that the actual work done by Verzàr on muscles had been of a more definite character. It consisted of lifting a weight and keeping it (or failing to keep it) at the height to which it had been lifted. Clearly this is a very complex process. The first portion of it is readily to be expressed in grm.-cms., the second is somewhat illusory and at once brings us to the brink of controversy. We must avoid the use of vague terms such as statical work. In some further, as yet unpublished, experiments Hill has shown that the heat produced by a sartorius muscle of the frog in maintaining for 1 second a tension of 1 gram weight in 1 centimetre of muscle length is, including recovery processes, about 25×10^{-6} gram-calories. This exceedingly large number corresponds to the oxidation of 6×10^{-9} grams of carbohydrate, which would correspond to an amount of O_2 used, $4{\cdot}4 \times 10^{-6}$ c.c. In Verzàr's experiment there was 0·5 c.c. of O_2 used, as a result of 25 sec. stimulus, or an average amount of 0·02 c.c. per sec. In a muscle 5 cm. long Hill's number should give about 22×10^{-6} c.c., so that if his result is comparable with Verzàr's, the gastrocnemius used by Verzàr would have exerted a tension of 1000 grams weight in an isometric contraction. It is a pity that Verzàr's experiment was not conducted isometrically and the tension exerted expressed in absolute units. In all future work on the subject, isometric contractions, as Hill has repeatedly urged, should be used. In any case it is very striking that the tension that could be exerted, according to Hill's figures, is exactly of the right order of magnitude. The gastrocnemius preparation of the cat, as used by Verzàr, could certainly lift about 1000 grams weight. It would be of the greatest interest to ascertain exactly the amount of O_2 used by a muscle in maintaining a tension, per second, per gram weight of tension maintained, per centimetre of muscle length. As Hill has urged, the tension exerted and not the work done is the fundamental quantity in the muscle: and therefore the O_2 used in maintaining unit tension for unit time on a muscle of unit length is the fundamental unit of oxidation.

The Heart. The pioneer work on this subject was carried out by

Dixon and myself[5]: it was a simple investigation into the question we are discussing, namely whether the oxygen consumption and the carbonic acid production of the heart varied with the functional activity. I call it the pioneer work because in the light of the beautiful researches which have since been performed on the subject by Rohde[6] and his colleagues in Heidelberg and by Evans[7] at University College, London, it seems now as I read it over but "poor stuff"; nevertheless, I remember, we were not a little proud of it at the time it was done, for to tell the truth it tested our powers to the uttermost and I can only claim for it what Dr Johnson claimed for the preaching of women, "Sir, a woman's preaching is like a dog's walking on its hind legs, it is not well done but you are surprised to find it done at all." However it did prove the point at issue in a primitive sort of way. Since many of the methods of altering the functional activity which we chose have not been tested in the more finished work of our successors and as the points which we raised are those which have been elaborated by them, it forms a suitable introduction to the later work.

The first point about the work in which it falls short of the ideal is that it is not strictly quantitative—by which we mean that while the gas measurements were quantitative there were no other records of the changes in activity than the obvious alterations shown by the graphic records of the heart's contraction. There is therefore no means of judging of the relation between the functional activity of the heart and the gaseous exchange, other than a comparison of the figures for the blood gas exchange with the tracing.

This comparison we therefore proceed to give.

(*a*) The first case is that of cardiac augmentation with adrenalin.

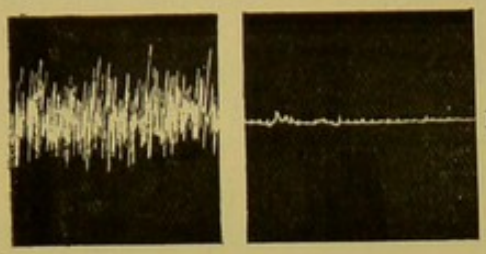
Oxygen used per gram per min.

Period I	Before adrenalin	0·010 c.c. 0·010 c.c.
Period II	After adrenalin	0·045 c.c. 0·045 c.c.

I II

FIG. 43.—Showing two periods, preceding and succeeding injection of adrenalin.

(*b*) Before and after pilocarpine.

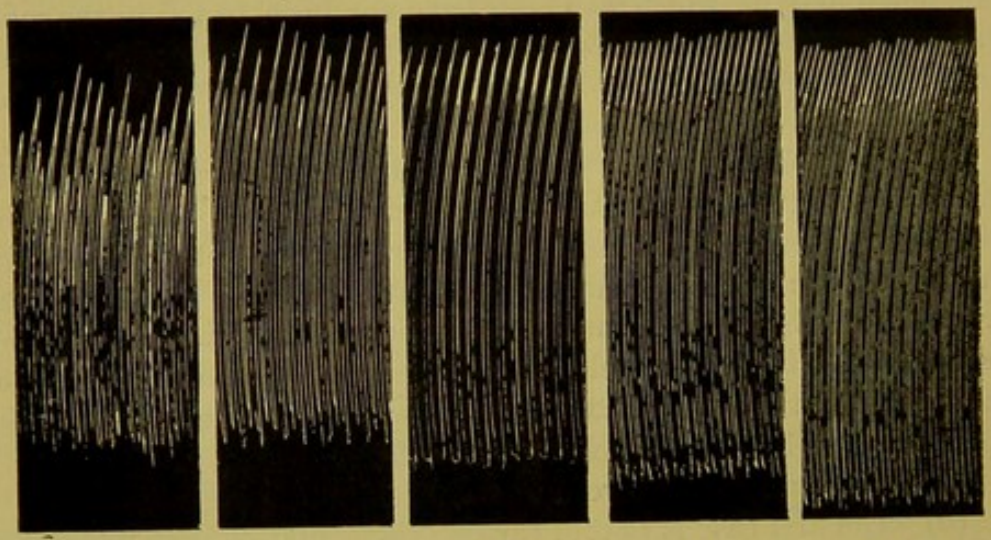


I II

Period	Gas per gram per min.	
	Oxygen used	CO_2 given out
I Before pilocarpine	0·050	0·048 c.c.
II After pilocarpine	0·010	0·003 c.c.

Fig. 44.—Pilocarpine injected between periods I and II.

(*c*) Effect of atropine following on that of pilocarpine.

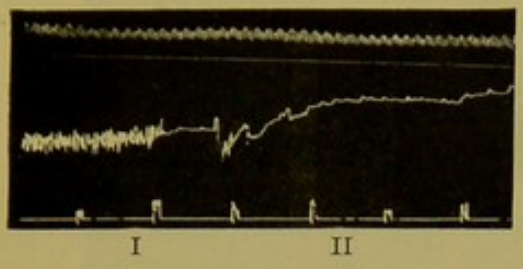


I II III IV V

Fig. 45.—Record of puppy's heart. Upstroke=systole. Period I is normal. Period II shows the effect after injecting 5 mgs. of pilocarpine and Period III after 20 mgs. Periods IV and V show the recovery of the heart after two successive doses of atropine.

Period		Gas per gram per min.	
		Oxygen	CO_2
I	No drug	·033	·041
II	After 1 c.c. 0·5 % pilocarpine	·014	·036
III	,, 1 c.c. 2 % pilocarpine	·009	·003
IV	,, 2 c.c. 2 % atropine	·015	·005
V	,, 3 c.c. 2 % atropine	·021	·008

(*d*) The effect of potassium chloride on a feebly beating heart.



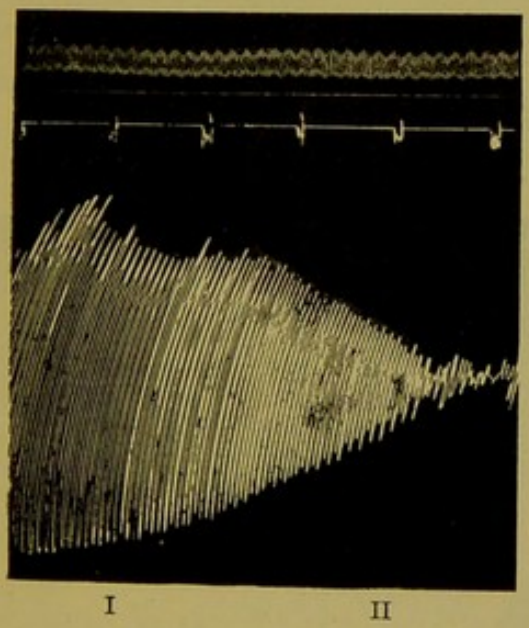
Period		Gas per gram per min.	
		Oxygen	Carbonic acid
I	Before KCl	·030	·061
II	After ,,	·027	·048
,,	Later	·020	·020
,,	Later	·012	·007

Fig. 46.—Before and after KCl.

(*e*) The effect of barium chloride following upon potassium chloride (the same heart as above).

	Oxygen per gram per min.
Fig. 46. Period II, under influence of KCl.....	·012
Fig. 47. Period I, after injection of $BaCl_2$	·040

(*f*) The effect of a further dose of barium chloride which induces contraction.



	Gas per gram per min.	
	Oxygen	CO_2
Period I	·040	·010
Period II.....	·030	·013

Fig. 47.—Showing the effect of barium chloride.

(*g*) Effect of chloroform.

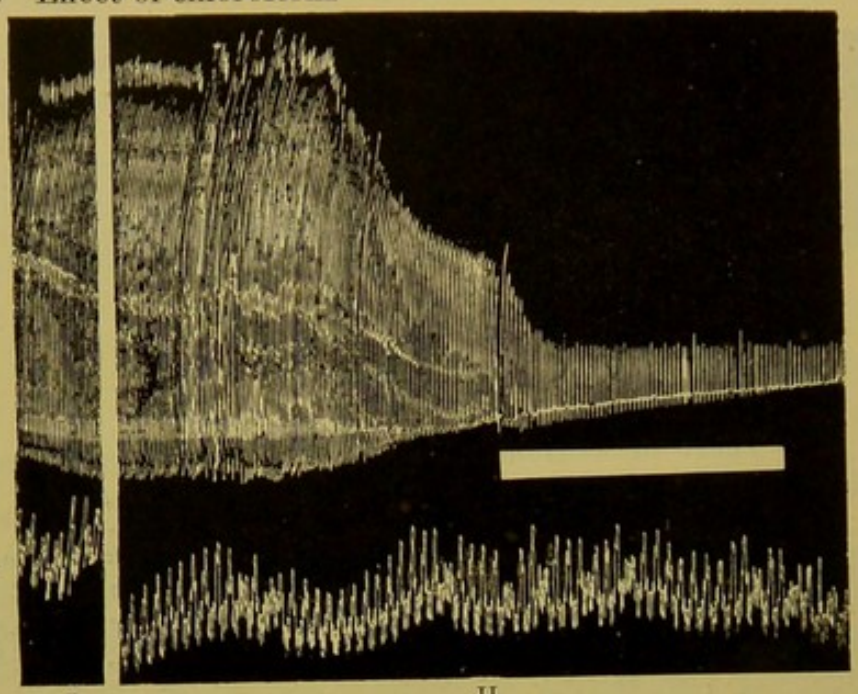


Fig. 48.—Upper tracing represents record of puppy's heart. Lower tracing = blood pressure of the perfusing animal. Period I = normal. Period II shows the effect of injecting 20 minims of $CHCl_3$ water. The signal mark represents the time during which the sample of blood was taken.

		Gas per min.	
		Oxygen	Carbonic acid
Period I.	Before injection of chloroform water ...	3·0 c.c.	8·8 c.c.
Period II.	After injection of chloroform water ...	0·37 c.c.	1·9 c.c.

(*h*) Stimulation of the vagus.

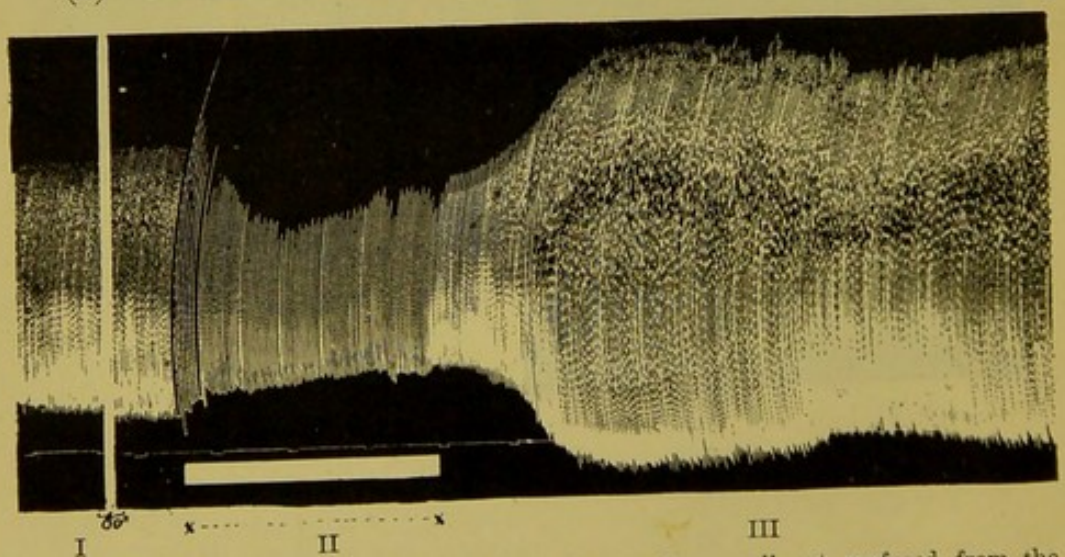
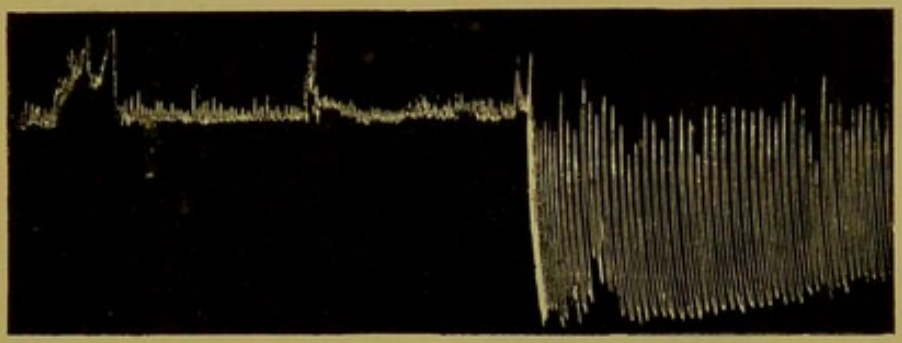


Fig. 49.—Record of the movements of the heart of a small cat perfused from the circulation of a large cat. Upstroke = systole. Period I = normal. In Period II the signal mark represents the time of vagus stimulation (coil at 10 cms.). The third period corresponds to the after effect and in this period the third sample of blood was taken.

		Gas per gram per min.	
		Oxygen	Carbonic acid
Period I.	Before stimulation	0·014 c.c.	0·038 c.c.
,, II.	During ,, ...	0·009 c.c.	0·005 c.c.
,, III.	After ,, ...	0·022 c.c.	0·015 c.c.

It is sufficiently evident from the examples which we have given that obvious changes in the activity of the heart run, roughly speaking, *pari passu* with changes in the call for oxygen.

There are two remaining points in the research which may here be considered and which I am inclined to emphasise more strongly than I did at the time, in view of the fact that they have both since been confirmed by the work of Evans [7].



I II

Fig. 50.—Record of puppy's heart. Period I shows the condition during the incompetence of the aortic semi-lunar valves (i.e. the isometric condition). Period II shows the recovery after a tube had been introduced into the left ventricle (i.e. the isotonic condition).

The first deals with a case in which the change in the activity of the heart is not evident. It is distended with blood to such an extent that it cannot contract. The condition of the heart may be expressed in more than one way; in our paper we stated that it was undergoing isometric contractions: in the light of the more recent work of Rohde [9] and of Hill [8], to which we shall shortly refer, we would say that each beat expended itself simply in a change of tension and not in a change of form.

The question then that was thus accidentally forced upon us was whether, in the absence of evident contractions, there was increased oxidation. The following is our description of this experiment:—"The cavities on the left side of the heart were much enlarged. The heart was endeavouring to contract but the resistance to the

outflow of blood (i.e. the pressure in the aorta) being greater than the force of contraction, it was performing a series of approximately isometric movements. At the point where the tracing changes its character the resistance to the outflow was abolished by introducing a tube through the wall of the left auricle into the left ventricle so that now the heart could drive its blood up the tube at each contraction. In the first period the heart bore some resemblance to an enlarged heart with incompetent semi-lunar valves: this is characterised by the rapid pulse and the failure to produce effective contractions. In the isotonic period the rhythm had returned to

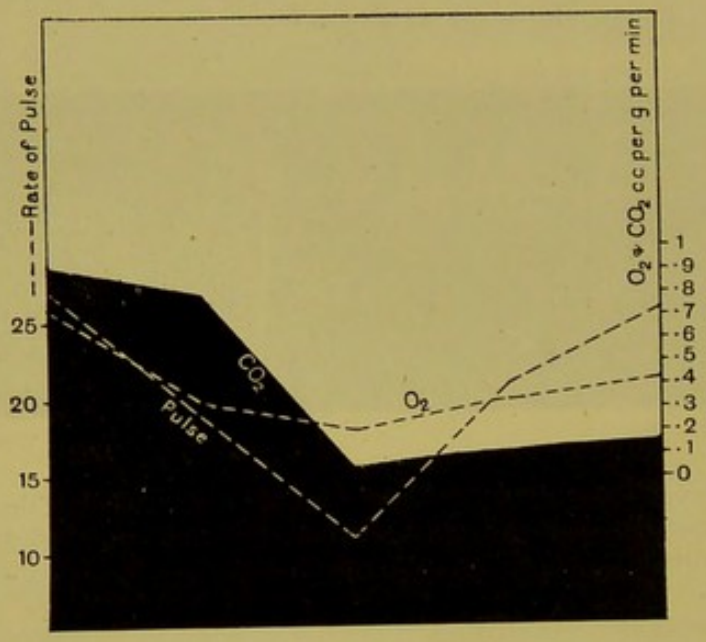


Fig. 51.

its normal rate and the contractions were well marked. The oxygen consumption in the first period was ·174 c.c. per minute, in the second ·169. Another point of interest arises in that the slightly greater oxygen consumption was associated with a reduced blood-flow. In the isometric period the blood passing through the coronary system was 1·9 c.c. per minute, in the isotonic it was 2·6."

The other point to which we would refer is the apparently much greater irregularity of the figures for carbonic acid than of those for oxygen. This irregularity is due to a number of causes, such for instance as the solubility of the gas in the blood and any change in the acidity (hydrogen ion concentration) of the heart itself, for it

has been made clear by Fletcher[9] that an evolution of CO_2 follows from a production of lactic acid in the frog's gastrocnemius.

Dixon and I came to the conclusion that the irregularity was only apparent and that in reality the changes in CO_2 production lagged somewhat in time after the changes in the oxygen intake; this "hysteresis" of CO_2 was also apparent in the kidney[10] and the intestine[11], and as we said above it has been confirmed in the heart by Evans.

Evans was interested in the effect of increased activity, not only on the call for oxygen, but also on the respiratory quotient of the beating heart. In a calculation of the ratio of the $\frac{CO_2 \text{ given out}}{O_2 \text{ taken in}}$ by the organ, this carbonic acid "hysteresis" proved fatal to the application to his problem of the method Dixon and I had employed. To obtain valid data it was necessary to integrate the whole of the oxygen taken in and of the CO_2 given out over a long time (20 minutes). For this purpose Evans had recourse to an ingenious device which likely enough will prove useful for other purposes. He made a heart-lung preparation something similar to that used by Stolnikoff for measurement of the output of the heart. He then circulated air through the lungs by an artificial respiration apparatus, and measured the amount of oxygen taken up and of CO_2 given out. After an allowance had been made for the gaseous exchange of the lungs themselves, these data furnished the respiratory quotient of the heart.

One of the merits of this preparation consists in the ease with which the work performed by the heart can be varied. Taking the work as being practically (the output) × (the pressure against which the heart is working), the work may be increased in either of two ways: (1) by feeding the right auricle with more blood, and (2) by increasing the resistance in the aorta. Evans obtained an absolute rise in the oxygen intake and in the CO_2 output when extra work was thrown upon the heart, but if the heart were in good condition he obtained a fall in the gaseous exchange per kilogram-metre of work performed and also a fall in the respiratory quotient. Judged as a machine the efficiency of the heart was extremely low—from two to ten per cent.

At the risk of going outside the title of my book I cannot refrain from an allusion to the work of Rohde, despite the fact that he worked on an excised heart perfused with Locke's solution; for on the work of Rohde I am an enthusiast. His technique may be considered

under two headings, (*a*) his method for gauging the functional activity, and (*b*) that for measuring the oxygen.

The research was performed upon the excised heart of the cat in every case. A rubber balloon was introduced into the cavity of the ventricle, and the balloon was distended with water at a known pressure. Thus it was possible (1) to estimate the pressure against which the heart was working, and (2) by taking a tracing to obtain a record of (*a*) the rise of pressure during systole, and (*b*) the rate of the pulse.

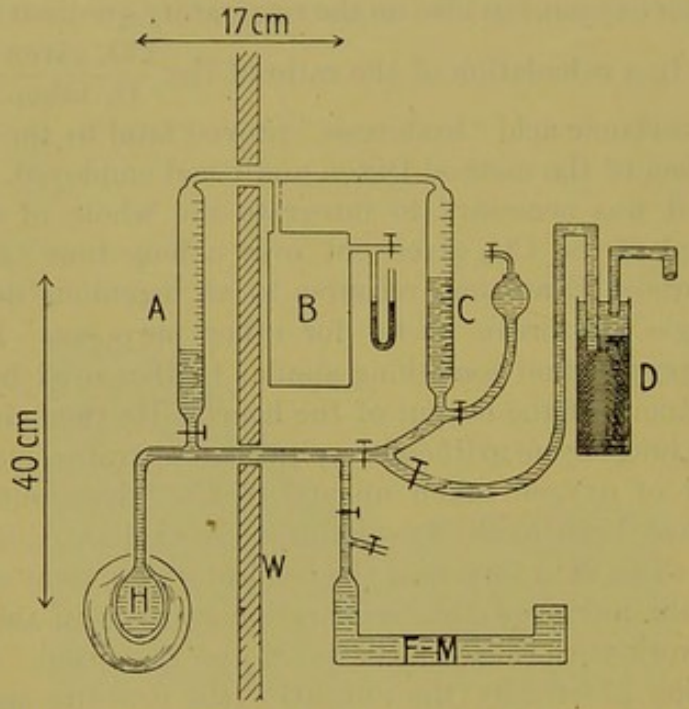


Fig. 52.—Rohde's arrangement for measurement of the functional activity of the warm-blooded heart.

H = rubber balloon in left ventricle. *A* = burette for measuring constant pressure. *B* = pressure bottle. *C* = burette for measurement of capacity of balloon during isometric contractions. *D* = quick-silver valve. *F-M* = Frank's "Feder manometer." *W* = wall of incubation chamber. Bore of tubing = 0·6—0·7 cm.

The apparatus is sufficiently described by the figure, from which it will be seen that it is possible, by appropriately turning the taps, (*a*) to make the heart beat against a known pressure isotonically, or (*b*) by preventing the fluid from leaving the rubber bag to make the beat isometric and that with any given volume of fluid in the bag.

The technique for measuring the oxygen was also very different from that used by us. In the first place the heart was perfused not

with blood but with oxygenated Locke's solution. Here again little explanation is needed beyond the statement of the general principle: the Locke's solution circulated round a closed circuit in which was placed among other things (1) the heart which deoxidised the solution, and (2) a chamber from which the fluid could take up another stock of oxygen before returning to the heart; the supply of oxygen in this chamber was kept up by the admission of fresh quantities of the gas from a reservoir, and the quantity of the gas so admitted was measured. In this way it was possible to find out how much gas the heart was using over considerable periods of time and thus to rid the calculations of momentary fluctuations. The method appears to give reliable results for time intervals of ten minutes and over.

Of the results which Rohde obtained I will first refer to that of a heart which, at the same initial pressure, was made to beat both isotonically and isometrically.

Period of observation	Kind of contraction	Initial pressure cm. water	Oxygen used	Pulse rate
10.5 —10.20 a.m.	isotonic	43	7·20	138
10.25—10.40 a.m.	isometric	43	7·80	138
11.00—11.20 a.m.	isotonic	43	7·55	138

The more interesting and important part of the work may be best gathered from the account of one of the 177 experiments which Rohde performed.

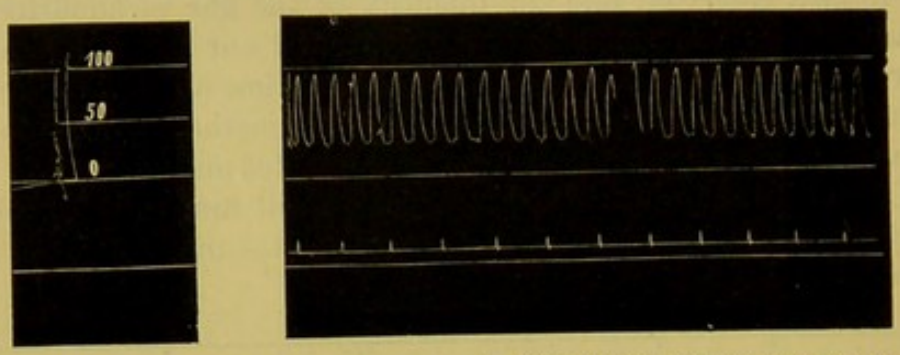
The functional activity of the heart was altered by altering the pressure in the pressure bottle.

Fig. 53 shows a series of tracings from a heart. The heart was beating isometrically; the tracings represent the changes of pressure as registered by Frank's manometer. Take the first portion which shows heart beats, what information does it give? It tells (1) the frequency of the beats, which is 159 per minute, (2) the initial pressure, which is 35 mm. of mercury, (3) the maximal increase of pressure, or as Rohde calls it the pulse pressure, which is 97 mm. In the other tracings each one of these is changed. The question then is, upon which of these does the oxygen used by the heart depend? The answer is, that it does not vary directly with any one, but with the product of the frequency and the maximal increase of pressure. To put the thing in another way, if Q is the oxygen used, T the

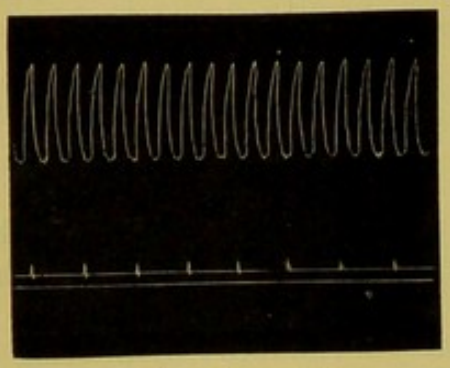
maximal increase of pressure in the beat and N the frequency, $\frac{Q}{NT}$ is a constant quantity.

Isometric curves from left ventricle of a surviving cat's heart during a 2 hours' research.

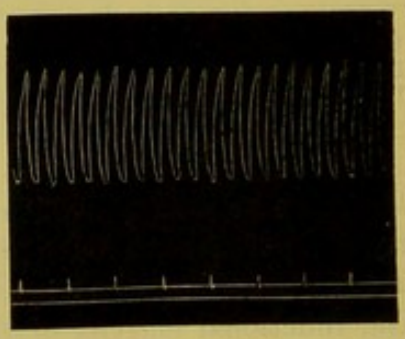
Time in seconds.



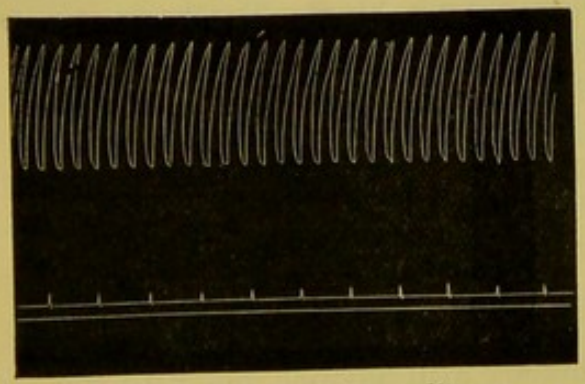
A quarter of an hour after commencement of circulation with Locke's solution.



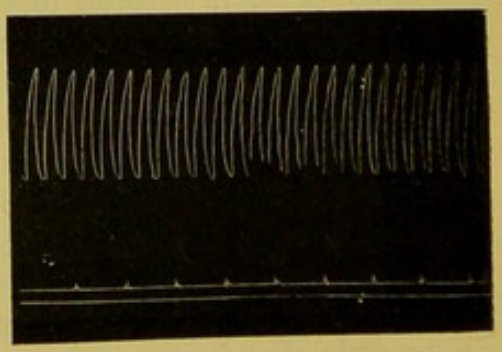
Half an hour ditto.



One hour ditto.



One and a quarter hours ditto.
Commencement of rise in pressure.



2 hours ditto.

FIG. 53.

The following data taken from the experiment in question illustrate this point:—

Period	Initial pressure	(Q) Oxygen used per min.	(N) Frequency of pulse	(T) Maximal pressure	$\frac{Q}{NT}$
9.50—10.10	35	9·45	159	97	613×10^{-6}
10.10—10.30	59	10·45	165	106	597 ,,
10.30—10.50	35	9·75	168	98	593 ,,

It is perhaps even more clearly shown by the following chart:—

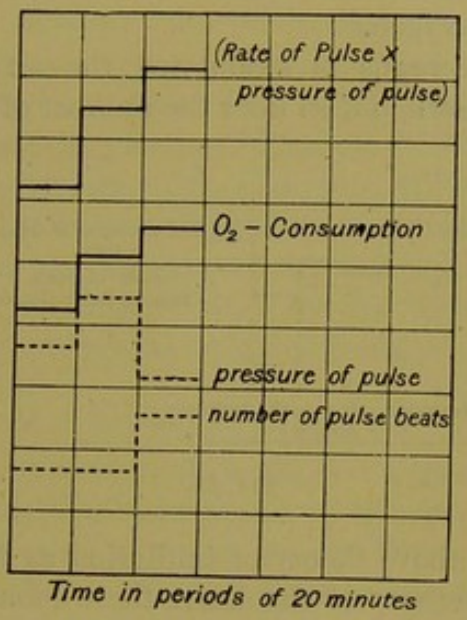


Fig. 54.—Note the correspondence between the increments of oxygen used and of the rate and maximal pressure of the pulse.

To the work of Hill[8] on the heat given out by frog's muscle, I have already referred; I must return again to it, for he came to the same conclusion about the heat given out as Rohde had done about oxygen taken in, namely that with any one contraction it varied directly with the maximal tension set up in the muscle.

For any heart, the law that $\frac{Q}{NT}$ is constant, has of course its limitations:—

(1) If subjected to very high pressures the amount of oxygen required begins to go up relatively, in other words the heart is losing its efficiency, it is being over taxed.

(2) In the natural process of death the same change takes place.

(3) In the case of hearts treated with drugs, numerous depressing drugs—chloral hydrate, atropine, KCN, muscarine and veratrine—also cause an increase in the ratio $\frac{Q}{NT}$.

On the other hand, strophanthin, adrenalin and—from the physiologist's standpoint most important of all—vagus stimulation produce their changes without any alteration in the ratio $\frac{Q}{NT}$.

The significance of this latter conclusion is very great, from the point of view of the theory of inhibition, for it has been held by Gaskell and others that the essential factor in the anabolic process was a storage of oxygen.

Now when the vagus is stimulated the absolute quantity of oxygen used goes down, but so does the number of contractions. One

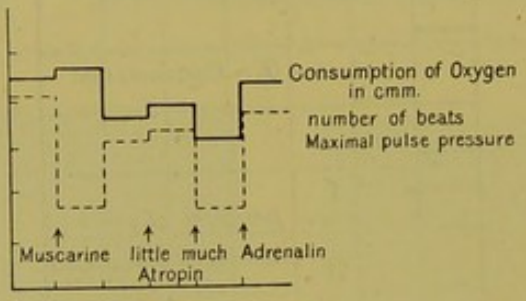


Fig. 55.

might express the above theory of inhibition as follows. Relatively to the functional activity of the heart there should be more oxygen taken up during vagus stimulation than at other times; the experiments of Rohde seem to show that this is not so.

We are tempted to enlarge upon the future that we see before the type of experiment which we have been describing; this however we will leave to Rohde himself and must now pass to another series of experiments conceived on the same lines.

The Kidney. Next to the contractile organs which we have just studied, probably the best attested case of functional activity going hand in hand with oxygen consumption is that of the kidney. There is, so far as I know, no case where the kidney does work in which there is not also an increased oxygen consumption by the organ. Now it is necessary here to use the words "does work" in a strictly physical sense, and not in the loose and general way which lends itself to

all manner of false deductions. There is only one method by which a measurement of the work can be made, viz. by the use of the second law of thermodynamics and the laws of osmotic pressure. Whenever a given solution (e.g. the blood), containing any number of substances dissolved, is separated into two or more separate parts of different concentrations (e.g. blood and urine), then work is done: and this work is always positive and can never be negative. It always requires the liberation of free energy outside to effect the separation of a solution into two different solutions.

In order to avoid confusion we must emphasise that actually and commercially (so to speak), to carry out the separation of the several bodies, far more work will probably be done than is theoretically necessary. In the same way the theoretical minimum work in foot-pounds, which it is necessary to do in order to carry bricks up a hill, is given by the weight of the bricks in pounds multiplied by their vertical rise in feet: in practice, however, it will inevitably be the case that very much more work than this will actually be done in carting the bricks up the hill, depending on the state of the road, the wind, the friction of the wheels, and the training of the horse, or the internal friction of the engine which carries them up. But, in spite of that, the only general and valuable estimate of the work to be done is the product

(weight of bricks) × (vertical rise in feet),

for the actual amount of work expended depends entirely on the method adopted, and the mechanism by which the work is done—and that of course we do not in general know. The secretion of urine may be regarded as the separation of one fluid, the blood, into two fluids of the same total volume, the concentrated blood and the urine. The *actual* energy used in carrying out the process of secreting a given sample of urine we cannot calculate, until we know the inner mechanism by which secretion is performed. All we can do is to calculate, from the "molecular concentrations" of the several salts in the urine and the blood, a certain quantity W, which is the minimum work which would have to be impressed on the blood in order to separate it into concentrated blood and urine. W is always positive *for every conceivable change*, as will be shown below, and is obtained on the hypothesis of reversible changes being carried out in concentrating the blood by means of semi-permeable membranes: there are many processes by which the separation can be carried out, but the second law of thermodynamics asserts *that whatever be the process*,

provided it be reversible, the work done in accomplishing it must be the same quantity W, while if the process be not reversible the work done will be greater than W.

Whatever then be the mechanism by which the secretion takes place, we may assert definitely, and beyond the possibility of error, that free energy to at least the value W must have been provided, presumably at the expense of oxidative processes.

How then can we calculate W, the least work which must be done in the secretion of a volume V litres of urine?

Let the blood be assumed to contain the several constituents $A_1, A_2, \dots A_n$ at molecular concentrations* $c_1, c_2, \dots c_n$, and the urine secreted from this blood to contain the same bodies in concentrations $c_1', c_2', \dots c_n'$. Then the minimum work which it is necessary to do to separate this urine from the blood is

$$W = VRT\left[c_1' \log \frac{c_1'}{c_1} - (c_1' - c_1) + c_2' \log \frac{c_2'}{c_2} - (c_2' - c_2) + \dots\right],$$

where R is the gas constant (approximately two calories) and T the absolute temperature.

Now each of the terms $c' \log \frac{c'}{c} - (c' - c)$, which may be written $c'\left[\log \frac{c'}{c} - \left(1 - \frac{c}{c'}\right)\right]$, can be shown mathematically to be positive for all values of c' and c. Hence whatever type of urine has been secreted W is the sum of a series of positive terms, and is therefore positive. Every constituent A, therefore, whose concentration is greater or less in the urine than in the blood, has added its quota to the total minimum work it is necessary to do to secrete that urine.

It has been assumed that the minimum work which is necessary to separate a given urine can be calculated merely from the freezing points of urine and blood. This is absolutely fallacious. These lowerings of freezing points give no clue as to the value of the expression for W given above: they merely tell us the value of the two expressions

$$c_1 + c_2 + \dots + c_n,$$

and

$$c_1' + c_2' + \dots + c_n',$$

and therefore of the part of the expression for W given by

$$(c_1' - c_1) + (c_2' - c_2) + \dots.$$

* Concentrations reckoned in gr. mols. per litre.

Of the remaining terms in the expression for W,

$$c_1' \log \frac{c_1'}{c_1} + c_2' \log \frac{c_2'}{c_2} + \ldots,$$

the freezing points give us absolutely no evidence. Their use for this purpose involves a very serious error, for these neglected numbers may be relatively very large: for example, if A_1 represents urea, c_1' the concentration of the urea in the urine is very large, while c_1 the concentration in the blood is extremely small. Hence the first term

$$VRTc_1' \log \frac{c_1'}{c_1}$$

is very large and cannot be neglected. Similarly if we consider some body occurring largely in the blood, and not so largely in the urine, c' is small while c is large, so that

$$VRTc' \log \frac{c'}{c}$$

may be finite and negative. In order therefore to calculate W we can only gain satisfactory results if we know the concentrations in blood and urine of each separate important constituent. Without this information, the results deduced from freezing-point measurements are completely fallacious.

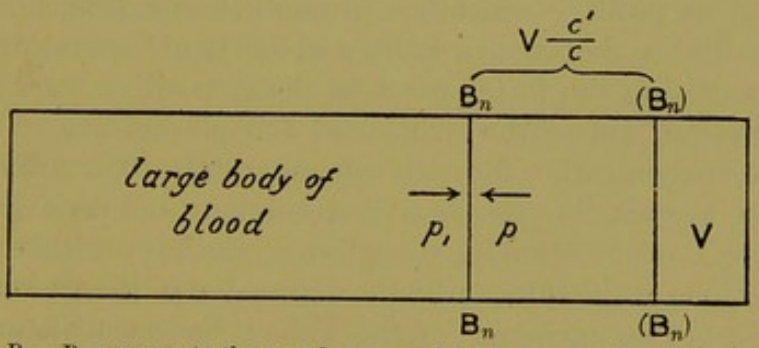


FIG. 56.—B_n—B_n represents the membrane used for concentrating the substance A_n. B_n initial position. (B_n) final position.

[An actual reversible process by which the separation might be carried out is as follows: (It should be noted that I am not proposing in any way a hypothesis of kidney secretion, but am merely describing a method by which it is possible to calculate the minimum work which it is necessary to do in order to separate the urine).

Let us suppose a membrane B_1 permeable to all the dissolved bodies except A_1: then the substance A_1 which, in the urine in volume V is at concentration c_1', and which was initially in the blood at concentration c_1, must have occupied a volume

$V\frac{c_1'}{c_1}$. Hence if we use the membrane B_1 to concentrate this body from a volume $V\frac{c_1'}{c_1}$ to a volume V, we have to do work against its osmotic pressure. On the membrane we have two osmotic pressures working, one each side, viz.: the fixed osmotic pressure p_1 of A_1 in the original solution, and the gradually increasing osmotic pressure p of A_1 in the gradually concentrating solution. The total work done is

$$-p_1\left(V\frac{c'}{c}-V\right)+\int_V^{V\frac{c'}{c}} p\,dv=-p_1 V\frac{c'}{c}\left(1-\frac{c}{c'}\right)+Vc'RT\log\frac{c'}{c},$$

according to the gas laws: or finally, since $pv=nRT$, where n is the number of molecules of gas considered, putting $p_1 V\frac{c'}{c}=Vc'RT$, we find the total work to be

$$=-V(c_1'-c_1)RT+Vc_1'RT\log\frac{c_1'}{c_1}$$

Using now a membrane B_2 permeable to all the bodies except A_2, we find we have to do work

$$-V(c_2'-c_2)RT+Vc_2'RT\log\frac{c_2'}{c_2},$$

in concentrating A_2; &c.

The total work W is the sum of all these terms, each of which must be positive*.]

The considerations which have just been applied to the problem preclude us therefore from drawing any comparison between the gaseous exchange of the kidney and the so-called work as calculated only from the freezing points of the blood and urine. The freezing points give us positive assistance in another direction, however, for they show that as between a kidney which is not secreting and one which is secreting the latter must be doing positive work in virtue of its secretion, unless the urine and the plasma are of the same crystalloid concentration for each salt separately. The urine secreted in response to such diuretics as sodium sulphate and urea is not of the same saline concentration as the plasma, as shown either by actual determinations of the salts, or by the depression of the freezing points. Since the gaseous exchange of the kidney has been determined in many of these experiments on sodium sulphate and urea diuresis, we may proceed to see whether there is a call for oxygen in such cases.

Let us be clear, however, how far we are relying on our freezing-point determinations; we are merely using them as an indicator to show that if the freezing points of the blood and the urine are different, the saline concentrations of one or more salts cannot be identical in the two fluids, and therefore work has been done. The

* The above discussion of the work performed by the kidney has been contributed by Mr A. V. Hill.

opposite case may of course exist, that in which the freezing points are the same, but in which nevertheless there must have been work done because the molecular concentrations of the salts, though jointly the same in each fluid, are severally different.

Data of four experiments on the work of the kidney.

Kidney secreting not at all or to a trifling extent		Kidney secreting		
Oxygen used	Urine	Oxygen used	Urine	Qualitative evidence of work
0·57 c.c.	0·00 c.c.	2·95 c.c.	0·85 c.c.	$\Delta_B = 0·54$, $\Delta_V = 1·41$
0·64 c.c.	0·07 c.c.	1·14 c.c.	0·34 c.c.	$\Delta_B = 0·61$, $\Delta_V = 1·17$
1·66 c.c.	0·00 c.c.	5·58 c.c.	1·53 c.c.	$\Delta_B = 0·62$, $\Delta_V = 1·08$
				Analysis of urine
0·04 c.c.	0·05 c.c.	0·09 c.c.	2·1 c.c.	NaCl = 0·22 % Sulphates as Na_2SO_4 = 1·25 % Urea = 0·28 %
0·03 c.c.	0·04 c.c.	0·09 c.c.	2·8 c.c.	NaCl = 0·39 % Na_2SO_4 = 2·0 % Urea = 0·25 %

Surgically, experiments of this character entailed a good deal of difficult operating, which was carried out in the first two cases cited above by Brodie, and if one followed the example of the surgical text-books one would call it "Brodie's operation." These operations were performed by him on dogs—the second two experiments having been upon cats. The operative procedure has not been materially altered in the numerous operations which have since been performed in the Cambridge Laboratory by Straub, Knowlton, Winfield, Neumann and myself, on the kidneys and suprarenals of cats and rabbits. The problem was to perform the necessary manipulations upon the kidney without having the experiment upset by the surgical shock involved in the exposure and manipulation of the intestines. Its solution lay in the fact that the shock might be obviated by getting rid of the intestines. The rectum was therefore tied in two places and cut between the ligatures; the same was done to the following structures, in order—the inferior mesenteric artery, the oesophagus, the coeliac axis, the superior mesenteric artery, and the portal vein. Of course any minor vessels must also be ligatured; by this procedure the whole of the intestines, spleen, pancreas and stomach may be removed, and with a little practice the blood pressure at the end will be as high or higher than at the beginning of the experiment. The animals were always anaesthetised, usually with urethane. The blood from the kidneys was withdrawn through a cannula placed in the vena cava, below the entrance of the renal

veins. It was then easy to divert the blood from these vessels into the cannula, and measure the rate of flow as it emerged.

The Salivary Glands. The evidence which exists with regard to the submaxillary gland is of the same general character as that which we have given for the kidney. In this case also the deficiency lies on the side of quantitative proof of the amount of work done. It is known however that saliva is always poorer in salts than is plasma, though the two differ when the secretion is less rapid than when it is slow. We can therefore affirm positively that whenever the gland secretes it must do positive work, inasmuch as the secretion differs in its salts from the plasma, though we are unable to state precisely even the minimal value of such work.

Of the salivary glands the submaxillary is that on which most work has been done.

The gross fact is that stimulation of the chorda tympani produces an increase in the metabolism of the gland. This could be shown even in the old days of the blood-gas pump. The following are some data from the dog.

Gaseous exchange in c.c. per minute[14].

	Resting		Active chorda		
Exp.	O_2	CO_2	O_2	CO_2	Saliva secreted per min.
II (1)	0·32	0·20	1·20	1·58	1·6 c.c.
,, (2)	0·30	0·20	0·93	0·60	1·6 c.c.
,, (3)	0·29	0·20	0·59	0·75	1·3 c.c.
IV	0·12	0·17	0·25	0·25	0·9 c.c.
V	0·12	0·12	0·54	0·60	1·0 c.c.
VI	0·56	0·60	1·06	1·86	1·0 c.c.

In the few experiments which have been performed the amount of oxygen used by the gland per cubic centimetre of saliva is more uniform than might have been expected.

Exp.	Rate of flow of saliva per min. in c.c.	Oxygen per c.c. of saliva
1	2·1	0·48
	3·2	0·45
	2·5	0·43
2	1·8	0·66
	1·55	0·60
	1·2	0·5
3	0·37	1·05
	1·05	1·00
4	1·4	0·50
	2·1	0·50
	2·5	0·44

It must however be remembered that these results were obtained by methods which are now quite out of date; moreover the presence of $CHCl_3$ in the blood may have caused some inaccuracy unsuspected at the time.

The more recent developments of the discussion of the relation of activity to oxygen used, as in the case of striped muscle, have

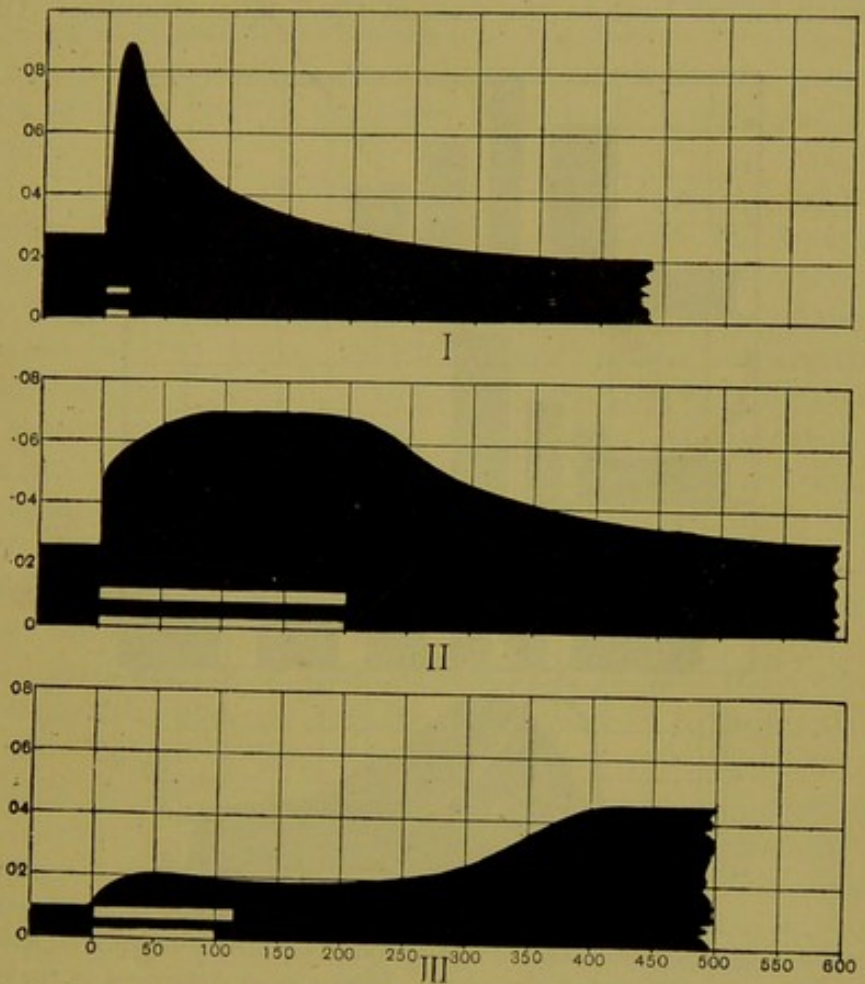


Fig. 57.—Ordinate = volume of oxygen used in c.c. per minute. Abscissa = time in seconds. Upper signal = duration of flow of saliva. Lower signal = duration of chorda stimulation. I, short stimulation. II, long stimulation. III, fatigued gland.

gone to show that the oxygen is not used entirely at the time of the secretion, but that it continues to be used for some little time afterwards. This is not only so in the case of stimulation of the chorda tympani, but it is also the case when saliva is elicited by the injection of adrenalin. The experiments have been done on the cat.

Fig. 57 I, II and III shows the relation in time between the flow

of saliva and the call for oxygen which is initiated by stimulation of the chorda tympani.

No. I (Fig. 57) gives the rate of oxygen consumption which follows a short stimulus, 20 seconds, while No. II shows the consumption following a stimulus of ten times the duration. Not only does the oxygen consumption long outlast the stimulus in each case, but it does so for a much longer time in the case of the longer stimulus. This is

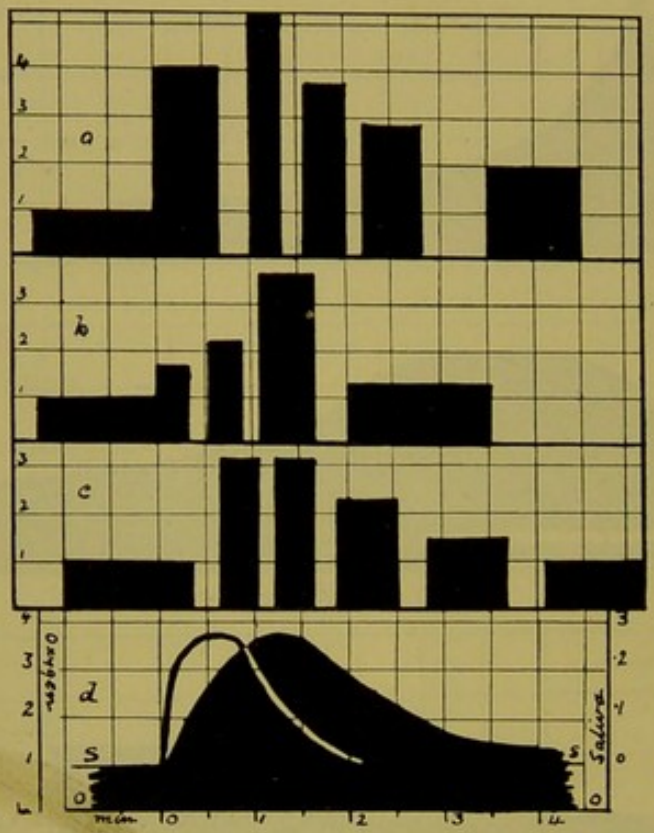


Fig. 58.—*a*, *b*, *c*, oxygen used in active as compared with resting glands in individual exps. *d*, line represents mean rate of salivary secretion in c.c. per minute in the three Exps. *S*—*S* = base line for saliva. Black area = oxygen used by the active as compared with the resting gland. *O*—*O* = oxygen base line.

probably a fatigue effect to some extent, for in No. III, in which the animal was in bad condition as a result of surgical shock, the call for oxygen only manifests itself very slowly and has scarcely passed its maximum at the right of the figure, that is to say almost six minutes after the stimulus has ceased.

Nor is it only as the result of chorda tympani stimulation that we have this prolonged call. In the cat it follows upon stimulation

of the sympathetic as well, at least on the accepted theory that adrenalin action is sympathetic secretion.

Fig. 58 shows the oxygen consumption which results from injection of 1 c.c. of $\frac{1}{10000}$ adrenalin into the jugular vein of a cat in three experiments. These ran an almost even course, so that it seemed allowable to average out the results. Their mean is given at the bottom of the figure. From it we can not only see that the oxygen use outlasts the secretion of saliva by some considerable time, but we can also calculate with a fair degree of accuracy the quantity of oxygen which is used as the result of a certain quantity of salivary secretion.

The Pancreas. Sufficient has probably been said about the submaxillary to show that a great deal of knowledge has been gained about it within the last ten years; nevertheless this has only sufficed really to open the door for a more exact investigation of the whole problem. We have done enough to convince our readers that the call for oxygen follows the functional activity of the gland, but we want a much more rigid definition of the latter. Of the other organs which have formed the subject of research, the pancreas[15], the liver[16] and the suprarenal[17] glands, there is little which need be said; in the case of the two former, experiments have been performed which clearly increase the activity of the glands, but we have no measurements in units of the increase.

The chief interest of these experiments perhaps lies in the very diverse stimuli which are used for the purpose of bringing about the increase. This is an important matter, because it is clear that if functional activity, when produced by stimuli of the most diverse kinds, evokes a call for oxygen, we are on a much safer footing in supposing that the oxygen want is the direct result of the functional activity.

So far as the pancreas was concerned the stimulus used was of course secretin. The experiments were performed in collaboration with Prof. Starling shortly after he and Prof. Bayliss discovered the mechanism of pancreatic secretion. The experiments consisted in isolating the tail of the pancreas in the dog from the rest of the organ; this may be done without upsetting the circulation. A cannula is so placed that the blood from this portion may be collected with ease, its rate of flow being measured at the same time and its gases analysed. The experiments were done in the early days, before the introduction of the differential method of blood-gas analysis, and

whilst the ferricyanide method was still on its trial. We therefore made a great point of checking the results obtained by the ferricyanide method by those obtained with the blood-gas pump. After arterial and venous samples had been collected for the determination

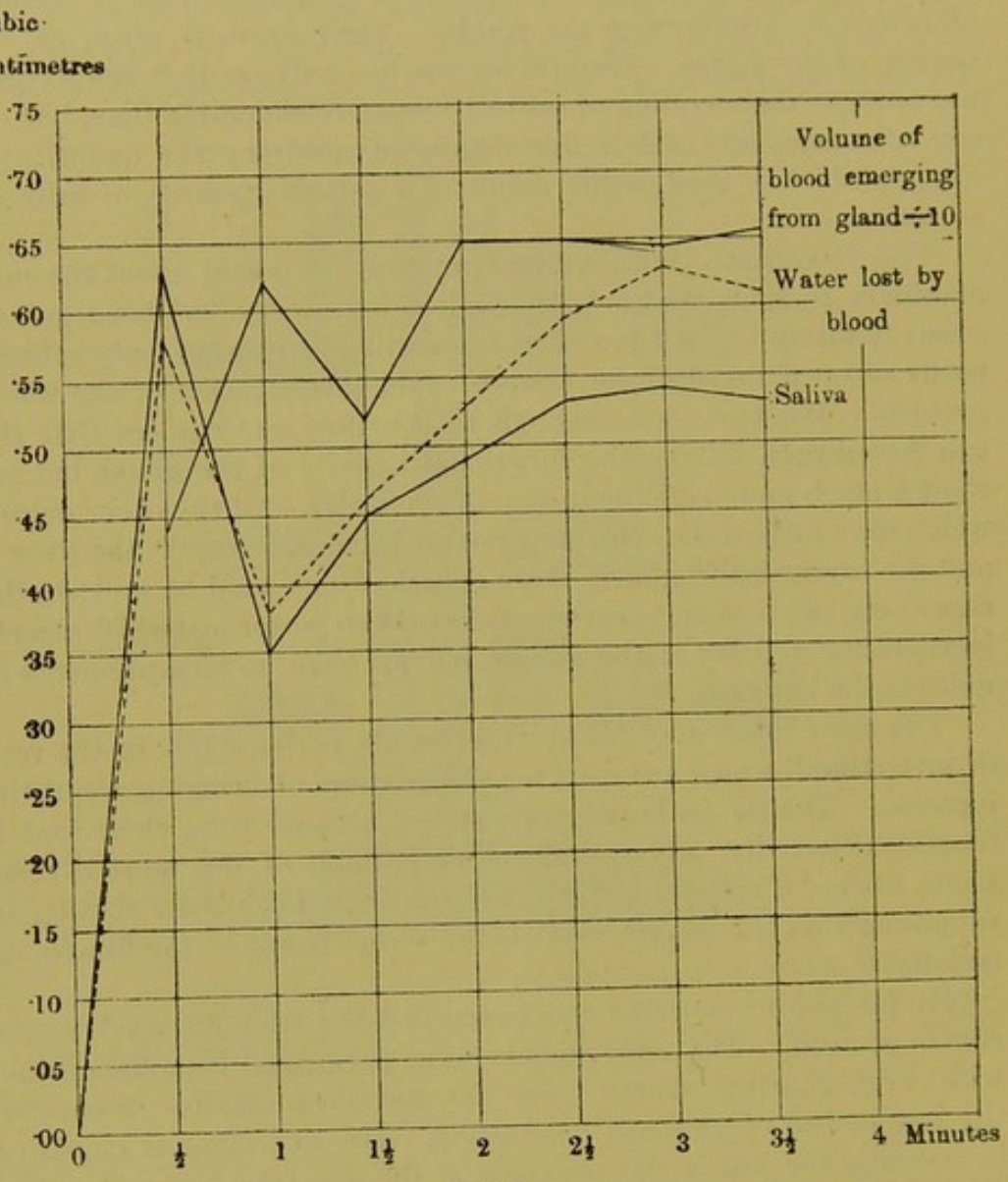


Fig. 59.

of the metabolism of the resting gland, an injection of secretin was made, and when the juice flowed another set of samples was taken. We made no allowance for the concentration of the blood as none seemed to be necessary. Perhaps as we have not referred to this

matter before we may therefore explain more particularly what we mean.

When a gland secretes, water is taken from the blood. The result is that the corpuscles in the venous blood are more numerous than in the arterial blood per cubic centimetre. To find out how much oxygen the blood has lost in its passage through the gland, it is necessary to know the sum total of the oxygen lost by each corpuscle; therefore the amount of oxygen in the venous blood must be subtracted not from the amount of oxygen in the same volume of arterial blood, but from the amount of oxygen in a volume of arterial blood which contains the same number of corpuscles (or the same amount of haemoglobin) as are contained in the venous blood collected.

In the case of the submaxillary gland exact measurements have been made. The following figure shows the relation of the volume of venous blood passing through the gland during stimulation of the chorda tympani, to the volume of the saliva secreted and to the amount of water lost by the blood. It will be seen that the two latter are almost equal in amount; the slight excess of the water lost by the blood over the saliva secreted is to be accounted for no doubt as lymph [18]. To obtain the volume of arterial blood from which a given volume of venous blood was derived one multiplies the volume of saliva by 1·1 and adds it to the volume of the venous blood.

If the conditions in the pancreas and the kidney be considered in the same way, it will be found, for experiments of the order of accuracy of those with which we have been dealing, that the volume of pancreatic juice or urine is so small as compared with that of the blood that no correction need be introduced for the concentration.

The following results for the oxygen used during rest and activity were obtained by the ferricyanide method:

	Oxygen absorbed per min.		
	Resting pancreas	Active pancreas	Response to injection
Exp. 1.	·49 c.c.	1·71 c.c.	
	·60 c.c.	3·50 c.c.	good
Exp. 2.	·25 c.c.	·51 c.c.	
	—	·34 c.c.	good
Exp. 3.	·12 c.c.	·28 c.c.	
	·11 c.c.	·34 c.c.	2·1 c.c. in 8′
Exp. 4.	·08 c.c.	·33 c.c.	
	·06 c.c.	·29 c.c.	·9 c.c. in 5′

With the blood-gas pump :

Oxygen absorbed per min.	
Resting pancreas	Active pancreas
·40 c.c.	·53 c.c.
·23 c.c.	·31 c.c.

The Liver. Lastly we come to an organ which is perhaps more obscure than any of which we have yet treated—the liver. It is possible to express the mechanical work performed by muscle for it is doing a definite thing ; it is possible in the case of the kidney to lay down lines for calculating at least the minimum work done by that organ ; but who shall express in units what the liver is about. Its functions are so manifold and in many cases so ill understood, and the evidence of those functions is so difficult to estimate even when they are understood, that at present there seems to be but little hope of getting any accurate notions of its work. All that we can do is to attempt to excite it by what we may regard as its normal stimulus, namely the presence of food in the intestine. It seems at least a fair assumption that the liver will increase in activity during digestion.

But the estimation of the oxygen used by the liver offers a very difficult surgical problem ; fortunately it fell into the hands of a skilful operator in the person of Shore, and it proved possible to attain reliable results in a very considerable percentage of the experiments.

The blood had to be collected from

(1) an artery,
(2) the portal vein,
(3) the hepatic vein,

in such a way that one could obtain one's samples and measure the rate of flow in each of the two last vessels, without upsetting the vascular conditions of the liver.

This is perhaps scarcely the place to describe the operative procedure in detail ; in a few words however the blood which runs into the inferior vena cava from all organs except the liver is conveyed in a hirudinised animal round to the superior vena cava. The vena cava inferior is then tied above the renal veins and a cannula inserted just above the ligature. By pinching the vena cava in the region of the diaphragm, the blood from the liver may be collected by the cannula. For the collection of the portal blood a cannula is introduced into the splenic vein. Into this cannula the blood may be

diverted. The greatest care must be taken to insure that the blood is collected at the pressure at which it normally is in the vein.

We performed two series of experiments. The determinations were made as a rule about noon; in the first series the animals were given a clear day without food having been fed the evening before that, there being therefore about 42 hours from the time of the last meal till that of the experiment. In the second series the animal, a cat in every case, was fed the evening before the experiment, i.e. about 18 hours previously. In neither case was much food found in the intestines and therefore it is not surprising that the difference in the metabolism of the abdominal viscera which drain into the portal system was trifling. But the metabolism of the liver was markedly higher in the case of the fed animals than in the case of the unfed ones, as the following figures show:

Animals fasting			Animals fed		
	Oxygen used per gram per min.			Oxygen used per gram per min.	
Exp.	Portal organs	Liver	Exp.	Portal organs	Liver
1	·012 c.c.	—	8	·011 c.c.	·034 c.c.
2	·013 c.c.	·005 c.c.			·045 c.c.
3	·012 c.c.	·005 c.c.	9	·018 c.c.	·050 c.c.
4	·011 c.c.	·007 c.c.	10	·018 c.c.	·030 c.c.
5	·013 c.c.	·017 c.c.	11	·013 c.c.	·034 c.c.
6	·013 c.c.	·012 c.c.		·015 c.c.	·029 c.c.
7	·008 c.c.	·018 c.c.	12	·016 c.c.	·024 c.c.
Average	·012 c.c.	·011 c.c.		·015 c.c.	·035 c.c.

In our discussion of the call for oxygen we have reviewed the activity of many organs of the body, muscle, heart, kidney, secreting glands and absorbing epithelium; these organs are excited by the most diverse forms of stimulus, electrical stimuli, hormones, drugs, &c., and evince their activity by doing work of the most diverse kinds; in one respect only do they resemble one another, namely that in no organ excited by any form of stimulus can it be shown that positive work is done without the blood supply having to respond to a call for oxygen.

REFERENCES

(1) Pflüger, "Ueber die physiologische Verbrennung in den lebendigen Organismen," *Pflüger's Arch.* x, p. 350, 1875.
(2) Chauveau and Kaufmann, *Comptes Rendus de l'Acad. Franç.* CIII, p. 1063 and CIV, p. 1765.
(3) Verzàr, *Journal of Physiol.* XLIII, p. 243, 1912.
(4) Hill, *Ibid.* XLVI, p. 28, 1913.
(5) Barcroft and Dixon, *Ibid.* XXXV, p. 182, 1907.
(6) Rohde, *Arch. f. Exp. Path. und Pharmacol.* Bd. 68, p. 401, 1912.
(7) Evans, *Journal of Physiol.* XLV, pp. 230, 231, 1912.
(8) Hill, *Ibid.* XLII, pp. 40 et seq., 1911.
(9) Fletcher, *Ibid.* XXIII, p. 10. Fletcher and Hopkins, *Ibid.* XXXV, p. 301.
(10) Barcroft and Brodie, *Ibid.* XXXIII, p. 67, 1905.
(11) Brodie and Vogt, *Ibid.* XL, p. 167, 1910.
(12) Fletcher and Hopkins, *Ibid.* XXXV, p. 247, 1907.
(13) Barcroft and Piper, *Ibid.* XLIV, p. 359, 1912.
(14) Barcroft, *Ibid.* XXVII, p. 31, 1901.
(15) Barcroft and Starling, *Ibid.* XXXI, p. 491, 1904.
(16) Barcroft and Shore, *Ibid.* XLIV, p. 296, 1912.
(17) Neuman, *Ibid.* XLIV, p. 188, 1912.
(18) Asher (for discussion in relation to lymph flow), *Biochem. Zeitschr.* XIV, p. 75, 1908.

CHAPTER VII

THE CALL FOR OXYGEN CONSIDERED AS A PHYSIOLOGICAL TEST

An advantage of the assurance that every increase in the activity of the cell means an instant call for oxygen lies in the fact that it furnishes a method of deciding whether in certain cases there is or is not increased activity on the part of the cell.

The most obvious instance in point is furnished by the kidney. When Ringer's solution is injected into the blood of a cat or a rabbit, there is an immediate increase in the amount of urine secreted. Yet so far as may be judged from the nature of the secretion there is no adequate reason to suppose either that there is or that there is not increased activity on the part of the cells of the kidney. It might be very plausibly supposed on the one hand that the mere fact of increased flow of urine was an index of increased cellular activity—a view which I myself held till a few years ago. On the other hand in this particular case the urine secreted is, so far as its crystalline constituents are concerned, of the same composition as the plasma. Therefore it is possible theoretically for the urine to be excreted as the result of some change in the vascular conditions, the energy necessary for the filtration being supplied by the heart.

We* at once asked ourselves, Is there a call for oxygen? The answer is sufficiently shown by the following experiment, a chart of which is given in Fig. 60.

The result is clear, there is no call for oxygen, no evidence of work done by the cells as would have been the case if for instance a solution of sodium sulphate had been injected, or some drug which essentially altered the composition of the urine. This fact is sufficiently shown by the following chart in which the oxygen used by the gland and the volume of urine secreted are shown both during a diuresis caused by sodium sulphate and one caused by Ringer's solution.

* Dr Hermann Straub and myself.

We need not however stop at this point. Nothing could be more unsatisfying than to prove that such a diuresis was due to mechanical

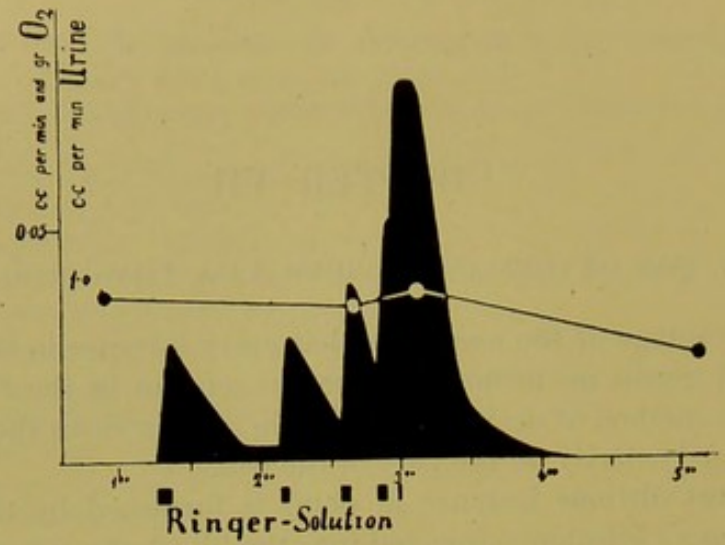


Fig. 60.—Line = oxygen consumption. Black area = urine excreted.

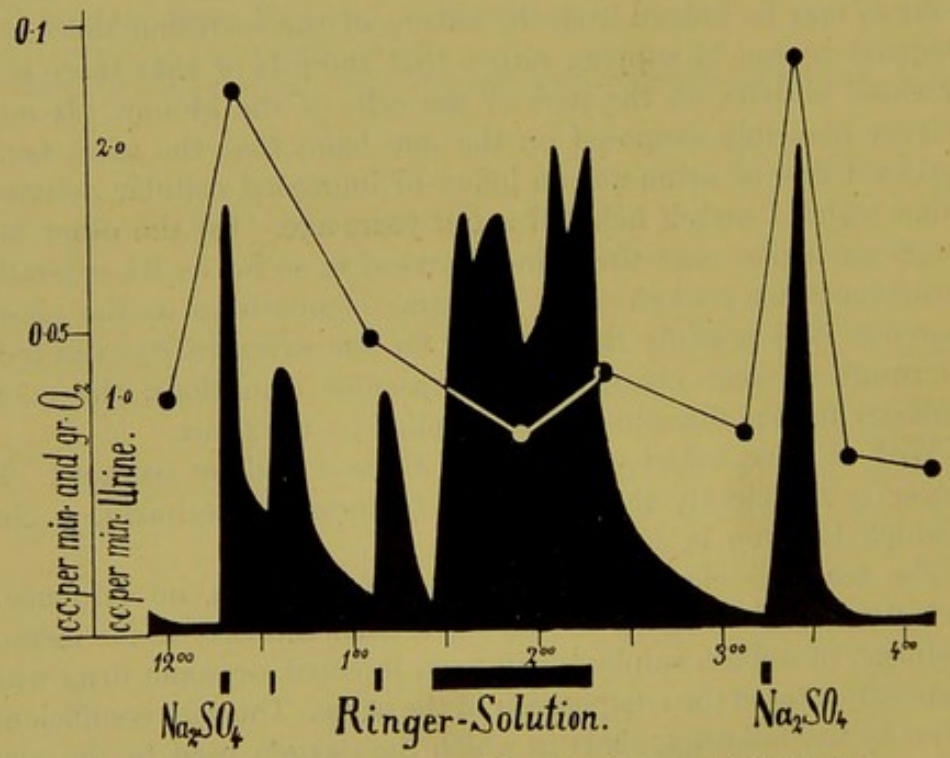


Fig. 61.—Line = oxygen consumption. Black area = urine excreted.

causes without making any effort to see what the mechanical causes at work might be.

In the experiment which I have quoted a number of changes took place in the vascular conditions, any one of which might easily have had an effect on the flow of urine, all of which may have conspired in this matter. There was for instance an increase in the general arterial pressure, an increase in the rate of flow, a decrease in the viscosity of the blood and a decrease in the concentration of proteins in the plasma. We can proceed to eliminate these one by one and see whether a diuresis follows, a diuresis which preserves its characteristic feature of taking place without any call for oxygen.

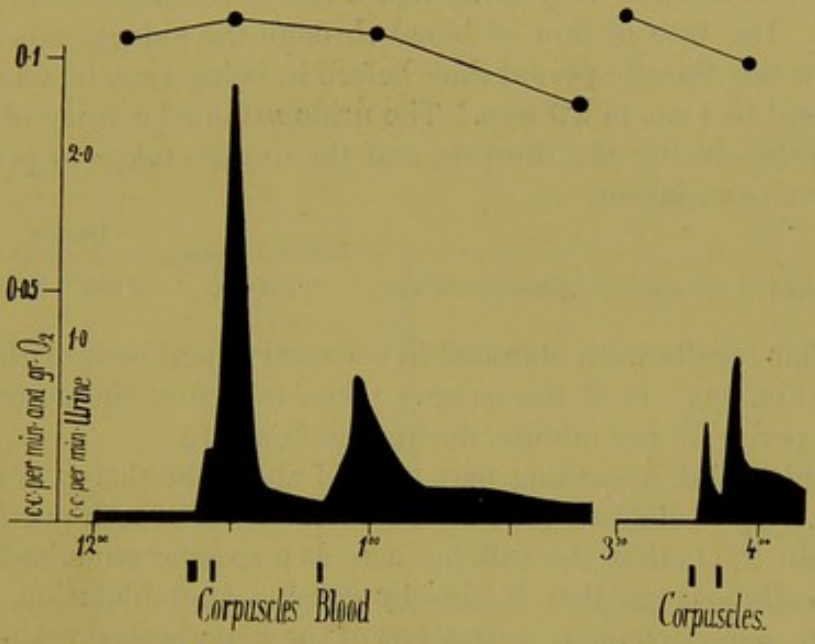


Fig. 62.—Rabbit.

Fig. 63.—Rabbit.

Oxygen and urine plotted as in Fig. 60.

The change in the blood-flow and the general arterial pressure may be eliminated together.

The method of performing the experiment which is least upsetting in every way is to suspend, in the Ringer's solution, red blood corpuscles, then to remove a certain quantity of blood and replace it by the suspension of corpuscles. If this is done, one gets a very considerable diuresis, the vascular conditions of the kidney remain practically unaltered, the composition of the blood remains unaltered as regards salts, and there is no tendency to anaemia. One factor only has been introduced, the plasma is less concentrated in protein : yet a copious flow of urine is at once set up ; the question faces one,

Is this or is it not due to increased activity on the part of cells of the kidney?*

The record of such an experiment is given in Fig. 62. The animal which was the subject of the experiment was secreting 0·05 c.c. of urine per minute, its blood pressure was 95 mm. of mercury; 22 cubic centimetres of blood were taken out and 25 c.c. of Ringer's solution were injected: the secretion at once rose to 0·4 c.c. per minute (see the notch on the record of the diuresis at 12.23), the arterial pressure being then but 52 mm. The suspension of corpuscles (25 c.c.) was put into the jugular vein, the arterial pressure at once rose to 84 mm., and the diuresis reached the very large figure (for a rabbit) of 2·35 c.c. per minute. The rate of flow of blood through the kidneys was slightly slower in the diuretic period than before it, being 1 c.c. in 4·3 seconds as opposed to 1 c.c. in 2·9 secs. The urine attained a value of 0·95 % of chlorides during the diuresis, and the oxygen taken in presented scarcely any variation.

	Before diuresis	During		After
Oxygen taken in per gram of kidney per minute	0·104 c.c.	0·108	0·105	0·09

Similar results were obtained in a second experiment of the same nature (Fig. 63). In it the oxygen taken in before the diuresis was 0·11 c.c. per gram per minute, during the flow 0·10.

At this point something may be said about the theory of caffeine diuresis. Up to the present time two theories have been put forward to explain it, (1) that the caffeine acts as a specific stimulant to the kidney cells and (2) that it acts by causing vaso-dilatation, accompanied to some extent by a paralysis of the hypothetical reabsorptive mechanism of the tubules.

The idea that mere dilution of the protein constituents of the plasma could cause a copious diuresis without any activity on the part of the cells of the kidney was to us so interesting that we felt bound to pursue it further. There seemed to be two possible ways of explaining it. The first of these was that owing to the decreased viscosity of the blood the pressure in the capillaries was greater than formerly, the arterioles not damping the pressure to their normal extent, and secondly, an explanation might be found by expanding a conception put forward some years ago by Starling[2]. To explain the fact that the flow of urine normally stops when the arterial pressure is abnormally reduced, Starling pointed out that the proteins

* The main points in the following discussion on the kidney have been confirmed by the independent work of Prof. Tangl of Buda-Pest.

in the blood exercised an osmotic pressure of about 25—30 mm. of mercury. In so far as filtration could account for the flow of urine, filtration could only take place and therefore urine could only flow when the capillary blood pressure was greater than 25 mm., since the proteins do not go through the wall of the glomerulus. In short the available pressure for filtration is the difference between the capillary blood pressure and the osmotic pressure of the proteins of the plasma. A necessary corollary to this clearly is that if you dilute the proteins and therefore lower their osmotic pressure you increase, other things being equal, the available pressure for filtration.

The issue between these two explanations was taken up by

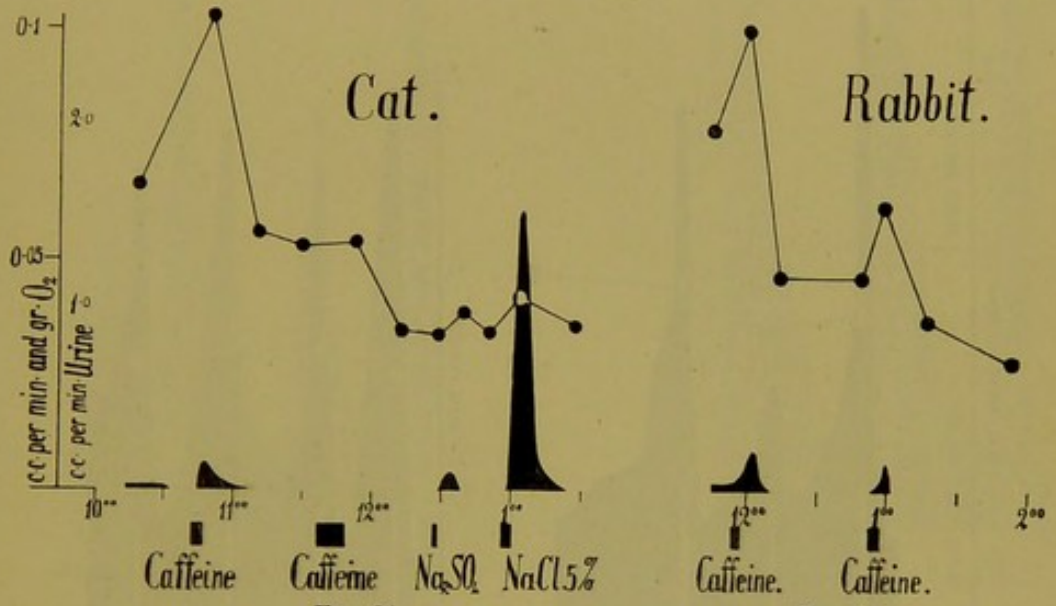


Fig. 64.

Fig. 65.

Oxygen and urine plotted as in Fig. 60.

Knowlton[3]. His experiments may be summed up very shortly; always obtaining a diuresis which produced no work on the part of the kidney. He showed:

(1) That if gelatine was put in the Ringer's fluid in such quantity that osmotic pressure of the gelatine was approximately equal to that of the proteins of the plasma, while the viscosity of the solution was approximately that of defibrinated blood, relatively little diuresis was produced. This is seen in the chart given in Fig. 66. Three injections were made:

(*a*) Ringer's solution without gelatine.
(*b*) Ringer's solution and 5 °/₀ gelatine.
(*c*) Ringer's solution with gelatine.

The oxygen used was the same in each of the three cases; therefore any difference in the quantity of urine was not due to a different degree of activity in the kidney. Yet in the second case there was almost no diuresis whilst in the first and third there was copious diuresis.

(2) In control experiments starch in place of gelatine was added to the Ringer's solution. The addition of soluble starch produces no

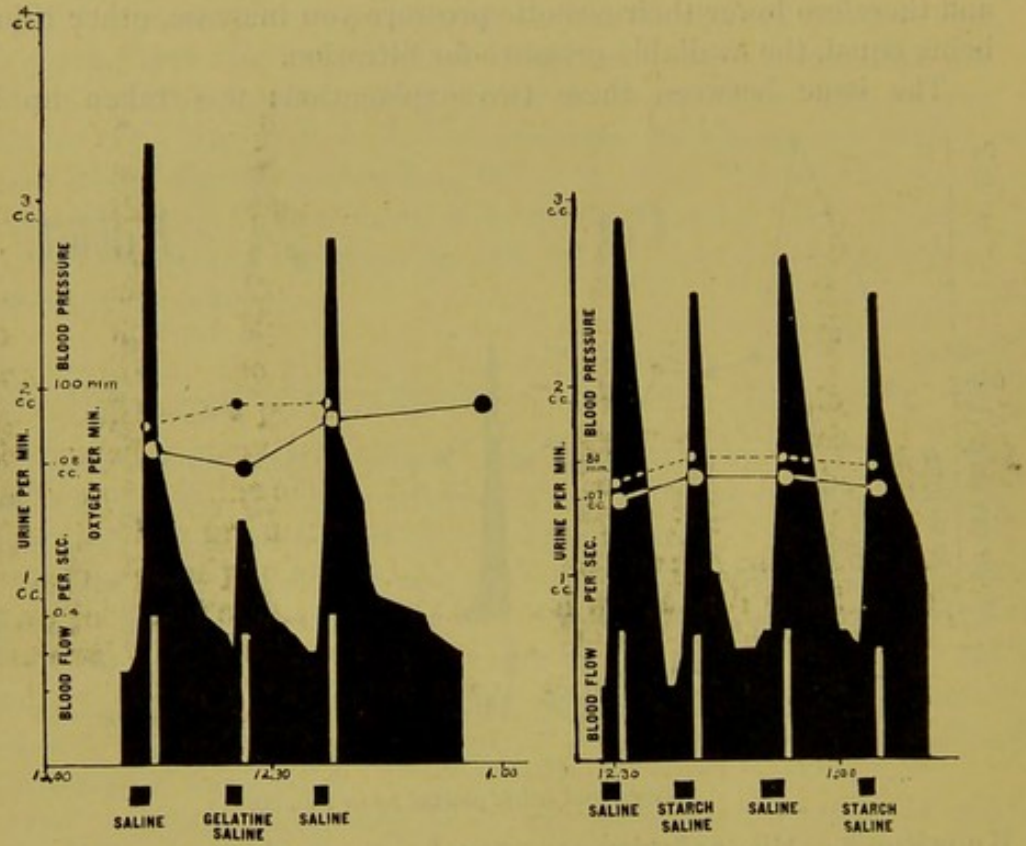


FIG. 66.—Urine = black area. Blood pressure = broken line. The black line just below the blood pressure shows the oxygen consumption by the kidney. Abscissae = time in hours.

increase in the osmotic pressure of Ringer's solution but the fluid has twice the viscosity of blood: yet when injections of Ringer's solution with and without starch were made alternately the diuresis was almost the same in every case.

(3) A 5 per cent. solution of gum acacia has almost no greater viscosity than has the saline solution, but on the other hand it has a considerable osmotic pressure though not quite equal to that of the plasma of the gelatine. The result was the same as that of injection

of the gelatine though corresponding to the slightly lower osmotic pressure; there was just a little greater diuresis than in the case of the starch, though still the diuresis was but small as compared with that of the Ringer's solution which contained no gum acacia.

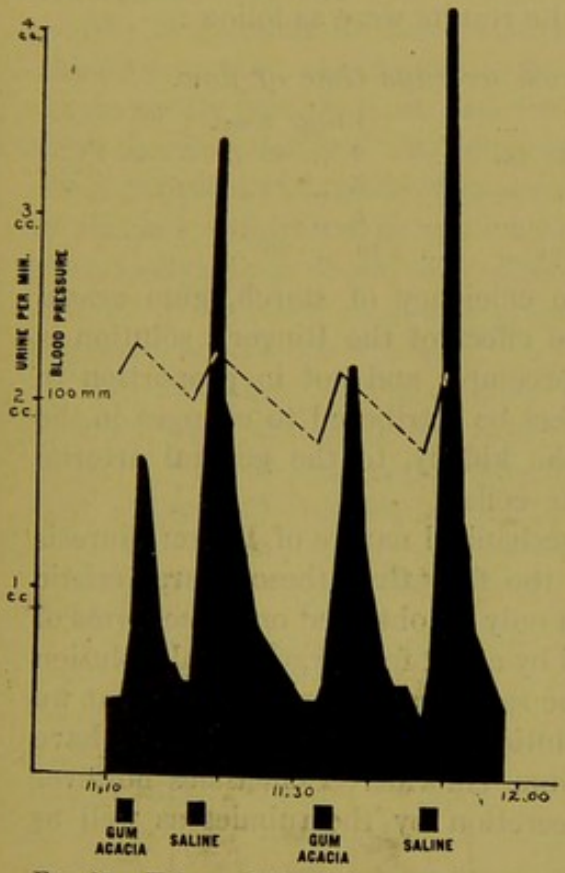


Fig. 67.—Urine and blood-pressure plotted as in Fig. 66.

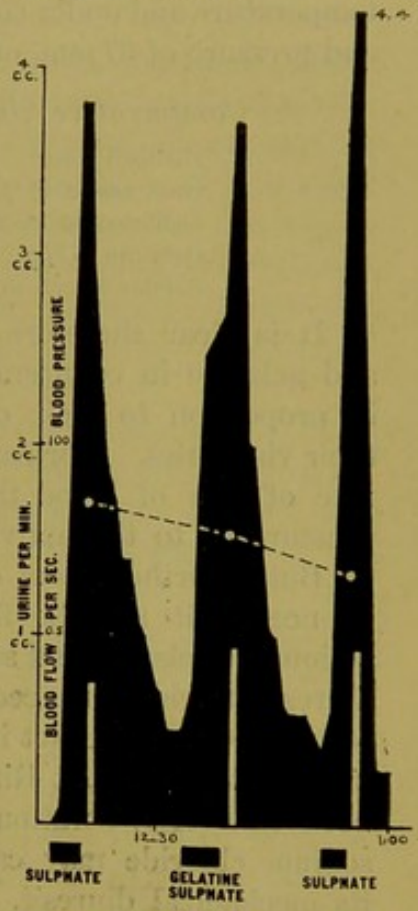


Fig. 68.—Urine, blood-pressure and blood-flow through the kidney plotted as in Fig. 66.

The following are the data of the osmotic pressure :

Solution	Temp.	Osmotic pressure of colloid
5 % gelatine in normal saline	37° C.	23 mm. Hg
5 % gum acacia in normal saline	37° C.	12 mm. Hg
3 % soluble starch in normal saline	37° C.	2 mm. Hg

These figures for gelatine and for gum acacia are considerably lower than the figures given by Moore and Roaf. This is to be explained in part by the fact that they were dissolved in Ringer's solution instead of distilled water and thus the "solution aggregates" of the colloids were altered.

The comparative viscosity of the solutions used was determined by measuring the time of flow of a given quantity through a tube of small bore. The apparatus used was kindly lent by Mr W. B. Hardy. It consisted of a capillary tube and arrangements for causing flow at constant temperature and under constant pressure. At a temperature of 37° C. and pressure of 37 mm. of water, the results were as follow:

Comparative viscosity from average time of flow.

Distilled water	1 min. 5 sec.
Gum acacia (5 %) in saline	2 ,, 22 ,,
Defibrinated blood	5 ,,
Gelatine (5 %) in saline	6 ,,
Soluble starch (3 %) in saline	10 ,, 20 ,,

It is clear therefore that the efficiency of starch, gum acacia and gelatine in counteracting the effect of the Ringer's solution is in proportion to their osmotic pressures and not in proportion to their viscosities. Nor can this effect be attributed to changes in the rate of flow of blood through the kidney, to the general arterial pressure or to the activity of the cells.

But if further proof of the mechanical nature of Ringer diuresis be needed it may be found in the fact that these characteristic actions of gelatine and starch can only be obtained on those forms of diuresis which are unaccompanied by a call for oxygen. Salt solution seems frequently to act in just the same way; in such cases what we have just said about Ringer's solution might with equal truth have been said of a solution of sodium chloride. Sometimes however sodium chloride may cause a secretion by the tubules as well as its mechanical diuresis.

We have already shown that diuresis obtained by sodium sulphate is not of this character. There is increased work done by the kidney as evidenced both by the call for oxygen and by the fact that the urine is, or may be, almost a pure solution of sodium sulphate.

The presence of gelatine in the sodium sulphate solution injected does not counteract the action of the sodium sulphate or at least it does so only to a trifling extent, for although sodium sulphate has a specific secretory action it must of course have a salt action as well.

The information which has been recounted in the preceding pages appeared to be in conflict with a statement which is often put forward as being one of the fundamental facts with regard to urinary secretion, namely that a kidney, the artery of which has been clamped, does not secrete urine for a long time. Winfield and I determined

therefore to test the matter for ourselves and find out if possible the limits within which this statement is true. It is clear at the outset that, while it may be perfectly true that such a kidney does not secrete, it is quite a different matter to suppose that it cannot be made to do so by appropriate methods.

We first tested the matter with Ringer's fluid and with sodium chloride on the one hand, these being diuretics which can act mechanically and elicit no call for oxygen. In contradistinction to these we used caffeine, this being as we have already seen a diuretic which acts solely in virtue of its stimulating effect upon the epithelium of the kidney and has no salt effect. The result was perfectly clear after asphyxiation of the cells of the kidney by clamping; the sodium chloride produced a copious diuresis, the caffeine on the other hand produced no diuresis whatever.

The following data are those of three experiments in which diuresis was evoked, in the case of Exps. 1 and 2 by injection of Ringer's fluid, in the case of Exp. 3 by injection of 10 c.c. of 5 per cent. sodium chloride, the animal in each case being a rabbit.

Exp.	Normal flow of urine c.c. per min.	Normal blood-flow c.c. per min.	Saline diuresis before clamping renal artery	Corresponding blood-flow	Length of time of clamping artery	Second saline diuresis	Corresponding blood-flow
1 (*a*)	0·1	—	1·75 c.c.	—	10 secs.	1·3 c.c.	—
1 (*b*)	0·1	—	2·2 c.c.	—	5 min.	1·3 c.c.	—
2	0·4	24	3·8 c.c.	24 c.c.	10 min.	1·3 c.c.	9 c.c.
3	0·05	48	1·3 c.c.	63 c.c.	18 min.	0·9 c.c.	24 c.c.

It is clear that any diminution which takes place in the diuresis is amply accounted for by the less satisfactory vascular condition, and in any case there is in each experiment a good diuresis after the artery has been clamped for periods varying from 10 seconds to 18 minutes.

On the other hand the following experiment will show how different is the effect of clamping upon the diuresis obtained by caffeine:

	Duration of clamping	Diuresis caused by ·01 grm. of caff. sod. ben.	Diuresis caused by 10 c.c. 5 °/₀ NaCl
Left kidney	20 minutes	0·00 c.c.	0·4 c.c.
Right kidney ...	not clamped	0·5 c.c.	1·2 c.c.

The statement then that the kidney does not secrete after being clamped is subject to certain limitations. If the word "secrete" is used in the specialised sense it is true, but it can excrete if the mechanical conditions are so altered as to promote increased filtration.

Another problem very similar to that which we have just studied, in which the "call for oxygen" has been used as a test of the activity of the cells, is furnished by the work of Brodie and Vogt. The question was, Does the passage of water through the cells of the intestine involve the activity of the intestinal epithelium?

First let me consider cases in which the fluid is passing from the lumen of the intestine to the blood, i.e. cases of absorption from the intestine.

When physiological saline is placed in the intestine and thence finds its way into the blood, work is not necessarily performed in order to satisfy the energy conditions of the system. In practice of course there is likely to be some, just as some expenditure of energy is necessary to drive even the most facile bicycle along the most level and perfect track. The absorption of distilled water is still more interesting for then work might actually be done on the system. There is therefore no *à priori* reason for supposing that the absorption of these fluids from the intestine either is or is not a process involving the activity of the cells.

The simple way of putting the matter to the test was to determine the effect on the oxygen used up by the intestine. In practice the experiment is far from simple, but in the hands of a physiologist who probably has no superior alive at the present time in the type of manipulative procedure involved, the experiments were satisfactorily carried out. The result was an increase in the oxygen consumption, which clearly showed that the absorption of water was an active process. The following are the data of a couple of experiments from Brodie and Vogt's paper.

1. *Absorption of Physiological Saline.*

11.25. Operation completed.
11.45. B.Fl. = 46·875 c.c. per min. = 0·433 c.c. per grm. per min. B.P. = 95.
11.49. Blood samples taken, Stage I.
11.55. *Injection of* 100 *c.c. of warm sodium chloride solution*, 0·93 %.
11.58. Rate of absorption 0·4 c.c. per min.
12.03. ,, ,, 1·0 c.c. per min.
12.06. B.Fl. = 46·875 c.c. per min. = 0·433 c.c. per grm. per min. B.P. = 130.

12.08. Blood samples taken, Stage II.
12.11. Rate of absorption 1·97 c.c. per min.
12.13. ,, ,, 2·0 c.c. per min.
12.24. B.Fl. = 28·85 c.c. per min. = 0·267 c.c. per grm. per min. B.P. = 92.
12.27. Blood samples taken, Stage III.
12.28. Rate of absorption 1·9 c.c. per min.
12·36. ,, ,, 2·0 c.c. per min.
12.45. ,, ,, 0·83 c.c. per min.
12.52. B.Fl. = 35·72 c.c. per min. = 0·331 c.c. per grm. per min. B.P. = 85.
12.53. Blood samples taken, Stage IV.
12.54. Rate of absorption 0·0 c.c. per min.

No fluid was recovered from the intestines. Weight of intestines 108 grams.

The analysis of the blood gases gave:

	Stage I	Stage II	Stage III	Stage IV
Time	11.49	12.08	12.27	12.53
O_2 absorbed by grm. per min.	0·0087	0·0194	0·0210	0·0068
Blood-flow ,, ,,	0·433	0·433	0·267	0·331
Rate of absorption c.c. per min.	0·0	1·97	1·9	0·0
Time after injection	—	13′	32′	58′

In percentages of the value in the resting organ:

O_2	100	224	241	78
B.Fl.	100	100	62	76

2. *Absorption of Distilled Water.*

In this group we have one experiment.

Dog. Intestines washed out with 300 c.c. warm saline solution. Artificial respiration. In this experiment the rate of absorption was followed.

11.10. Operation completed.
11.48. B.Fl. = 35·71 c.c. per min. = 0·331 c.c. per grm. per min. B.P. = 105.
11.50. Blood samples taken, Stage I.
11.55. *Slow injection of* 100 *c.c. of distilled water at* 37° C.
11.59. Commence to determine rate of absorption.
12.00. Absorbed 2 c.c. = 2 c.c. per min.
12.01. ,, 2·5 c.c. = 2·5 c.c. per min.
12.02. ,, 3·5 c.c. = 3·5 c.c. per min.
12 04. ,, 2·6 c.c. = 1·3 c.c. per min.
12.05. B.Fl. = 35·71 c.c. per min. = 0·331 c.c. per grm. per min. B.P. = 75.
12.06. Blood samples taken, Stage II.
12.09. Absorbed 12 c.c. = 2·4 c.c. per min.
12.11. ,, 4·3 c.c. = 2·15 c.c. per min.
12.14. ,, 6·0 c.c. = 2·0 c.c. per min.
12.21. Second set of observations of rate of absorption.
12.24. Absorbed 3·0 c.c. = 1·0 c.c. per min.
12.25. B.Fl. = 25 c.c. per min. = 0·232 c.c. per grm. per min. B.P. = 76.
12.26. Blood samples taken, Stage III.

12.29. Absorbed 3·2 c.c. = 0·64 c.c. per min.
12.31. ,, 1·7 c.c. = 0·85 c.c. per min.
12.34. ,, 2·5 c.c. = 0·83 c.c. per min.
12.37. ,, 4·0 c.c. = 1·33 c.c. per min.
12.43. ,, 7·1 c.c. = 1·18 c.c. per min.
12.53. B.Fl. = 30 c.c. per min. = 0·278 c.c. per grm. per min. B.P. = 87.
12.55. Blood samples taken, Stage IV.
1.02. Experiment stopped.

13 c.c. of fluid were recovered from the intestine. It was alkaline in reaction and gave a fair precipitate with silver nitrate. It did not contain any appreciable amount of mucin. Weight of the intestines 108 grams.

	Stage I	Stage II	Stage III	Stage IV
Time	11.50*	12.06	12.26	12.55
O_2 per grm. per min.	0·0056	0·0177	0·0120	0·0121
B.Fl. per grm. per min.	0·331	0·331	0·232	0·278
Blood-pressure	105	75	90	87
Rate of absorption	0·0	2·4	0·64	1·2
Time after injection	—	11′	31′	60′

* Injection at 11.55.

Or as percentages of the values for the resting organs:

O_2	100	316·1	214·3	216·1
Blood Flow	100	100	70·1	84·0

An endeavour was made to test the further question whether the absorption of peptone involved activity of the cells; in this Halliburton [5] and Miss Cullis took part. Their experiments showed that such an activity took place in the experiments, it is not however clear that the activity was greater than during the absorption of a corresponding quantity of the normal saline in which the peptone was dissolved.

We may now pass to the other phase of the question, namely the investigation of what happens when the fluid passes from the blood to the lumen of the intestine. This of course is a matter of great interest to the pharmacologist, and inasmuch as it was performed from his point of view, the presence of a strong solution of magnesium sulphate in the intestine was used for the purpose of evoking a flow of fluid. The result of the experiment seemed to show that in this case the activity of the cells was not invoked but that the action of the magnesium sulphate was a purely mechanical one.

Dog of 14 kilos weight. Intestines washed out with warm saline. Artificial respiration maintained throughout the experiment.

12.42. Operation completed.
1.09. B.Fl. = 25 c.c. per min. = 0·357 c.c. per grm. per min. B.P. = 135.

1.10. Blood samples collected, Stage I.
1.18. *Injected* 50 c.c. $MgSO_4$ *solution* (4·697 °/₀).
1.25. Increase of fluid in intestine 0·5 c.c. per min.
1.32. ,, ,, ,, 0·5 c.c. per min. No movements to be seen.
1.39. B.Fl. = 27·8 c.c. per min. = 0·397 c.c. per grm. per min. B.P. = 110.
1.40. Blood samples taken, Stage II.
1.46. 15 c.c. of fluid removed from the intestine.
1.51. Increase of intestine volume 0·25 c.c. per min.
2 09. ,, ,, ,, 0·01 c.c. per min.
2.10. B.Fl. = 27·8 c.c. per min. = 0·397 c.c. per grm. per min. B.P. = 105.
2.11. Blood samples taken, Stage III.
2.15. Animal killed.

64 c.c. of fluid were recovered from the intestine. The fluid was very viscid and had much mucin in it. Alkaline in reaction and slightly blood stained. It contained 2·48 °/₀ $MgSO_4$. The fluid removed at 1.46 was clear and contained 4·04 °/₀ $MgSO_4$. The intestine weighed 70 grms. The mucous membrane was very distinctly injected throughout especially over all Peyer's patches. It was covered with thick mucus. The solution injected contained 2·35 grm. $MgSO_4$. That withdrawn 0·61 grm. and that recovered at the end of the experiment 1·59 grm. This leaves 0·15 grm. unaccounted for. This probably was partly absorbed, partly adherent to the mucous membrane although the intestine was washed out and the sulphate estimated in the washings. The minimum amount of water secreted into the intestine was 29 c.c.

The samples on analysis gave the following results:

	Stage I	Stage II	Stage III
Time	1.10*	1.40	2.11
O_2 per grm. per min. ...	0·0191	0·0203	0·0166 c.c.
Blood Flow ,, ...	0·357	0·397	0·397 c.c.
Time after injection ...	—	22′	53′

* Injection at 1.18.

Or in percentages:

O_2	100	106	87
Blood Flow	100	111	111

REFERENCES

(1) Barcroft and Straub, *Journal of Physiol.* XLI, p. 145, 1910.
(2) Starling, *Ibid.* XXIV, p. 317, 1899.
(3) Knowlton, *Ibid.* XLIII, p. 219, 1911.
(4) Brodie and Vogt, *Ibid.* XL, p. 135, 1910.
(5) Brodie, Cullis and Halliburton, *Ibid.* XL, p. 173, 1910.

CHAPTER VIII

THE METABOLISM OF THE BLOOD ITSELF

One of the subtler problems in physiology, and one which has made a considerable appeal to some minds, is that of the extent to which the blood itself can be regarded as a living organ of the body. In some works blood is described as one of the "connective tissues," the essential difference between it and for instance cartilage being that the matrix is fluid instead of solid. This question brings us back to a statement of what we regard as the essential criteria of life. Without indulging in any general statement on the subject it is fairly obvious that any tissue which we regard as alive must have a metabolism of its own, and for that reason various workers have from time to time tried to estimate the amount of oxygen used up by blood per minute and the amount of carbonic acid given off by it. If such a metabolism were proved to exist, it would be natural to discuss the extent to which the plasma, the red corpuscles and the white corpuscles respectively participated in it.

The early work on this subject was of course wholly vitiated by ignorance of the growth of microorganisms, the so-called metabolism of the blood being simply evidence of bacterial action.

Within the last few years, the problem has been taken up afresh by two workers, Warburg[1] and Morawitz[2], both at that time working at Heidelberg, though in different laboratories. As will be seen they worked from different points of view.

Before describing their work in greater detail there is one point which should be made clear. The oxidative processes in the blood, in so far as they exist, are of two quite different natures which must not be confused. In the first place there may be substances which are readily oxidisable, which have found their way into the blood in small quantities from the tissues and which take up a certain

quantity of oxygen; these incomplete products of metabolism have been studied by Pflüger and more recently by Krogh and others*; once oxidised they are done with; they have nothing to do with the subject which we are about to discuss. In the second place we have a call for oxygen which may be attributed to the life of the corpuscles of the blood itself. This is a steady oxidation which goes on from hour to hour.

As regards the life of the red corpuscles a natural line of inquiry was that adopted by Warburg, namely to get some idea of the importance of the nucleus to the metabolism of the corpuscle. He therefore compared the metabolism of healthy human blood with that of avian blood.

The method was very simple. The blood was obtained aseptically (as shown by cultures in bouillon), centrifugalised in sterile salt solution and received in two glass bottles of 3 c.c. each. In these bottles were some glass beads. The corpuscles and the salt solution were thoroughly mixed by shaking, the oxygen in the blood of the one was estimated at once, the other was incubated for a given time.

The following results were obtained for human blood: the degree of reduction is obtained by dividing the difference between the oxygen in the two samples by the oxygen in the original blood, and then multiplying by one hundred.

	No. 1		No. 2		
Duration of incubation ...	3	15	17	20	hours
Degree of reduction	3 °/₀	5 °/₀	9 °/₀	10 °/₀	

In rabbit's blood the rate of reduction is considerably greater.

Duration of incubation ...	2½	2½	8	7	8½	12½	hours
Degree of reduction	8 °/₀	8 °/₀	22 °/₀	19 °/₀	31 °/₀	29 °/₀	

As compared with these it will be seen that the metabolism of the nucleated corpuscles of the goose is very great, and is maintained at a fairly even level.

Duration of incubation ...	150	102	150	minutes
Fluid in which corpuscles are suspended	plasma	Ringer without $NaHCO_3$	Friedenthal	
Reduction per *hour*	33 °/₀	32 °/₀	33 °/₀	

* Whilst this book has been in the press a very instructive paper on this subject has appeared by Evans and Starling, *Journ. of Physiol.*, XLVI.

These observations were so controlled as to make it clear that the effects were not in any way due to leucocytes.

The reaction just described between oxygen and the nucleated corpuscles of the goose has been used by Warburg as a typical reaction by which to test the action of narcotics upon living matter. Having determined the rate at which reduction takes place in the manner we have described he went on to determine the rate at which it is inhibited by various substances. The results of a single set of determinations will show the general plan of the research.

Suppose the problem be to determine the effects of various strengths of potassium cyanide upon living matter; the corpuscles were suspended in ·9 °/₀ NaCl solution containing the required strength of potassium cyanide and incubated for 5 hours (in the case of other organic substances usually 2 hours). The degree of reduction which was found would then be expressed as a percentage of what it would have been had the potassium cyanide not been present.

The following figure will show the elegance of the results which may be obtained by this method.

In contradistinction to the small amount of metabolism which normal blood exhibits, a most interesting fact was discovered by Morawitz[2], namely that the blood of anaemic animals had a very considerable metabolism.

The technique is simple. Healthy rabbits were given daily injections of phenylhydrazine hydrochloride until they became anaemic. When the haemoglobin content had reached about 20 °/₀ of its normal value, the blood was withdrawn from the carotid or other artery as the result of an aseptic operation, defibrinated by shaking with glass beads and thoroughly shaken with oxygen. One portion of the blood was analysed at once in the Barcroft-Haldane apparatus, the remainder, about 3 c.c., was placed in a glass bottle from which air was excluded, and kept in a water-bath at known temperature and for a known time. The oxygen present was then analysed. An example will perhaps make the method most clear.

Rabbit No. 3. Made anaemic by injections of phenylhydrazine between 10 May and 4 June. Haemoglobin value sank to 18 °/₀.

Bled and killed on June 4. Maximal oxygen capacity of 1 c.c. of blood 0·043 c.c. After two hours at room temperature—oxygen in blood nil.

Morawitz performed numerous control experiments, and the series yielded the following data :

(1) That the oxidation is a function of the corpuscles, since it is just as evident in a suspension of them which has been removed from the plasma and washed three times over in normal salt solution.

(2) That it has an optimum temperature equal roughly to that of the body.

(3) That it cannot be accounted for by nucleated corpuscles, either red or white.

(4) That it is in short due to the young unnucleated red cor-

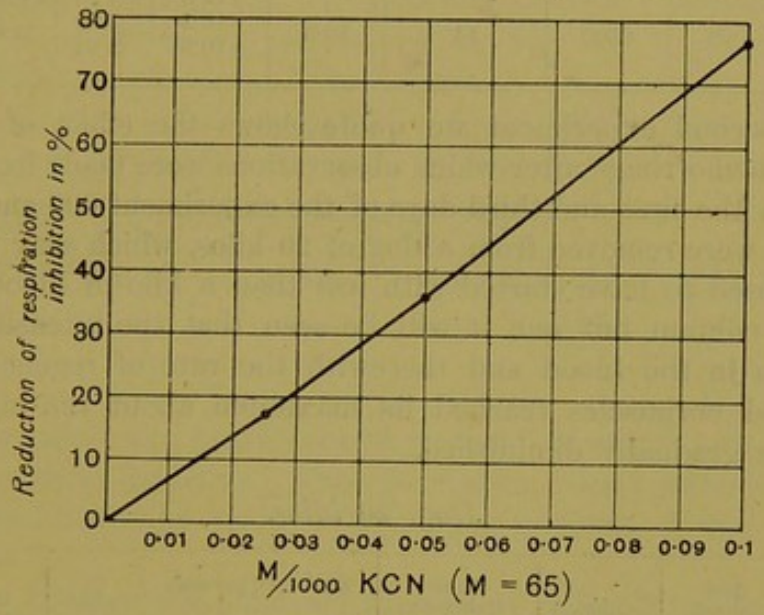


Fig. 69.

puscles which are present in large numbers in the blood of the anaemic rabbit.

The work was continued by Itami[3], who followed the whole course of numerous cases of anaemia produced experimentally both in dogs and rabbits. The anaemia was in some cases post-haemorrhagic, in some cases induced by phenylhydrazine. The technique of the blood-gas analyses was the same as that of Morawitz.

Let us consider some abstracts of typical experiments. The first shows the influence of continuous bleeding. It is evident from the last column but one that as more and more new corpuscles were produced the oxygen consumption of the blood itself became greater and greater in amount, corresponding to the increased number of young corpuscles present.

Rabbit, Male, 2900 grams.

Date after bleeding (days)	Red blood corpuscles Millions	Nucleated elements	Hb by Haldane's haemoglobinometer per cent.	Maxim. O_2 per cent.		O_2 after 5 hours per cent.	O_2 used up per cent.	Bleeding c.c.
				Estimated from haemoglobinometer reading	Observed			
1	5·21	6500	82	15·2	15·4 15·3	14·2 14·0	7·8	20
3	4·79	3250	74	13·7	14·1 13·9	12·2 12·0	13·6	20
5	4·45	4850	68	12·6	12·8 12·9	10·1 10·0	21·1	30
7	4·06	5000	62	12·5	11·9 12·0	3·7 4·0	60·0	35
9	3·29	6500	54	10·0	10·2 10·3	2·5 2·6	75·0	—

The second experiment we quote shows the effect of a single violent haemorrhage after which observations were made from day to day. On the first and third days of the experiment 600 and 400 c.c. of blood were removed from a dog of 20 kilos, which may therefore be supposed to have started with less than a kilo of blood. From the last column but one it will be seen that the intensity of the oxidation in the blood and therewith the rate of regeneration of red blood corpuscles reached its maximum about the eighth day and then gradually diminished.

Dog, 20 kilos.

Date after bleeding	Red blood corpuscles Millions	Nucleated elements	Hb by Haldane's haemoglobinometer per cent.	Maxim. O_2 per cent.		O_2 after 5 hours per cent.	O_2 used up per cent.	Bleeding c.c.
				Estimated	Observed			
1	6·93	14000	110	20·4	20·4 20·2	18·8 19·0	17·0	600
3	5·03	10000	78	14·4	14·7 14·6	11·3 11·2	22·8	400
5	4·35	20000	68	12·6	12·8 12·6	4·7 4·8	62·7	—
8	4·18	19500	66	12·2	12·4 12·5	4·3 4·2	65·3	—
12	4·42	17500	76	14·1	14·3 14·2	10·4 10·5	26·0	—
15	5·26	14000	80	14·8	14·9 14·8	12·2 12·1	18·1	—

In the third experiment given here he again controls the question of whether the increased oxidation in the blood can be attributed to the nucleated elements. For this purpose he divides the blood

into two portions. One of these, B, is rapidly defibrinated by vigorous shaking, the other, A, is defibrinated very slowly. In the latter case blood-counts showed that there were three times as many nucleated corpuscles as in the former, yet the percentage of reduction was not very different in the two cases.

Experiment on dog.

	Red blood corpuscles Millions	Hb by Haldane's haemoglobinometer per cent.	Nucleated elements	Maxim. O_2 per cent.		O_2 after 5 hours per cent.	O_2 used up per cent.
				Estimated	Observed		
B	3·32	50	6000	9·3	9·2 9·4	5·0 4·8	46
A	3·96	54	18500	10·0	10·1 10·3	5·0 4·7	52·9

Experiment on rabbit.

B	2·60	40	2250	7·4	7·6	1·5 1·5	80
A	2·73	41	7500	7·6	7·7 7·7	1·1 0·9	87

A short while ago I came across an instance of the type of blood which Morawitz describes. The observation is not without interest in itself and it may be useful as a warning to some. I will therefore describe it.

In the *Biochemical Journal* Moore and Wilson[4] published some observations on the alkalinity of the blood in cancer. It occurred to me that such changes in reaction could be observed by exposing the blood to a known oxygen pressure and observing whether the haemoglobin became more or less saturated with oxygen than would normally have been the case.

Dr Hopkins was kind enough to give me the blood of numerous rats, which he was killing at the time and which had grown sarcomata. The result of the experiment seemed interesting beyond my expectation, for the rats with sarcomata possessed blood which reduced to a much greater extent than did the blood of normal rats. Indeed it was possible to draw a very presentable curve showing that the quantity of $\frac{\text{reduced haemoglobin}}{\text{total haemoglobin}} \times 100$ or percentage reduction of the blood exposed to 17 mm. oxygen pressure followed the weights

of the tumours. The interpretation of this would have been—the bigger the tumour the more acid the blood. My suspicion was aroused by the great degree of reduction which some of the blood underwent. This caused me to do control experiments in which I treated some of the same blood with air instead of 17 mm. of oxygen; these control experiments led us to suspect that the blood reduced itself.

A second series of experiments were undertaken for the object of testing this point. The technique was simple. The rats were killed by cutting the throat. The blood was whipped with feathers, thoroughly saturated with air and placed in a 1 c.c. glass syringe. Care was taken to prevent air entering the syringe which was placed in an incubator at 37° C. for ¾ hour. The blood was then expelled into the bottle of a differential apparatus under ammonia. The percentage saturation was measured in the usual way, and as a known quantity of blood was used the total oxygen capacity was also obtained.

Many of the samples showed a great degree of reduction. In the series there were two classes of animals, those with tumours of 5 grams and under and those with tumours of 13 grams and over. The blood which reduced itself to a great degree during the 45 minutes incubation came in each case from the rats with large tumours, but the degree of reduction followed the total oxygen capacity of the blood more closely than the weight of the tumour. Roughly speaking the more anaemic the rat the greater was the amount of reduction in the blood. The following table gives the data.

Number of rat	71	71 A	72 A	73	73 A	74	74 A	75	Control sheep's blood
Weight of tumour (grams)	1	0+	23	13	34	1	5	14	—
Percentage saturation of blood after ¾ hour incubation	85	89	49	0	64	84	80	66	98
Oxygen capacity: c.c. of O_2 per c.c. of blood	·14	·111	·070	·044	·088	·134	·132	·069	—
Oxygen capacity: Gower-Haldane haemoglobinometer ...	75	60	38	24	47	78	77	38	—

Fig. 70 represents these data in the order of the haemoglobin value showing how close is the correspondence between the degree of anaemia and the power of the blood to eat up its own oxygen.

It is therefore necessary to control many researches by testing for self-reduction in the blood. With this warning let me pass to a wholly different subject for which this property of young corpuscles has been used as a test.

Armed with the discovery that freshly formed red cells have an appreciable metabolism, Morawitz and Masing[5] sought to solve a problem which has been something of a stumbling-block to physiologists, namely whether or no there is an increased formation of red blood corpuscles at high altitudes.

When they attacked the problem it was capable of statement as follows: It was known that life at increased altitude caused an increase in the number of red blood corpuscles per cubic millimetre

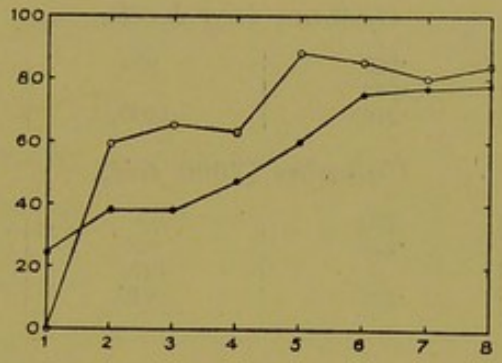


Fig. 70.—Shows the degree of anaemia and the degree of self-reduction of the blood in each of eight rats which had sarcomatous tumours. o = Percentage saturation of blood after incubation. • = Haemoglobin value (100 corresponds to ·185 c.c. oxygen per c.c. of blood). The figures along the abscissa denote the rats.

of blood taken from the finger or ear. This increase was accounted for in three different ways by different authors.

(1) That there was an actual increase in the formation of red corpuscles.

(2) That there was a diminution in the plasma leading to a greater concentration of the corpuscles.

(3) That the corpuscles became unevenly distributed, being increased relatively to the plasma in the peripheral blood and decreased relatively to the fluid elements of the blood in the viscera.

It is not easy to arrive at a judgment as to whether or no there is an increased formation of haemoglobin in man. The most obvious path along which to seek a solution is that of measuring the total blood volume by the carbon monoxide method, either as described by Haldane[6] or by Plesch[7]. Indeed measurements of this sort have

been made since the experiments of Morawitz and Masing which we are about to describe. Douglas[8] in Teneriffe obtained the following data upon my own blood.

Date	Hb °/₀	Oxygen capacity in c.c. per c.c. of blood	Total oxygen capacity of blood in body	Grams of haemoglobin	Volume of blood
		Cambridge.			
Feb. 5	100	·185	1017	140	5500
,, 6	100	·185	1053	146	5700
,, 21	100	·185	1025	142	5550
Mean for.....	100	·185	1032	143	5583
		Orotava (sea level).			
March 21	103	·191	995	144	5220
,, 23	105	·194	—	—	—
,, 25	113·5	·210	1000	157	4770
		Cañadas (7000 feet).			
March 29	108	·200	—	—	—
April 2	108	·200	950	142	4750
,, 4	—	—	1075	—	—
,, 11	111	·205	940	145	4580

The last two columns indicate that in my own case there was no increase in the amount of haemoglobin, but from some cause or other a concentration of the blood.

To return however to Morawitz and Masing, they showed that an appreciable rise took place in the metabolism of the blood itself, as the result of bleeding to the extent of 400 c.c. To put this in another way, if the body is called upon suddenly to make haemoglobin to the extent of 8 °/₀ of the haemoglobin in the body, the formation of young corpuscles with high metabolism will be sufficient to be appreciated by their desire for oxygen. The experimental procedure was to shake the sterile blood, incubate it for 5 hours, at the end of which time the oxygen in the blood was measured by a gas analysis apparatus. Normal blood would have lost less than ·01 c.c. of oxygen per c.c. of blood, while that from the patient after bleeding would have lost more than ·01 c.c. of oxygen.

If then there were at high altitudes a formation of new blood, erythrocytes corresponding in quantity to those in 400 c.c. of normal blood, the blood would acquire the power of a considerable degree of

self reduction. The following table gives the results of Morawitz' and Masing's experiments at Col d'Olen (10,000 feet) from which it will be seen that they were able to discern no sudden increase in the haemoglobin formation. The respiratory activity of the blood itself was appreciably less in the case of "P.M." as the result of his climb to Col d'Olen than in the result of losing 400 c.c. of blood, whilst in the case of "E.M." there was no greater evidence of young corpuscles on Monte Rosa than at Heidelberg.

I. *Subject of research, P. M.*

Date	Hb in per cent.	No. of red corpuscles per cub. mm.	No. of white corpuscles per cub. mm.	O_2 capacity of blood in volume per cent.		O_2 diminution in volume per cent.	
				Freshly shaken	After 5 hours incubation		
April 12	114	5,200,000	5200	21·1	20·2	0·9	
,, 13	—	—	—	—	—	—	
,, 15	110	5,100,000	5200	20·3	19·1	1·2	Bled 300 c.c.
July 29	115 (113)	(5,600,000)	—	21·5	20·6	0·9	Heidelberg (115 m.)
Aug. 2	114	(5,400,000)	4000	21·3	20·3	1·0	
,, 6	—	(5,400,000)	—	—	—	—	
Aug. 13—17	—	—	—	—	—	—	Small journeys
Aug. 19	—	—	—	—	—	—	Climb to Col d'Olen
,, 21	116	5,300,000 (4,800,000)	4800	21·8	21·0	0·8	
,, 23	(115)	(5,000,000)	—	—	—	—	
,, 24	119	5,600,000	5000	22·2	21·3	0·9	Col d'Olen (3000 m.)
,, 26	121	5,700,000	6500	22·1	22·2	0	
,, 28	120	5,700,000	4500	21·9	21·0	0·9	
Sept. 8	118	5,500,000	—	—	—	—	Bled 400 c.c.
,, 9	117	5,300,000	6400	21·6	20·1	1·5	Heidelberg (115 m.)

II. *Subject of research, E. M.*

July 28	(108)	5,300,000	—	20·4	19·5	0·9	
,, 31	113	—	4100	20·7	20·2	0·5	Heidelberg (115 m.)
Aug. 12	112	5,500,000	(6000)	—	—	—	
Aug. 13—17	—	—	—	—	—	—	Small journeys
Aug. 19	—	—	—	—	—	—	Climb to Col d'Olen
,, 22	117	6,000,000 (5,000,000)	5700	22·3	21·5	0·8	
,, 23	—	(5,800,000)	—	—	—	—	
,, 25	118	6,100,000 (5,100,000)	5200	21·8	21·2	0·6	Col d'Olen (3000 m.)
,, 27	122	5,300,000	5300	23·0	22·2	0·8	

When I was working at the Col d'Olen laboratory the question of the increase of red corpuscles was the subject of research by Cohnheim[9] and his collaborators. They arrived at the same

conclusion as Morawitz, namely that in spite of all that has been said and written on the subject they could detect no considerable increase in the number of corpuscles or the amount of haemoglobin in the blood.

At the time we were at Col d'Olen, Haldane and his collaborators Douglas, Yendell Henderson and Schneider[10], were investigating the same problem on Pikes Peak (14,000 feet high). They went into the matter with great care; estimating (1) the number of corpuscles by two different types of haematocytometer, Bürker's and Thoma-Zeiss'; (2) the oxygen capacity of 1 c.c. of blood by a haemoglobinometer and by direct determinations with ferricyanide; (3) the total blood

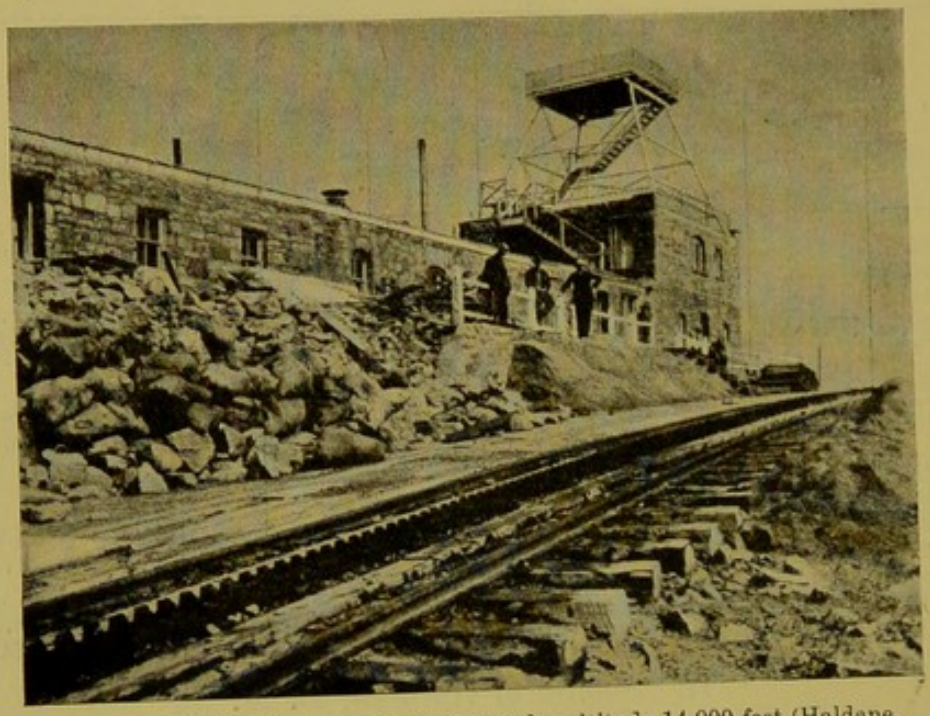


Fig. 71.—Pikes Peak observatory, Colorado, altitude 14,000 feet (Haldane, Douglas, Henderson and Schneider).

volume and total oxygen capacity of the body by the CO method already described. The following charts show clearly that the results which they obtained for the various members of the party were very uniform.

In each case there was a gradual rise in the total oxygen capacity which reached its maximal value, only after some time, about three weeks after the ascent. This perhaps is the essential point, for it proves quite clearly that the body reacts to the altitude either by producing increased quantities of haemoglobin or by retaining what would otherwise be broken. This at least is a positive reaction.

The change in the blood volume is much less considerable, it is also less constant in different individuals; for instance, in the first week Douglas' blood volume went down, Haldane's remained practically constant, those of Henderson and Schneider rose a trifle. The factor which perhaps underwent the most constant change was the percentage of haemoglobin in the blood. Cutting out the daily

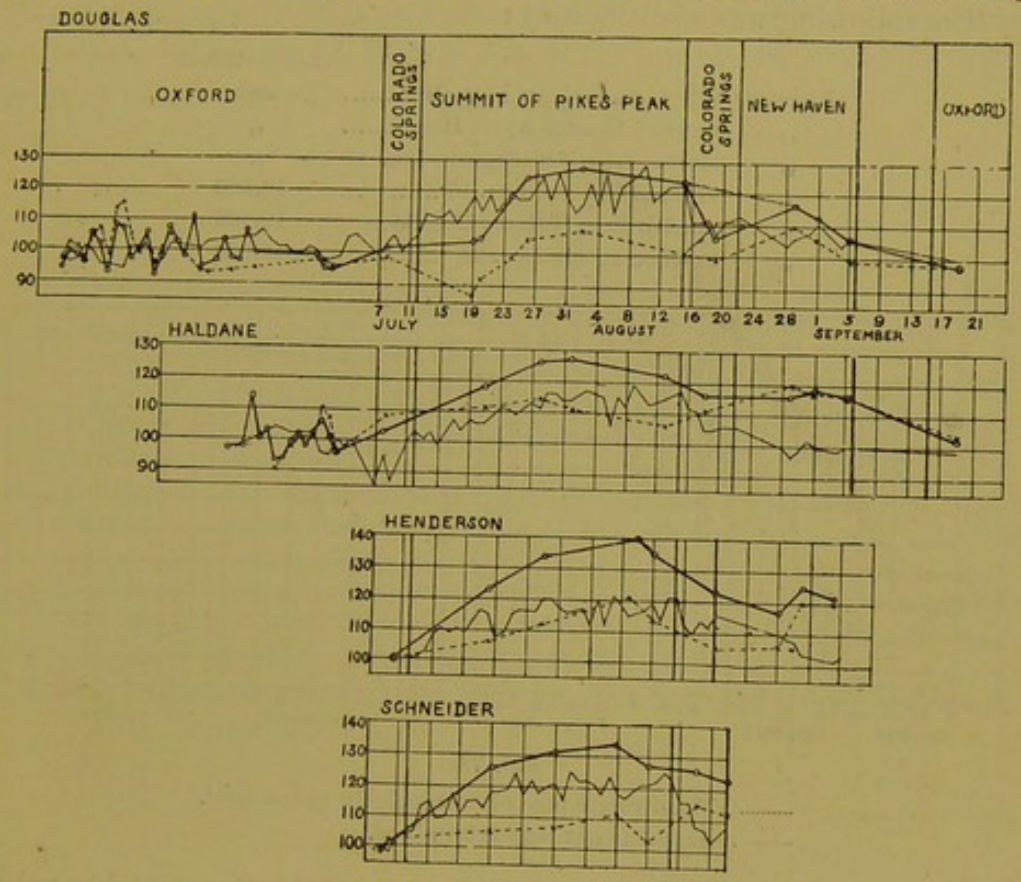


Fig. 72.

Abscissae and ordinates on the same scale in each case.
Ordinates represent percentages of the average values obtained before ascending the Peak (Oxford and Colorado springs) on the particular subject.
——= percentage of haemoglobin.
·····= blood volume.
——= total oxygen capacity or total amount of haemoglobin.

variations, which were considerable, this reached a maximal value of about 120, in each case taking its original value as 100, but here again it must be observed that the rise was but a gradual one.

A very complete series of results on the oxygen capacity of the blood have been performed by Mr J. Richards[11], the manager of a

tin mine at Pazna in Bolivia situated at an altitude of 15,000 feet. As coal gas was not available for the purpose of operating the Haldane's haemoglobinometer, he used one containing picrocarmine jelly which was standardised by Haldane before and after the series of observations and found not to have varied more than 1 %. The readings have been reduced to those of the Haldane's standard instrument.

The following was his itinerary :

		Level in feet
Richards	left Liverpool on Nov. 10.....	sea
,,	reached Buenos Ayres Dec. 5.....	,,
,,	started from Antofagasta Dec. 15	,,
,,	reached Tatoral Dec. 17	12,500
,,	started from Tatoral Dec. 24.....	12,500
,,	reached Pazna Dec. 24	15,000

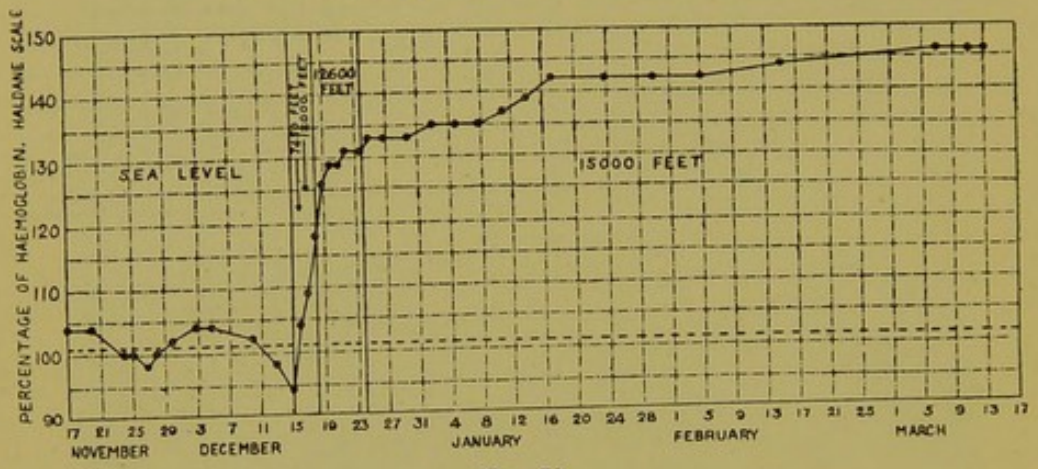


Fig. 73.

.....= Mean percentage of haemoglobin at sea level.

——= Actual percentage determined.

The chart shows the very definite rise in the haemoglobin-value of the blood and that it did not reach its maximum till after two and a half months' residence at the altitude of 15,000 feet.

What then is to be made of the very conflicting results which have been obtained by different observers, each of unassailable position as a physiological expert, and even of the same observer (Douglas) working at the different places but with the same method ?

For what it is worth, my view of the matter at the present time is this. Col d'Olen (10,000 feet) and the Cañadas (7000) are too low to get much result, at all events in the short time that has been at the disposal of the workers. In a fortnight at the Cañadas the total oxygen capacity of my body practically remained the same. In later

chapters in this book it will appear that in other respects I was also unchanged—in fact the altitude had not as yet touched me. At Col d'Olen even, changes such as diminution of alveolar CO_2 pressure are, in some individuals, very slight. This consideration will explain why Douglas got negative results at Las Cañadas, and why Cohnheim and possibly Morawitz also got negative results at Col d'Olen, whilst Zuntz, Haldane and others obtained positive results at higher altitudes.

It may of course be that in a longer time there would be well-marked positive results at lower altitudes.

REFERENCES

(1) Warburg, *Hoppe Seyler's Zeitsch.* LXVI, p. 305, 1910; *Ibid.* LXXVI, p. 331, 1912.
(2) Morawitz, *Archiv f. exp. Pathol. und Pharmacol.* LX, p. 298.
(3) Itami and Morawitz, *Deutsch. Arch. f. klin. Med.* C, p. 191.
(4) Moore and Wilson, *Biochem. Journal*, I, p. 326; Mathison, this book, chap. V.
(5) Morawitz and Masing, *Deutsche Med. Wochenschr.* 1910, Nr. 8; *Deutsche Archiv f. klin. Med.*
(6) Haldane and Lorrain Smith, *Journal of Physiol.* XXV, p. 331, 1900.
(7) Plesch, *Münchener Med. Wochenschr.* LVII, 8, p. 406.
(8) Douglas, *Journal of Physiol.* XL, p. 472, 1910.
(9) Cohnheim, Kreglinger, Tolber and Weber, *Zeitsch. f. Physiol. Chem.* LXXVIII, p. 70, 1912.
(10) Douglas, *Phil. Trans. of Roy. Soc.* Ser. B, Vol. 203.
(11) Richards, Supplement to Report of Pikes Peak Expedition. *Phil. Trans. of Roy. Soc.* Ser. B, Vol. 203.

CHAPTER IX

THE REGULATION OF THE SUPPLY OF OXYGEN TO THE TISSUES

From the consideration of the call of the tissues for oxygen, I pass to the mechanism by which that call is met. The information about the passage of oxygen from the blood to the organs may be considered under two main headings: firstly the provision for bringing to the organ the quantity of oxygen which is required, and secondly the physical process by which the oxygen is transferred from the capillary blood vessel to the cell. In the case of most organs of the body the sole channel by which the organ receives its oxygen is its nutrient artery. One organ, the liver, has two channels of supply; it is by no means obvious whether the portal vein or the hepatic artery provides the essential oxygen supply. I shall clear the ground by first considering this question of the liver, then I shall pass to the functional fluctuations in the oxygen supply of organs generally and the mechanism by which these fluctuations are brought about.

Concerning the liver, then, let me quote two text-books of physiology currently read by students: (1) "The liver has a double blood supply: the portal vein which supplies a rich capillary anastomosis round every liver cell and carries venous blood from the alimentary canal, and the hepatic artery, which carries oxygenated arterial blood and supplies chiefly the connective tissue surrounding the bile ducts and blood vessels in the division between the lobules, known as Glisson's capsule." (2) "The hepatic artery, the chief function of which is to distribute blood for the nutrition of Glisson's capsule, the walls of the ducts and blood vessels, and other parts of the liver." No one reading these statements would have been surprised had a quantitative investigation of the oxygen supply of the liver shown that the hepatic artery brought but a trifling quantity of blood to the liver and found oxygen merely for the accessory structures. Yet

this proved to be exactly the reverse of what takes place. The fact is[1] that the blood in the hepatic vein leaving the liver on the average contains about as much oxygen as does that in the portal vein. The comparison is shown in the following table :

Oxygen in 1 *c.c. of blood entering the liver by the portal vein and leaving it by the hepatic vein.*

										Total	Mean of 10 exps.
Portal vein	·041	·049	·114	·038	·033	·033	·017	·032	·039	·094	·490	·049
Hepatic vein	·042	·052	·088	·017	·074	·029	·000	·004	·056	·088	·450	·045

It is impossible to discuss the fate of the individual corpuscles on their way through the liver. We only have the data furnished by a knowledge of the initial and final conditions of the blood with regard to the organ. When we speak of the oxygen which it receives from the hepatic artery we mean the amount of oxygen in 1 c.c. of the arterial blood minus the oxygen in 1 c.c. of blood from the hepatic vein multiplied by the amount of blood entering the liver from the artery per minute ; and similarly with regard to the portal blood, the quantity of oxygen which the liver acquires from this channel is regarded as the oxygen in 1 c.c. of portal blood minus that in 1 c.c. of hepatic venous blood, multiplied by the number of c.c. of blood entering the liver by the portal vein per minute. By the addition of these two quantities (the oxygen acquired from the hepatic artery and that acquired from the portal vein) we arrive at the total quantity of oxygen used by the liver.

The following example which is typical will indicate what we mean :

Oxygen in 1 c.c. of blood in hepatic artery ·128 c.c.
,, ,, ,, portal vein ·033 c.c.
,, ,, ,, hepatic vein ·029 c.c.
Rate of flow of blood in hepatic artery 16 c.c. per minute
,, ,, portal vein 20 c.c. per minute
Oxygen taken from hepatic artery (·128 – ·029) × 16 = 1·58
,, ,, portal vein (·033 – ·029) × 20 = ·08
Oxygen used by liver per minute = 1·66

In the majority of experiments the whole amount of oxygen brought to the liver by the portal blood would not suffice for its needs, whilst that brought by the hepatic artery would more than suffice as the following figures show.

Oxygen in c.c. per minute.

Oxygen used by the liver	Oxygen brought to liver by	
	Portal vein	Hepatic artery
·35	·85	·47
·41	·47	·82
·77	1·85	·59
1·61	1·09	1·00
·91	·66	3·00
1·76	·66	2·06
5·81 c.c.	5·58 c.c.	7·94 c.c.

Indeed the oxygen used by the liver is unaffected if the portal vein be ligatured altogether and its blood diverted into the general circulation without first passing through the liver. So much for what we may call the "resting" liver; by that we mean the liver of an animal which had not been fed for 36 hours before the experiment.

Important as is the arterial supply to the resting liver, it is much more important in the case of the liver which is using up relatively large quantities of oxygen. In animals which were fed 18 hours before the experiment the quantities of oxygen brought to the liver by the portal blood are of the same order as in those experiments which we have already quoted; the excess of oxygen brought comes along the hepatic artery. From the following four experiments the reader can gather the extent to which the liver would be starved of oxygen did the hepatic artery perform no more important function than that of supplying Glisson's capsule, and the walls of the blood vessels and ducts.

Oxygen in c.c. per minute.

Oxygen used by the liver	Oxygen brought to liver by	
	Portal vein	Hepatic artery
3·8	·28	3·53
2·17	·58	1·56
1·92	·78	3·67
1·72	2·25	3·02
9·61 c.c.	3·89 c.c.	11·78 c.c.

The mechanism by which the arterial supply of blood is increased during digestion is unknown, it may be to some extent local and to some extent reflex. This at least is known from the work of Burton-Opitz[2]: the branches of the hepatic artery are richly supplied by vaso-constrictor nerves; these have been denied to the

branches of the portal vein. We have therefore a complete scheme before us. The regulation of the portal blood depends upon the intestine and on the processes going on in the intestine. So far as the liver is concerned the portal blood is the raw material on which it works, but it has also a blood supply proper to itself for the needs of its own metabolism.

Now to turn to the general question of the fluctuations which occur in the blood supply of the organs of the body. We are not concerned with such fluctuations as are brought about by the central nervous system without reference to the needs of the organ for the purpose of maintaining the general arterial pressure. These do not touch the respiration of the organ. In Chapter V we have emphasised the great variations which occur at different times in the need of the organ for oxygen. Unless the tissue when at rest is to be flooded with large quantities of unnecessary blood, the variations in its blood supply must be of the same order as those of the oxygen required. And for our present purpose we must regard the question of blood-flow from the point of view of the oxygen itself.

Let us take the pancreas as an instance. It is excited to activity by a stimulus which acts directly upon its cells; the gland at once requires four or five times as much oxygen as before: how is this to be obtained? Is there a reflex mechanism by means of which the pancreas can beg a supply of blood from the vaso-motor centre? We know of none. Does the secretion act on the vaso-motor centre itself? We have no reason to suppose that its action is anything but local. The broad question which is to be answered is this, Is there evidence that each organ of the body is so far master of its own metabolism that it can force the vascular system to give it the oxygen which it requires?

This is the sort of question which used to be put by Gaskell to his class when I was a student; since then the evidence on the subject has increased many fold.

Let me start then by accepting (1) the conclusions of Severini[3], Gaskell[4], and other more recent workers, in so far as they show that certain products of metabolisms, to wit acids including CO_2 and lactic acid, have the power of distending blood vessels, and (2) those of Dale[5] and his colleagues to the effect that β-iminazolylethylamine, a body so closely bound up with the physiology of protoplasm that it is liberated by the splitting off of CO_2 from histidine, will produce a powerful dilatation of the vessels. So powerful indeed is the effect

that the blood pressure of a monkey will fall to a little over half its former value if but 0·0005 gram of the drug is injected intravenously. How incredibly small a quantity of "β-I" would suffice to produce dilatation in an organ of a few grams weight, such as the monkey's pancreas, were the drug produced in the organ itself.

Truly no great effort of the imagination is necessary to conjure up such a mechanism as I have outlined. Our first concern is to inquire whether there actually exist cases of dilatation of which there is no other satisfactory explanation than that of a local chemical action. The chain of evidence appears to be most complete in the case of the submaxillary gland.

In the cat stimulation of the cervical sympathetic nerve is known to give a greater secretion of saliva in relation to the size of the submaxillary gland than in other animals. The cervical sympathetic also contains constrictor fibres running to the blood vessels of the

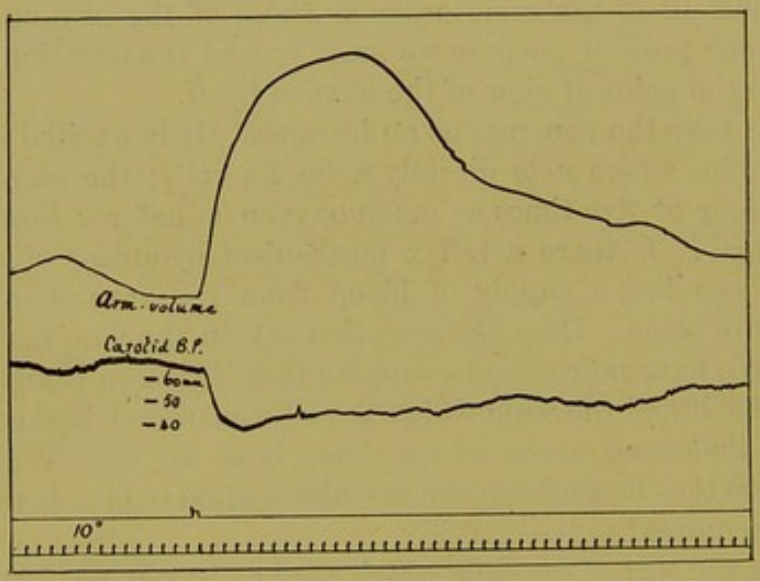


Fig. 74.—Monkey. A.C.E. Arm volume and blood pressure. Effect of 0·5 mgm. β-I.

gland. Stimulation of this nerve produces in succession the following effects upon the blood-flow from the gland: (1) decreased, (2) increased, and (3) decreased blood-flow. The second period or period of dilatation comes immediately after the secretion of the saliva. In Fig. 75 is shown the rate of flow of blood through the submaxillary gland in a typical experiment. If we seek the cause of this dilatation, which may be obtained in an even more striking fashion by the injection of adrenalin, we shall see to start with that it cannot be ascribed to mere loss of tone of the arterial wall, such as might be

obtained by section of the cervical sympathetic; for this constrictor nerve is cut throughout the experiment, and was actually being stimulated at the very time that the dilatation was observed. Clearly the dilatation takes place in spite of this stimulation; the cause of the dilatation, whatever it may be, is something which overrides the excitation of the vaso-constrictor fibres.

Perhaps the most natural assumption to make is that there are dilatator fibres mixed with the constrictor fibres in the sympathetic. This assumption was made by Carlson and his colleagues, Greer, Becht and McLean[6], who, independently of us, observed the vaso-motor phenomena which we have described. The second of the two papers on the subject which appeared from his laboratory, in its closing words states the issue as between true dilatator fibres, such as are

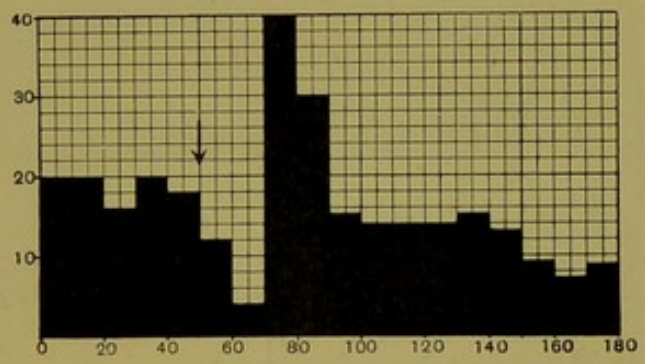


Fig. 75.—Ordinate = Rate of blood-flow through the submaxillary gland. ↓ = Stimulation of cervical sympathetic commences and lasts throughout. The figures represent graduation of a tube along which the blood from the gland flowed. ·0021 c.c. = 1 graduation of tube. Abscissa = time in seconds.

assumed in the chorda tympani and local metabolic products. "If there are some vaso-dilatator fibres...we should be able to show their presence by cutting out the vaso-constrictors by chrysotoxine and stimulating the vaso-dilatator apparatus by adrenalin, as chrysotoxine seems to affect the vaso-constrictors, not the vaso-dilatators."

The same issue had been pointed out to me at a meeting of the Physiological Society by Dale; in effect it is this: Ergotoxine (or chrysotoxine) does not paralyse the chorda tympani dilatator fibres, but does paralyse the sympathetic constrictor fibres; if therefore there are fibres in the sympathetic which are functionally chorda tympani fibres, then after the administration of ergotoxine and paralysis of the constrictor fibres, stimulation of the sympathetic will produce the same effect on a small scale as stimulation of the

chorda tympani, i.e. dilatation. Now this is precisely what does not take place. Administer ergotoxine in a suitable dose, stimulate the cervical sympathetic either with an induction current or with adrenalin, and you do not get dilatation; the effect of the excitation of the nerve upon the vessels of the gland is *nil*. Nor is there any secretion of saliva. The assumption that this dilatation is due to dilatator fibres has therefore broken down. The rival theory holds the field.

To leave the matter at this point would be unsatisfying. It is desirable that the theory of chemical, or as I prefer to call it

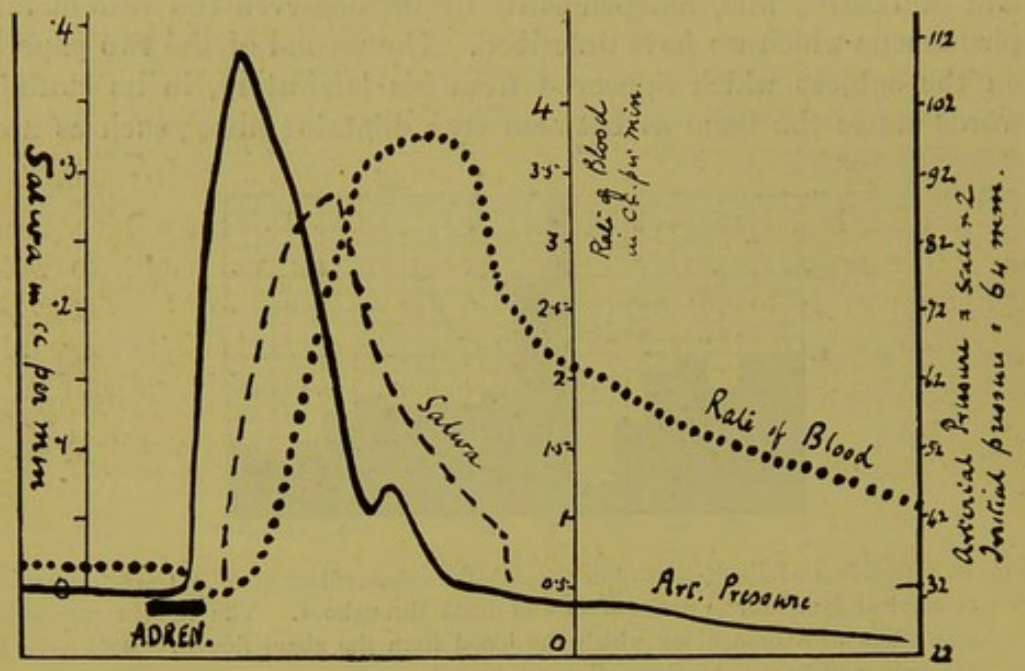


Fig. 76.

——— = Arterial pressure.
– – – = Rate of flow of saliva in c.c. per minute.
••••• = Rate of blood-flow through vessel of submaxillary drawn to one-tenth of the scale of the saliva curve.

"functional," dilatation should stand not merely by default. There should be actual proof that (1) the metabolism of the submaxillary gland is increased when the sympathetic is stimulated, and (2) that the actual metabolic products present are such as have the power of dilating vessels.

What evidence, then, is there of increased activity of the tissues in the gland when the sympathetic is stimulated?

The answer can best be obtained when the sympathetic is stimulated by means of intravenous injection of adrenalin, for when a

faradic current is used, the issue is complicated by the fact that the blood is almost entirely cut off from the gland at times, but with stimulation by means of adrenalin all is in our favour; we get a good flow of saliva, a good dilatation, and the effect of the primary constriction, as far as rate of blood-flow is concerned, is largely counterbalanced by the increased arterial pressure. The experiments were performed on cats. A graduated tube was fixed into Wharton's duct; an observer registered the rate of flow of saliva on the kymograph with a signal and tapping key. The blood from the gland was collected

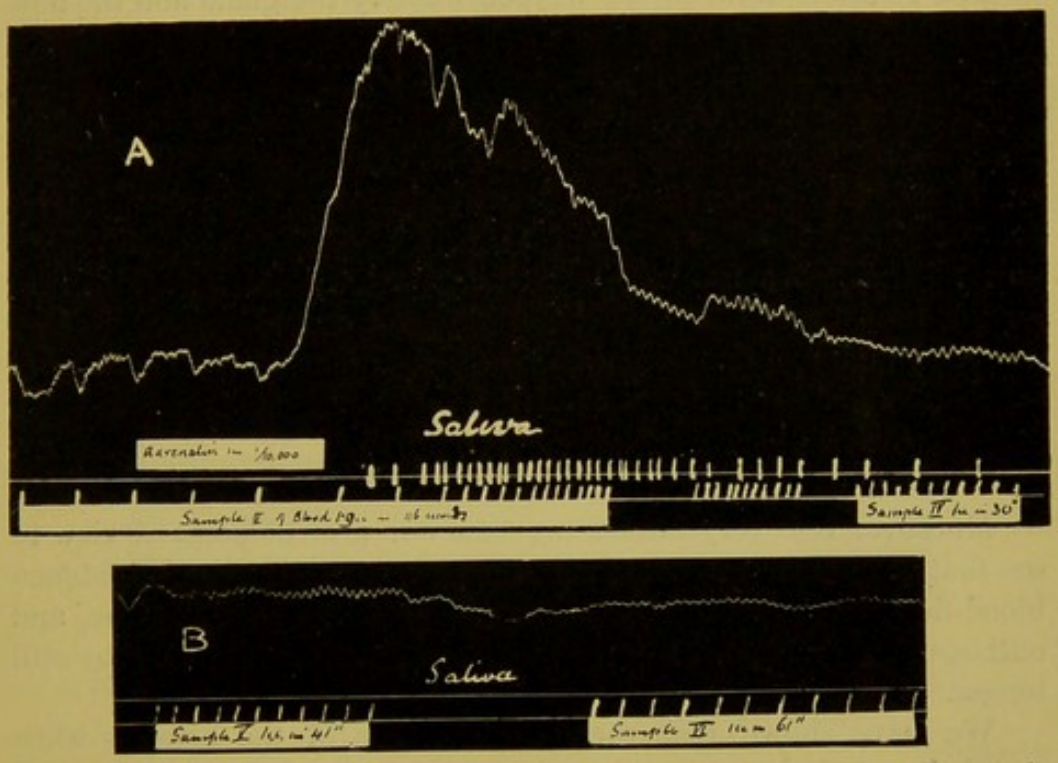


FIG. 77.—*A* and *B* form continuous portions of a single tracing. From above downwards (1) arterial pressure, (2) time of injection of adrenalin, (3) each mark of the signal signifies the flow of 0·01 c.c. of saliva, (4) blood signal marks correspond to 0·1 c.c.

in a cylindrical 1 c.c. pipette placed horizontally, the pipette was graduated in tenths of a cubic centimetre and the moment at which the blood meniscus passed each graduation was also recorded. The general arterial pressure was registered from the femoral artery. Samples of arterial blood for comparison with the blood from the gland were obtained from the other femoral artery. The figure given below shows the general time relations of the experiment, while we have seen in Chapter VI that there is a great increase in the metabolism of the gland as shown by the oxygen used up.

The following are the data yielded by a couple of experiments on this subject.

	Oxygen used up in c.c. per gram of gland per minute	
	Resting	Secreting (adrenalin)
Exp. 1	0·028	0·052
Exp. 2	0·026	0·050

Speaking roughly the gland uses up about the same volume of oxygen as it secretes of saliva.

The relation between the oxygen used by the gland and the flow of blood and of saliva may be studied in rather greater detail in such an experiment as that represented in Fig. 77.

The variations of oxygen consumption were estimated at different times during and subsequent to the secretion of saliva produced by an injection of adrenalin—the moment of taking the samples for analysis, the rate of flow of the blood in each case, and the rate of flow of the saliva are all shown in the figure. The quantities of oxygen used were as follows. Taking the amount of oxygen used by the resting gland (Sample I) as unity, the amounts used at different times indicated in the figure are found by analysis to be

Sample	II	IV	V	VI
Ratio of oxygen used to that of resting gland ...	1·2	2·1	3·7	1·3

Moreover it is very interesting from our present point of view to see that the heightened metabolism of the gland, like the heightened blood-flow, outlasts the flow of saliva by a considerable time, and outlasts the obvious direct vascular effects of the adrenalin by still longer.

We have shown that there is dilatation of the arteries when metabolism is induced by adrenalin, can we as a control experiment prove that adrenalin produces constriction and constriction only when there is not increased activity of the cells? The following facts may be cited in this connection. In the surviving gland, as in other perfused organs, the effect of drugs on the vessels survives the effect on the organ itself. Adrenalin added to the saline which perfuses the vessels of the submaxillary, a couple of hours after the death of the animal, produces no secretion of saliva and protracted constriction of the vessels ensues without the least vestige of dilatation; this effect may even be obtained on the following day.

The following example may be cited of a cat's submaxillary gland, which was perfused directly through the submaxillary artery.

Cat killed with chloroform about 11 a.m. perfused with warm Ringer's fluid.

11.48	30, 30, 30 drops per half minute.
11.50	Inject 1 c.c. pilocarpine.
11.53	44, 44, 45. Saliva.
12.22	37, 37. Saliva stops.
12.25	38
12.31	36
12.34	36 — No saliva.
12.45	36
12.49	36
12.55	Inject 1 c.c. adrenalin.
12.55·30	9, 10, 10, 10. No saliva.

The same gland next day:

10.19 20, 18, 18, 16, 17. Inject adrenalin. 4, 3, 3, 4, 3, 3.

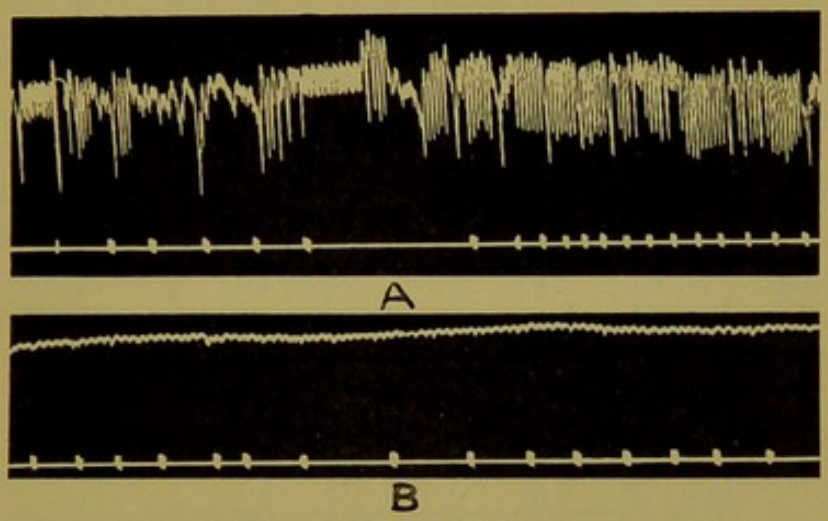


Fig. 78.—Effect of injecting adrenalin on the rate of blood-flow of submaxillary gland. *A* normal, *B* after clamping artery for 1 hour and 50 minutes.

We need not, however, have resort to artificial perfusions in order to find out that adrenalin does not cause dilatation but constriction when the gland is incapable of secreting. It is only necessary in the living animal to clamp the submaxillary artery, wash out the gland with cold saline, and leave it so for a couple of hours; at the end of this time the circulation of blood is reestablished. On the injection into the arterial stream, close to the origin of the submaxillary artery, of 0·5 c.c. of $\frac{1}{100000}$ adrenalin*, we find that constriction only ensues and that no saliva is secreted; under normal circumstances saliva

* The adrenalin comes out from the gland with the blood whose rate of flow is being measured, and so never reaches the general circulation.

would have flowed freely, and the constriction would rapidly have been overridden by dilatation.

It remains for us to see whether there are as a matter of fact bodies secreted into the blood which are capable of dilating the vessels. The bodies which are capable of causing dilatation may be divided into two groups from our present point of view: (1) acids, (2) organic bases, such as those isolated by Dale and Barger. The latter act in extremely minute quantities, and nothing would appear more probable than that such bodies should be produced in small quantities in the breakdown of tissues. It is a commonplace that extract of almost any organ produces such substances; their very commonness

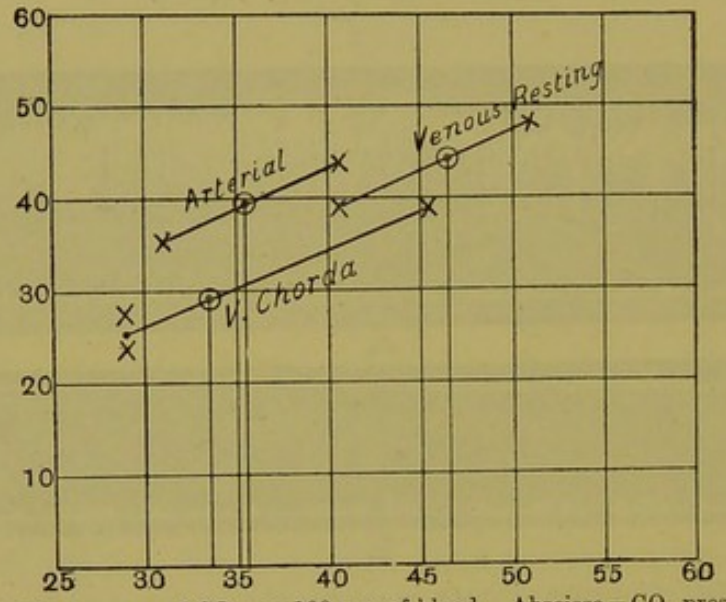


Fig. 79.—Ordinate = c.c. of CO_2 per 100 c.c. of blood. Abscissa = CO_2 pressure in mm.

has caused them to be largely overlooked in comparison with pressor substances. It is certain that such substances may be obtained from the submaxillary gland.

On the other hand, as regards the reaction of the blood, there are special reasons why the fluid bathing the cells should be less alkaline when the gland is secreting than when it is not doing so. The secretion of the submaxillary is, of course, strongly alkaline. This means that the bases are taken from the lymph and secreted whilst the acids are thrown back into the blood. We can test the question experimentally. We find that the blood in its passage through the gland does actually change its hydrogen ion concentration, and it would therefore seem that the lymph in equilibrium with it cannot but dilate the vessels.

That the "chorda blood" is richer in acids as compared with alkalis, than either the arterial blood or the venous blood, is doubly manifest. The first method of demonstration is essentially that used by Morawitz—namely, analysis shows that in equilibrium with carbonic acid at a given pressure the chorda blood contains less CO_2 than the ordinary venous blood, which in turn contains less than the arterial blood, showing of course that the bases are more completely saturated by other acids.

Fig. 79 shows the quantity of CO_2 contained in each of the three samples of blood when in equilibrium with the pressures of CO_2 at which the tests were carried out. For a given CO_2 pressure, say 41 mm., 100 c.c. of the chorda blood contains 36 c.c. of CO_2, the resting venous blood 39 c.c., whilst the arterial blood takes up 44 c.c.

The second method of showing that the chorda blood is the more acid is by the effect on the affinity of its haemoglobin for oxygen. The curves are shown in Fig. 88.

To summarise our case so far, stimulation of the submaxillary gland by means of adrenalin furnishes us with a case of local dilatation as the result of metabolic products, which dilatation can be shown to be complete in every particular: the direct effect of the drug on the vessels is constriction. This constriction is overridden when, and only when, the gland secretes, in that case bodies which are known to cause dilatation are demonstrably produced.

I do not think anyone can actually see a demonstration of the phenomenon which I have just described without asking himself whether this functional dilatation does or does not explain the whole phenomenon of dilatation as observed in the salivary glands. In other words, is it possible to demonstrate that the vaso-dilatation, which follows upon stimulation of the chorda tympani, involves a definite neuro-muscular vaso-dilatator mechanism?

Claude Bernard supposed that when the chorda tympani was stimulated three sets of fibres were thrown into action—the calorific, the vaso-dilatator, and the secretory. The evidence of the calorific was of the most direct kind, namely that heat was demonstrably produced when the nerve was stimulated; the evidence of the vaso-dilatator fibres was of the most direct kind, namely that the blood stream was demonstrably accelerated; and the evidence of the secretory fibres was of the most direct kind, namely that the saliva was seen to flow.

The conception of the calorific fibres has long since gone; the

heat is due to the liberation of energy caused by the stimulation of the secretory fibres; the challenge has come to the dilatator fibres. Are there really such things, or is the dilatation a "functional dilatation"? This question must arise from general considerations and from special considerations. Generally there is reason to believe that a functional dilatation accompanies functional activity; particularly the proven functional dilatation of the vessels when adrenalin is injected raises the question whether the same explanation does not fully account for the chorda dilatation.

I say "fully" because I think it will scarcely be challenged that, even if there were no vaso-dilatator fibres in the chorda tympani, there would be some degree of dilatation of a functional nature when it is stimulated. The question then really is this: Is the dilatation due to stimulation of the chorda tympani the result of two mechanisms or of one? If the dilatator fibre in the chorda is put on its trial, can it prove its title?

Were I to act as its counsel and attempt this proof I would be well advised to seek for some instances of dilatation, produced by chorda tympani stimulation, which do not appear to involve the functional activity of the gland. Two such may be cited.

(1) The most obvious case for consideration is that of the atropinised gland. The experiment is one of the best attested in physiology, namely, that if atropin is administered intravenously in certain doses saliva will not flow on stimulation of the chorda tympani, but dilatation takes place. With smaller doses of atropin saliva is obtained; with larger ones dilatation is not evoked, but between these extremes you may get the dilatation without the secretion. If the flow of saliva is the true index of the functional activity of the gland, the title is proved. But it is not a foregone conclusion that some subliminal degree of functional activity may not take place unless it finds its expression in a flow of saliva. Therefore it is necessary to measure the metabolism of the gland. The results of numerous experiments of this character are summed up in the table on p. 147.

In every case there is some degree of increased metabolism when the chorda tympani is stimulated. Certain criticisms of the experiments which we have quoted may rise in the mind of the reader. The first is that on comparing atropinised with unatropinised glands the increased metabolism elicited by the stimulation is considerably less in the former than in the latter. That is true, but it is also true that the dilatation is not maintained in the atropinised glands in the

Effect of stimulation of chorda tympani on the atropinised submaxillary gland.

Exp.	Animal	Unstimulated		Stimulated			
		Oxygen used c.c.	Blood flow c.c.	Oxygen used c.c.	Blood flow c.c.	Increase in oxygen %	Increase in blood flow %
1	Cat	0·024	0·40	0·027	0·36	11	40
2	Dog	0·026	0·94	0·034	3·2	31	240
3	Cat	0·018 0·016	0·25 0·34	0·026 0·023	0·67 0·56	44	106
4	,,	0·018	0·41	0·023	0·82	27	100
5	,,	0·020	0·60	0·031	2·6	55	333
6	,,	0·020	0·43	0·031	0·87	55	102
7	Dog	0·011	0·49	0·021	0·81	91	65
8	Cat	0·024	0·41	0·050	2·4	109	488
9	,,	0·026 0·022	0·25 0·25	0·046 0·049	2·2 1·7	50	812

same degree as it would be in the normal gland. The second assurance that must be given is that this increased metabolism is not a fictitious one caused by the increased rapidity of the flow. This was a matter which greatly exercised me as it proved very difficult to devise satisfactory control experiments; however after testing numerous methods of producing vaso-dilatation artificially, Prof. Franz Müller[7] drew my attention to the possibilities of yohimbin for this purpose, and in collaboration we tested the matter and found that even the very great amount of dilatation which can be induced by injecting small quantities of this drug directly into the submaxillary artery does not cause increased oxygen to be used by the gland.

(2) Bayliss[8] and Asher[9] pointed out another direction in which it might be possible to find the necessary evidence for the proof of the "title" of the vaso-dilatator fibre. They showed that when the central end of the depressor nerve is stimulated on the one side, say the right, the vessels of the left submaxillary gland dilate, whether or no the sympathetic is cut, provided that the chorda tympani is intact. The deduction is that a reflex stimulation of the chorda tympani takes place which produces vaso-dilatation, and here it should be said that there is no flow of saliva; on the other hand it must also be said that the phenomenon is only obtainable in a fraction of the experiments.

Here again an appeal may be made directly to measurements of the oxygen used by the gland to give an answer as to whether or no

there is increased chemical action taking place in the gland. My experience has been that in those cases in which I have been able to obtain the reflex there has been increased oxidation, whilst in the cases in which I have obtained no increased blood flow the oxidation has remained normal. But the experiments which I performed were few.

The answer then to the question, "Is it possible to demonstrate that the vaso-dilatation which follows upon stimulation of the chorda tympani involves a definite neuro-muscular vaso-dilatator mechanism?" is "It is not possible on the evidence at hand either to prove it or disprove it." The functional dilatation involved may be held to account for all known cases of dilatation in the submaxillary, but it is not proved to do so. The dilatation which takes place on stimulation of the atropinised gland is of relatively short duration. It is not impossible that under normal circumstances dilatation may be instituted by dilator fibres and maintained by metabolic products.

The pancreas. The transition from the submaxillary gland to the pancreas is a natural one. Adrenalin does not cause any flow of juice however from the pancreas; we must therefore give the appropriate stimulus, namely secretin. The effect of secretin on the vessels of the pancreas formed the subject of a research by Otto May [10], who showed by plethysmographic tracings that there was an increase in the volume of the pancreas when it was secreting and also that there was an increase in the "pulse volume" on the tracing. May attributes the dilatation to metabolic products acting on the vessels, and it is certain that there is ample evidence of increased chemical activity taking place in the pancreas itself inasmuch as the amount of oxygen which the pancreas uses is increased about four-fold when secretin is eliciting a flow of juice. The proof in the case of the pancreas is however less satisfactory than in the case of adrenalin in the submaxillary gland, because solutions of secretin usually, and in the case of May's experiments admittedly, contained depressor substance. The "depressor" substance is depressor in virtue of the fact that it dilates the vessels all over the body.

Therefore the question really is this: Does the solution containing secretin and depressor substance produce a greater degree of dilatation in the pancreas than the same solution would do if the secretin were absent? The answer to this question is given by May in the following words: "...there was an expansion of the small

intestine and of the pancreas. But whereas that of the former soon reached its maximum, and assumed its normal volume in less than a minute, the vascularity of the pancreas continued to increase gradually for some minutes; indeed it was maintained as long as the secretion of the gland (which began about two minutes after injection) continued—usually a period of 10—16 minutes." Direct experiments upon the outflow of the blood from the pancreatic vein have given very variable results.

Before I leave the subject of secretin I must make some allusion to the suggestion of Bayliss and Starling[11] that specific dilatation materials existed for specific organs, for instance extract of jejunum causes dilatation of the intestine but has no effect on the kidney. Their statement of results however was rather of the character of a preliminary communication.

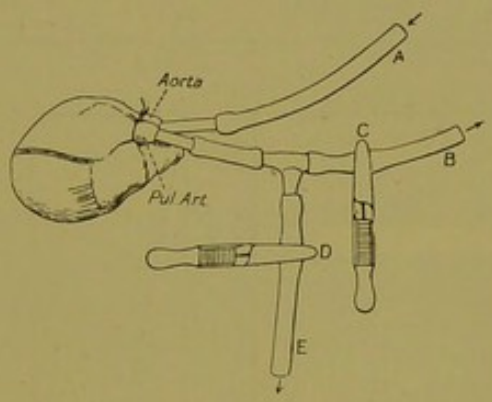


Fig. 80.—Connexions of heart perfused from another animal. *A*, tube leading from carotid artery of perfusing animal, into this drugs may be injected. *B*, tube leading to jugular vein of perfusing animal. Samples from the heart are taken at *E*. The clip is then removed from *D* and placed at *C*.

The heart. The heart presents a very close analogy in some respects to the submaxillary gland; this analogy has in fact been one of the current themes of physiological discussion, its tissue like the secreting tissue of the submaxillary gland has a double nerve supply; by playing upon this it is possible to increase or to decrease the activity of the heart: we are face to face with the question, Do these changes in activity alter the blood flow through the coronary system and, if so, by what mechanism are the alterations brought about?

In our research on the gaseous exchange of the heart, Dixon and I[12] arrived at some data with regard to the functional regulation of its blood flow. The arrangement of the experiment was as follows:

Two animals were used, a large and a small one of the same species, a cat and a kitten or a dog and a puppy.

The smaller animal's heart was perfused. The perfusion took place after the method of Haymans and Kochmann[13]; the blood went from the carotid artery of the large animal to the aorta of the small one, its course was thence through the coronary arteries, capillaries of the heart-muscle and coronary veins back into the

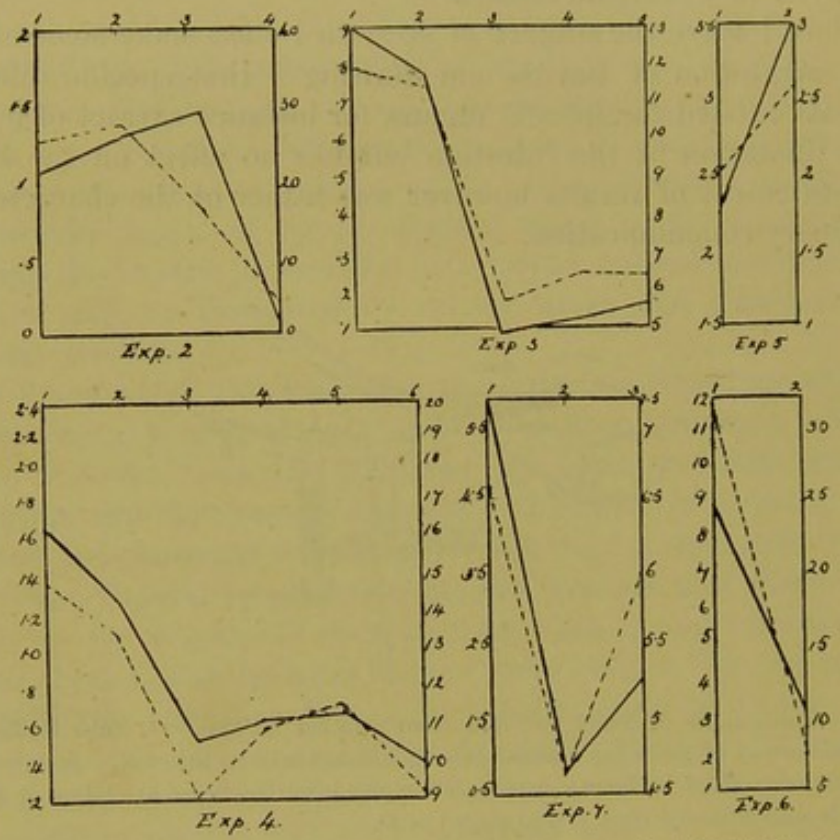


Fig. 81.—Shows the rate of flow through the coronary vessels (dotted line); and the output of carbonic acid (continuous line). The figures along the ordinates are in each case c.c. per minute, those on the left-hand side of each curve refer to the continuous line, those on the right-hand side to the dotted line. The periods are arranged along the abscissae.

right side of the heart, out by the pulmonary artery and back into the jugular vein of the perfusing animal. Between the pulmonary artery of the perfused heart and the jugular vein of the perfusing animal there was a T-tube. When one wishes to collect blood for analysis one attaches a graduated pipette to the T-tube, opens the clip and closes the tube at *C*. The blood runs along the horizontal pipette and its rate of flow can be measured directly.

The activity of the heart was altered very much in degree in different experiments, and in various ways, by the administration of adrenalin, pilocarpine, atropine, chloroform, vagus stimulation, etc. ; one general fact which emerged from the experiments was the close relationship between the output of carbonic acid and the rate of flow of blood. This is shown for the series of experiments in Fig. 81. The correspondence between the two is extraordinarily complete. It is incredible that such a coincidence should happen merely by chance, but whilst it must be admitted, I think, that the

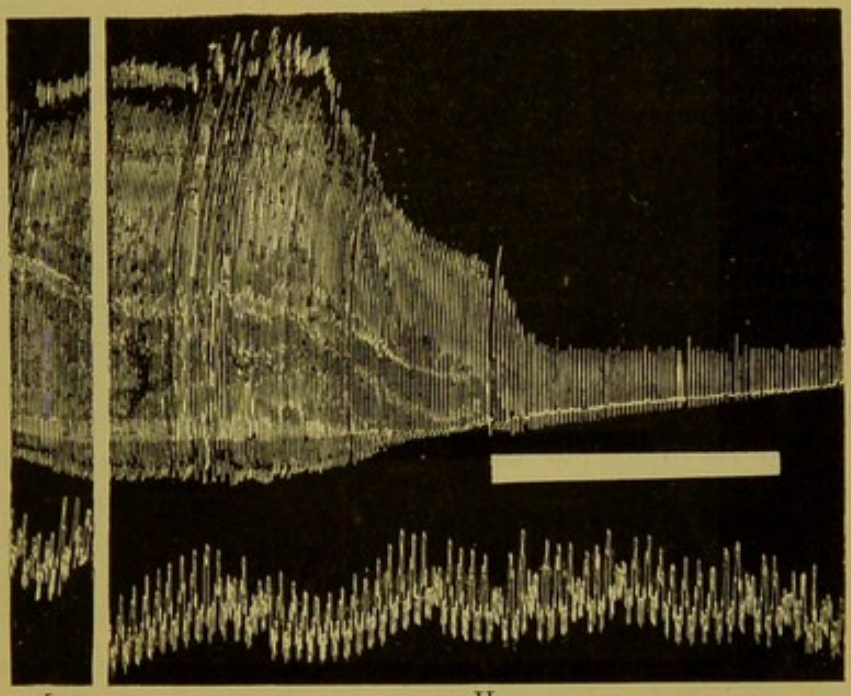


Fig. 82.—Upper tracing represents record of puppy's heart. Lower tracing = blood pressure of the perfusing animal. Period I = normal. Period II shows the effect of injecting 1·2 c.c. of $CHCl_3$ water. The signal mark represents the time during which the sample of blood was taken.

blood flow and the CO_2 output are in some way related, an analysis of this relationship is necessary before the assertion that the change in the cross-section of the coronary vessels was caused by the changes in the metabolic activity of the perfused heart is warranted.

The other possibilities are as follows :

Drugs which tend to increase the activity of the perfused heart, adrenalin for instance, would also tend to increase the activity of the perfusing heart; this would tend to raise the general arterial pressure in the perfusing animal and so alter the blood flow through

the perfused heart. This line of argument does not account for the changes in blood flow. The drugs were always injected in the tube leading straight to the perfused heart and in such small quantities that whilst they affected it profoundly they had little or no effect upon the general circulation of the perfusing animal. For example take Exp. 6 (Fig. 81), in it there are two periods one before and one after the activity of the perfused heart has been reduced by the injection of 20 minims of chloroform water into the tube leading to the perfused heart. In Fig. 82 both the tracing of the perfused heart and that of the general arterial pressure are shown in the two periods of the experiment. The general arterial pressure is scarcely altered, the metabolic activity of the heart is much reduced. This reduction is shown by the reduction in amplitude and frequency of the heart's beats as seen in the tracing; it is shown also by the change in the metabolism.

Period I	Oxygen taken in	CO_2 given out
Before chloroform injection ...	3 c.c. per min.	8·8 c.c. per min.
Period II		
After chloroform injection ...	0·37 ,,	1·9 ,,

As the actual effect of the chloroform in the coronary vessels themselves would be dilatation, we might therefore have expected that, other things being equal, there would have been a more rapid flow of blood through the coronary system of the perfused heart. Nevertheless this was not the case, the blood flow in the first period was 30 c.c. per minute, in the second 9 c.c.

Now that the effects of changes in the general arterial pressure have been excluded and in some experiments the local effect of the drug upon the vessels, what possibilities remain? The direct action of drugs on the vessels of the perfused heart may be still further eliminated, for in some of our experiments the rate of flow was not altered as the result of drugs but as the result of stimulation of the vagus of the perfused heart; this clearly does not affect the general arterial pressure of the perfusing animal, nor is it claimed that the vagus carries constrictor fibres to the coronary vessels; therefore the changes in blood flow which we get as the result of vagus stimulation cannot be regarded as due to either of the causes which we have considered. The rate of blood flow however follows the CO_2 output very closely.

In the experiment, a tracing of which is given, there were three periods: (1) before vagus stimulation, (2) during vagus stimulation, and (3) after vagus stimulation.

Period	Condition of heart	Rate of flow through coronary vessels c.c. per min.	Oxygen consumed per gram per min.	CO_2 given out per gram per min.
I	normal	6·7	·014	·038
II	vagus	4·6	·009	·005
III	after vagus	6·0	·022	·015

Another possible cause of the changes in the rate of flow through the coronary system is the rhythmic contraction of the cardiac muscles, which may be held to promote a more rapid flow of blood along the coronary system.

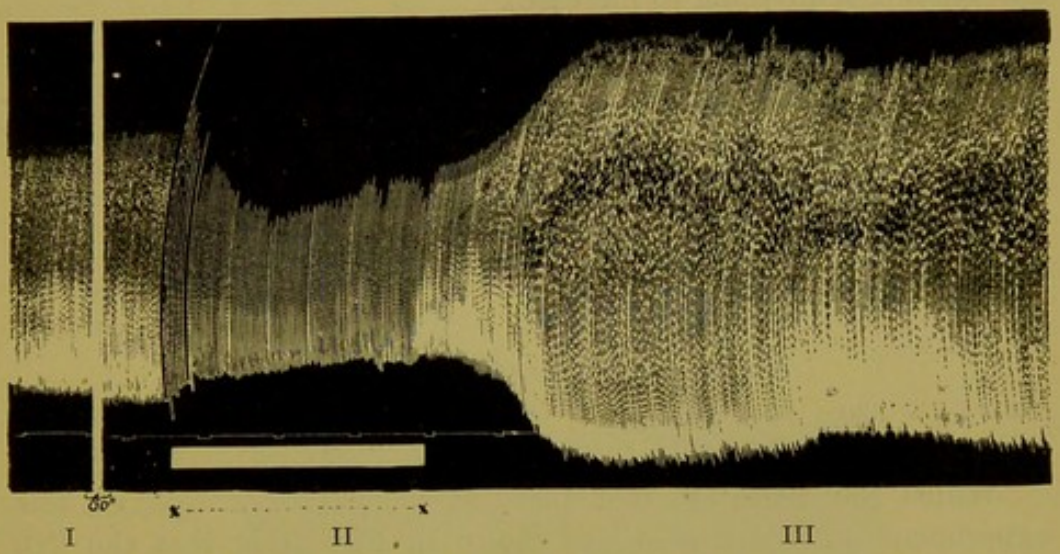


Fig. 83.—Record of the movements of the heart of a small cat perfused from the circulation of a large cat. Upstroke = systole. Period I = normal. In period II the signal mark represents the time of vagus stimulation. The third period corresponds to the after effect and in this period the third sample of blood was taken.

At first sight it seemed difficult to analyse the complex of processes into the elements necessary for a decision whether the variations in the activity of the heart produce their effect on the blood flow by the mechanical method of squeezing the blood intermittently along the vessels, or by the chemical method for producing dilator substances. Fortunately nature has performed the analysis for us. The output of CO_2, as I have already indicated in Chapter VI, lags to some extent behind the actual variations of the frequency and power of the contractions. The time relations of the activity and the CO_2 output are shown in Fig. 84, also those of the changes in blood

flow. The alterations in the rate of flow keep company with the CO_2 output and like it lag after the changes in pulse rate. The simultaneous "hysteresis" of the vascular dilatation and of the CO_2 production shows that the increased blood flow caused by increased activity is not due to a mechanical propulsion but to a chemical event.

In order to test the effect of metabolites on the coronary circulation more completely Dixon and I determined to induce partial asphyxia by restriction of the respiratory orifice of the perfusing animal. The carbonic acid accumulates in the blood and as the arterial blood gets darker the flow through the coronary vessels of the supplied heart increases, at the same time the heart beat slows it becomes less efficient[14] (the call for oxygen being unabated and tending if anything to increase).

Heart of puppy perfused from Dog.

The occlusion of tracheal tube commenced between periods I and II and ended after period III.

Period	Oxygen in art. blood °/₀	CO_2 in art. blood °/₀	Blood flow c.c. per min.	O_2 used per g. per min. c.c.	CO_2 used per g. per min. c.c.
I	16·0	28·3	11·4	·027	·043
II	13·6	34·9	11·4	·027	·035
III	**8·2**	**44·9**	**23**	**·036**	**·008**
IV	15·3	21·7	1	·018	·012

Kitten's heart perfused from Cat.

I	12·1	26·6	1·75	·010	·005
II	**5·4**	**41·1**	**5·7**	**·011**	**·002**

Whilst the dilatation took place at the height of asphyxia this experiment differed from those shown in Fig. 81 in that the CO_2 output in this period was particularly small. A possible explanation of this (though not the only one) was that the asphyxiated blood contained products of incomplete oxidation which likely enough were more active than CO_2. Indeed Verzàr has since shown that the blood coming from the titanised gastrocnemius contains measurable quantities of acid products[15]. We performed further experiments in which the effect of CO_2 was eliminated whilst that of other products remained. The asphyxia was produced by making the perfusing animal breathe a mixture of nitrogen and oxygen which became increasingly poor in the latter. When the oxygen sank to about 4 °/₀ the heart beats began to fail. Period II corresponds to this.

Period	Oxygen in art. blood °/₀	CO_2 in art. blood °/₀	Blood flow per min.	O_2 used per g. per min.	CO_2 prod. per g. per min.
I	15·4	35	8	·036	·025
II	**2·1**	**36·2**	**48**	**·033**	—

From this experiment which we have repeated on several occasions it would appear that CO_2 is not the only, nor is it the most powerful dilating metabolite.

Before leaving the subject of the automatic supply of blood to the tissues there is one aspect of it which must not be overlooked. The metabolic products continue to exercise their influence for some little time, amounting perhaps to minutes, after the functional activity of the organ has passed off. Whether we call this a happy accident or a beautiful mechanism it matters not, so long as we understand the true inwardness of the facts which we are studying. For the demand of the organ for oxygen is essentially something

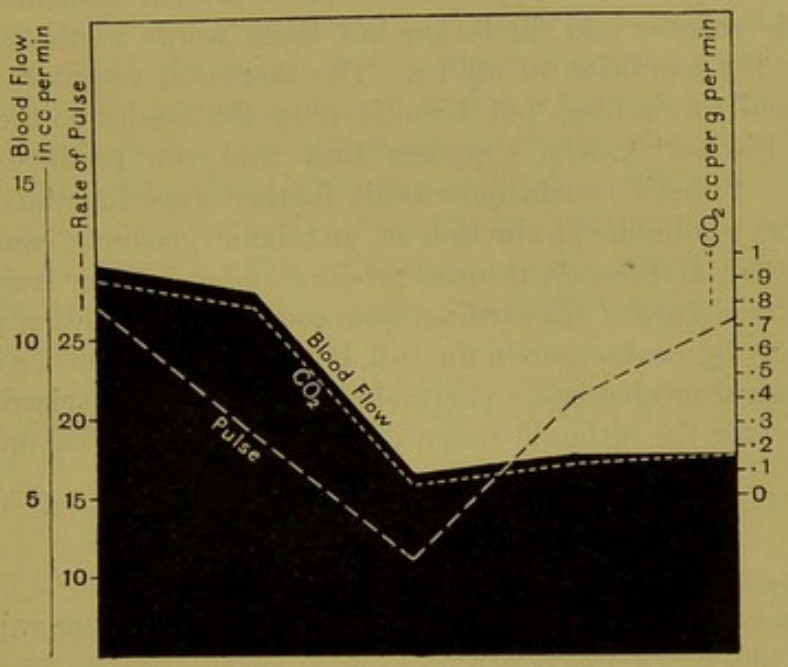


Fig. 84.—Rates of pulse and CO_2 excretion and blood flow from the coronary system.

which follows upon the activity of the organ, not something which causes the activity nor yet something which absolutely synchronises with it. The time relations of the activity of an organ and the oxygen used up in it make a story which I have already told. So far as can be gleaned from muscle and from the submaxillary gland, organs which by means of nervous stimuli can be thrown instantaneously into violent activity, activity which can be suspended almost as rapidly by a cessation of the stimuli, it would appear that contraction of the muscle or the secretion in the gland is not itself a manifestation of oxidation in the sense that the work of an internal combustion engine is a direct manifestation of the oxidative

explosion in the cylinder. The order of the processes is reversed; the contraction of a muscle is more like the running down of an alarum clock. The clock is already wound, at a given moment the potential energy of the spring is released and the alarm sounds. It has then to be laboriously rewound. In some such way the energy of muscle must be reinstated. During the period of reinstatement oxidation is increased and blood is required. It is then automatically supplied.

The very orderliness of the mechanism which I have described has sometimes made me picture what would happen if it became disordered; if some cataclasm should take place in the cell as the result of which it shed its products broadcast. The immediate effects would be redness and dilatation but these words would but feebly describe the condition of affairs. The increased vascular dilatation might lead to swelling, but I would refer the reader to the work of Martin Fischer[16], who supposes that acid can produce swelling directly. Fisher's conclusions await further experimental support.

If the wholesale production of metabolic products can produce *rubor*, *calor*, and *tumor*, it must produce *dolor* in their train, and so we get the picture of the cardinal symptoms of inflammation produced not merely by a cataclasm in the cell, but by a perversion of a beautiful physiological mechanism, a perversion which has been seized upon by "nature" for the ultimate removal of the cause of the calamity.

REFERENCES

(1) Barcroft and Shore, *Journal of Physiol.* XLV, p. 296, 1912.
(2) Burton-Opitz, *Quarterly Journal of Exp. Physiol.* III, p. 297, 1910; IV, p. 113, 1911.
(3) Severini, quoted from Gaskell (4).
(4) Gaskell, *Journal of Physiol.* III, p. 48, 1880.
(5) Dale and Laidlaw, *Ibid.* XLIII, p. 182, 1911.
(6) Barcroft, *Ibid.* XXXV, p. 19, 1907 and XXXVI, p. 53, 1908; Carlson, Greer and Becht, *Amer. Journ. of Physiol.* XX, p. 180, 1907; McLean, *Ibid.* XXII, p. 279, 1908; Barcroft and Piper, *Journal of Physiol.* XLIV, p. 359, 1912.
(7) Barcroft and Müller, *Ibid.* XLIV, p. 259, 1912.
(8) Bayliss, *Ibid.* XXXVII, p. 256, 1908.
(9) Asher, *Zeitschr. f. Biologie*, LII, p. 325, 1909.
(10) May, Otto, *Journal of Physiol.* XXX, p. 400, 1904.
(11) Bayliss and Starling, *Ibid.* XXVIII, p. 357, 1902.
(12) Barcroft and Dixon, *Ibid.* XXXV, p. 182, 1907.
(13) Haymans and Kochmann, *Arch. Pharm. et de Thérap.* XIII, p. 379, 1904.
(14) *Report of the Brit. Assoc. for the Advancement of Science*, 1907, pp. 401—2.
(15) Verzàr, *Journal of Physiol.* XLIV, p. 256, 1912.
(16) Martin Fischer, *Oedema.*

CHAPTER X

THE UNLOADING OF OXYGEN FROM THE BLOOD

THE transference of oxygen from the red corpuscles to the tissue cells involves at least two quite separate processes, firstly the chemical breakdown of the oxyhaemoglobin and secondly the diffusion of gas from the blood to the cells, through the capillary wall and through the lymph. It is not very easy to separate these two processes so completely that each can be considered apart from the other. Nevertheless it will be useful to start from a definite point of view with regard to the principles involved in the maintenance of an efficient diffusion; by this we mean a flux of oxygen which will provide for the maximal needs of the organ, not merely for its metabolism during quiescent periods.

The point of view is simple enough—for the maximal flux the maximal pressure-head is required. Herein lies physiological significance of the factors which have been set out in our consideration of the physical chemistry of haemoglobin. Let me place before the reader the following picture, which when he has considered he may vary to his liking. A certain quantity of haemoglobin is passing through the vessels of an organ—it must needs impart a given quantity of oxygen to the organ—in so doing it becomes reduced to the extent of 50 per cent. of its oxygen, the haemoglobin is in a pure salt-free solution and the temperature is 16° C. Can we discover the available pressure-head of oxygen in the blood leaving the capillary? It will be found from the dissociation curve of dialysed haemoglobin at 16° C. that the pressure corresponding to 50 % saturation is 0·3 mm. As the pressure in the tissues cannot be less than zero, the figure 0·3 mm. stands for the pressure-head which is available for the maintenance of the diffusion current through the capillary wall just at the point where the haemoglobin is parting with the last traces of oxygen which it loses. In the following paragraphs I will focus the consideration of the reader on the

difference of pressure which for the sake of simplicity of expression I will call the "final capillary pressure-head." To what extent have the effects of (1) rise of body temperature, (2) the presence of salts in the blood, and (3) the presence of CO_2, contributed so as to admit of the reduction of the blood at a higher pressure? We may take these in any order. Each of these factors—temperature, salts and CO_2—has a general and a local effect, a general effect because the whole blood of the body is at 37° C. and is impregnated with salts and CO_2, and a local effect because the blood in the capillaries rises in temperature, acquires carbonic acid, and in some cases at all events alters in its saline content as it traverses the tissue.

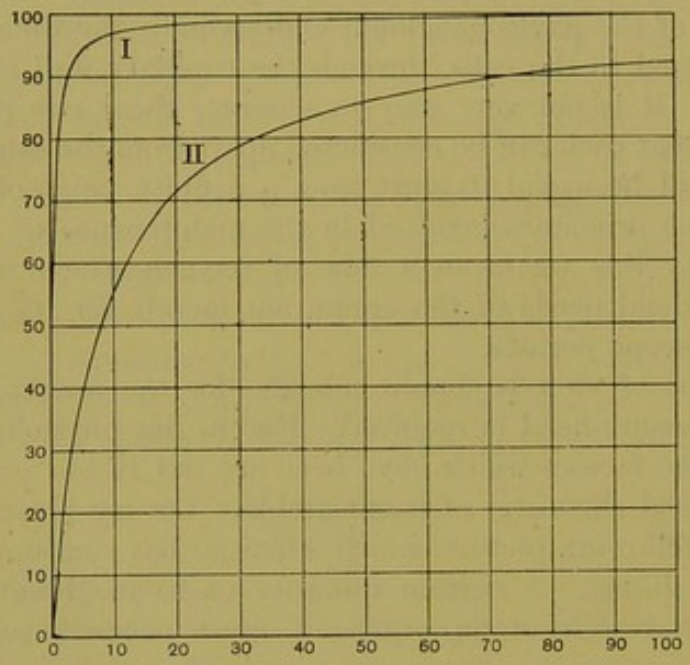


Fig. 85.

It will be best to consider the general effects of temperature, salts and acids first. Let us take them in reference to the case described above. Rise of temperature does not alter the mathematical form of the curve, it merely alters the scale on which it is drawn. From Fig. 85 which we have just been considering we can see the effect of temperature: haemoglobin at 38° C. when 50 % reduced is in equilibrium with oxygen at a pressure of about 7·5 mm. of mercury; this then becomes the "final capillary pressure-head," it is twenty-five times higher than it would have been at 16° C., hence oxygen can diffuse at 7·5 mm. pressure at least out of the vessels into

the tissue and therefore at twenty-five times its former velocity. Let us take the influence of salts next. The effect of salts is to aggregate the molecules of the haemoglobin; this aggregation introduces the double contour into the dissociation curves, causing the blood to give out its oxygen much more readily at low oxygen pressures whilst it takes it up more readily at high ones. Compare, for instance, the thick lines in Fig. 86 *A* and *B*. The former is the dissociation curve of dialysed haemoglobin at 37° C., the latter, that of human blood in the absence of CO_2 at the same temperature, is very close to that of haemoglobin in a solution of potassium chloride isotonic with blood.

The salts not only produce a clumping of the haemoglobin molecules so that each clump has on the average 2·5 molecules, but they have a very important effect in maintaining the state of aggregation at this level in spite of physiological changes in the reaction of the blood. The effect of CO_2 in moderate quantities in the presence of the salts is therefore to alter the value of K in the equation

$$\frac{y}{100} = \frac{Kx^n}{1 + Kx^n},$$

without more than a negligible alteration in n; the effect of 40 mm. CO_2 pressure on the dissociation curve of blood is to replace the thick line in Fig. 86 *B* by the dotted line, thereby raising the "final capillary pressure-head" to 25 mm.; indeed in the blood of many persons the oxygen pressure corresponding to 50 °/₀ saturation is as high as 30 mm. of mercury. This marvellous thing has therefore happened as the result of the combined effect of temperature, salts and carbonic acid; the "final capillary pressure-head" has become elevated about 100 times, making provision for oxygen to diffuse into the tissues at 100 times the speed—and this without sensibly reducing the percentage saturation of the blood in the pulmonary alveoli. For the sake of simplicity we have only discussed the capillary pressure of oxygen at one point in the capillary, namely the point at which the blood leaves it. The reader must recollect that diffusion of oxygen is taking place all along the capillary and he may work out for himself the effects of temperature, salts and carbonic acid at any particular percentage saturation of oxygen which he selects.

The effect of clumping produced by electrolytes is best grasped by a comparison of the effect of a decrease in the equilibrium constant (K) in clumped and unclumped solutions. A decrease in K alone

would simply spread out the curve. Compare the curves given in Fig. 86. In both cases *A* and *B*, the dotted line shows a large pro-

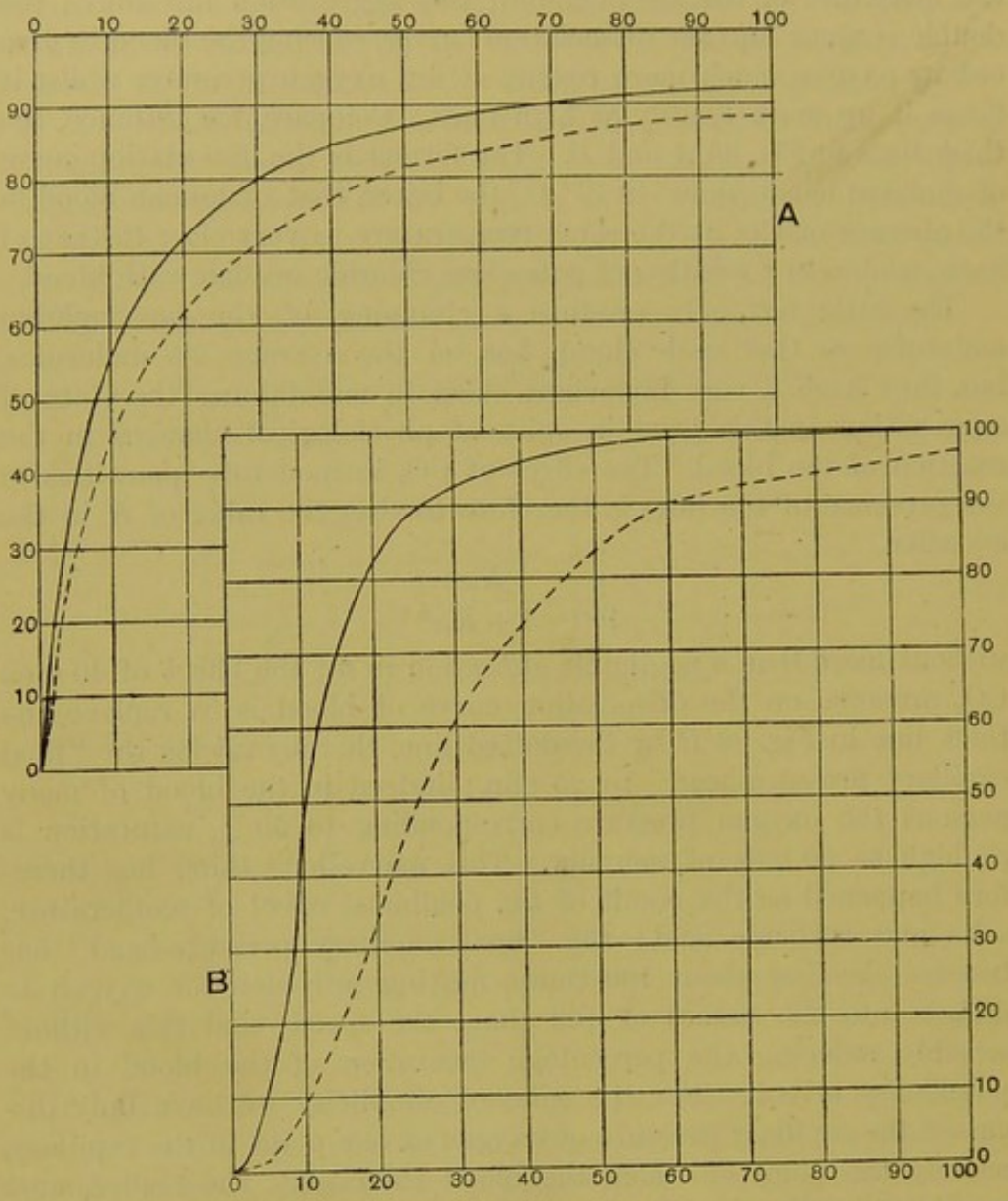


Fig. 86.—*A*, dissociation curves of haemoglobin solution. —— = dissociation curve of haemoglobin at 38° C. – – – = the effect which CO_2 would produce if there were no aggregation of the molecules. *B*, dissociation curves of blood. —— = without CO_2. – – – = 40 mm. CO_2 pressure. Ordinate = percentage saturation with oxygen. Abscissa = oxygen pressure.

portional increase in the pressure at any given percentage saturation as compared with the thick line. At low percentage saturations,

however, the absolute pressure in the case of haemoglobin solutions containing unaggregated molecules is so small that a considerable proportional increase does not produce much absolute rise; with blood, however, the opposite is the case. This is most apparent near the bottom of the curves. In the absence of CO_2 at 20 % saturation the corresponding pressure of oxygen would be 2 mm. in the case of the haemoglobin solution and 6 in the case of the blood; suppose enough CO_2 to be present to double each of these, the pressure of oxygen in the case of the unaggregated haemoglobin solution becomes 4 and of the blood 12, the blood gains greatly in the matter of millimetres pressure. The effect of the salts in steadying the degree of aggregation of colloid molecules is probably very great, for in the few observed cases where the change of reaction has been sufficient to produce a measurable change in aggregation, in spite of the salts present, the person observed has been in a very abnormal condition, either very much distressed as the result of exercise at high altitudes, or practically moribund as some of the cases referred to in the last chapter of this book.

We have discussed the physiological significance of temperature, acids and salts in their general aspect, but in regard to any particular organ they have a special as well as a general aspect. The blood is not the same when it leaves the organ as when it enters it; in the first place it is warmer; in the second case it has acquired carbonic acid and perhaps other acids; in the third its saline content may have been altered; each of these will have its effect upon the pressure at which the blood holds its oxygen. Of the salts we know nothing in this connection, nor have we data which enable us to make any allowance for the rise of temperature in the blood. It may be an important factor in the case of actively contracting muscle. We must therefore confine ourselves to the consideration of the effect of the acids thrown into the blood in raising the head of oxygen pressure and thus promoting rapid diffusion.

It remains for some physiologist in the future systematically to determine the influence of acid and other factors which we have enumerated in the various tissues of the body. He would then be able to present a statement of the pressure of oxygen in the vein leading from each of the organs which he had studied.

At present a single example must suffice to illustrate at once the principles which are involved, and the order of oxygen pressure which exists in the capillaries. The actual example forthcoming is

furnished by the submaxillary gland. We start with (1) the arterial blood, (2) the venous blood from the resting gland, (3) the venous blood from the secreting gland. Can we tell the oxygen pressure in all three of these?

Analysis gave the following data:

(1) The arterial blood was 94 °/₀ saturated with oxygen.

(2) The venous (resting) blood was 59 °/₀ saturated with oxygen.

(3) The venous (active) blood was 66 °/₀ saturated with oxygen.

From these we could infer the pressures of oxygen if we knew the dissociation curves. The curves are probably all different since the carbonic acid pressures may be supposed to be different, and possibly

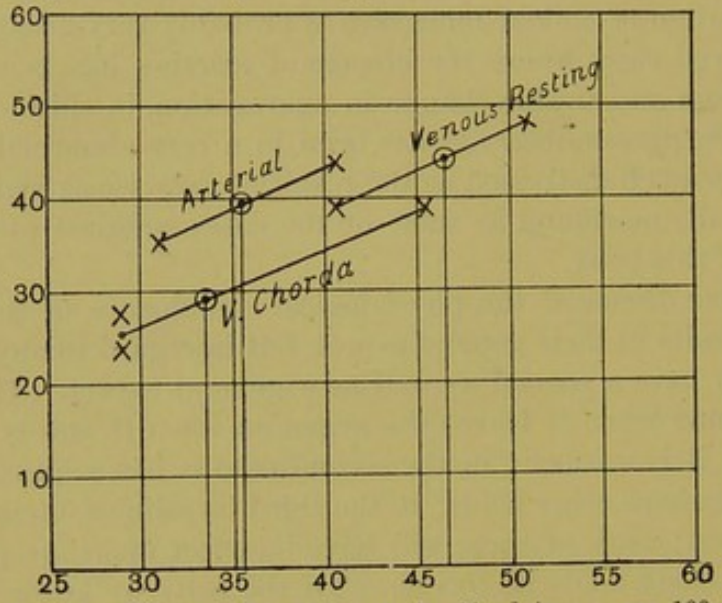


Fig. 87.—Ordinate = quantity of CO_2 taken up by blood in c.c. per 100 c.c. of blood. Abscissa = pressure of CO_2. The points ⊙ correspond to the actual quantities of CO_2 found in the samples of arterial venous and resting bloods. From these points the CO_2 pressures are inferred.

other acids are introduced in varying quantities. We do not know the carbonic acid *pressures* nor the concentrations of other acids present—we have available data however concerning the *quantity* of CO_2 in each sample of blood which is as follows:

(1) Arterial 100 c.c. blood contains 36 c.c. CO_2.

(2) Venous (resting) „ „ „ 44 c.c. CO_2.

(3) Venous (active) „ „ „ 29 c.c. CO_2*.

* The venous blood coming from the active submaxillary gland is often anomalous in that it yields less CO_2 when treated with acid than the arterial blood. The large quantity of CO_2 and carbonates which are being discharged from the gland in the saliva account for this phenomenon.

Our procedure is to expose each sample of blood to suitable known pressures of CO_2 in a tonometer at 37° C. and to analyse the blood in order to ascertain the amounts of CO_2 which it holds at these pressures. The crosses in Fig. 87 represent such analyses. A graph is made for each blood, relating the quantity of CO_2 to known pressures of the gas; from this graph we can read the CO_2 pressure corresponding to the quantity held by each sample of blood. Were the samples alike in the matter of other acids one graph would serve for the three, but no assumption of that kind can be made, indeed it was known that the reverse was the case.

These three graphs are shown in Fig. 87. From them it appears that the carbonic acid pressures in the three samples of blood were approximately:

(1) Pressure of CO_2 in arterial blood 36 mm.
(2) Pressure of CO_2 in venous (resting) blood 46 mm.
(3) Pressure of CO_2 in venous (active) blood 34 mm.

We now proceed to determine the oxygen-dissociation curves for the three samples of blood at or near these CO_2 pressures. For this purpose the following data were obtained:

(1) Oxygen pressure 36 mm. percentage saturation 60, 62 %, mean 61 %. $K = \cdot 000200$.
(2) Oxygen pressure 43 mm. percentage saturation 62, 65 %, mean 63·5 %. $K = \cdot 000147$.
(3) Oxygen pressure 40 mm. percentage saturation 54, 54 % mean 54 %. $K = \cdot 000116$.

The curves corresponding to these values of K are given in Fig. 88. The arterial blood has been assumed to be the same throughout the experiment, the curves given for it in the two portions of Fig. 88 are identical. On the curve may be read off the pressures corresponding to the observed percentage saturations of the blood with oxygen, we thus find that the oxygen pressure is:

(1) In the arterial blood 93 mm.
(2) In the venous (resting) blood 39 mm.
(3) In the venous (chorda) blood 49 mm.

The two latter form the "final capillary pressure-head" for the diffusion of oxygen from the capillary into the tissue under the circumstances of rest and activity respectively.

This completes our survey of the general and the local mechanisms for securing an efficient oxygen pressure in the capillary circulation. In the examples which we have taken from the submaxillary gland,

general considerations applied to the blood as a whole have determined a rise of pressure from about 0·3 mm. to 34 mm. in the blood leaving the resting gland and 29 mm. in the blood leaving the active gland (these

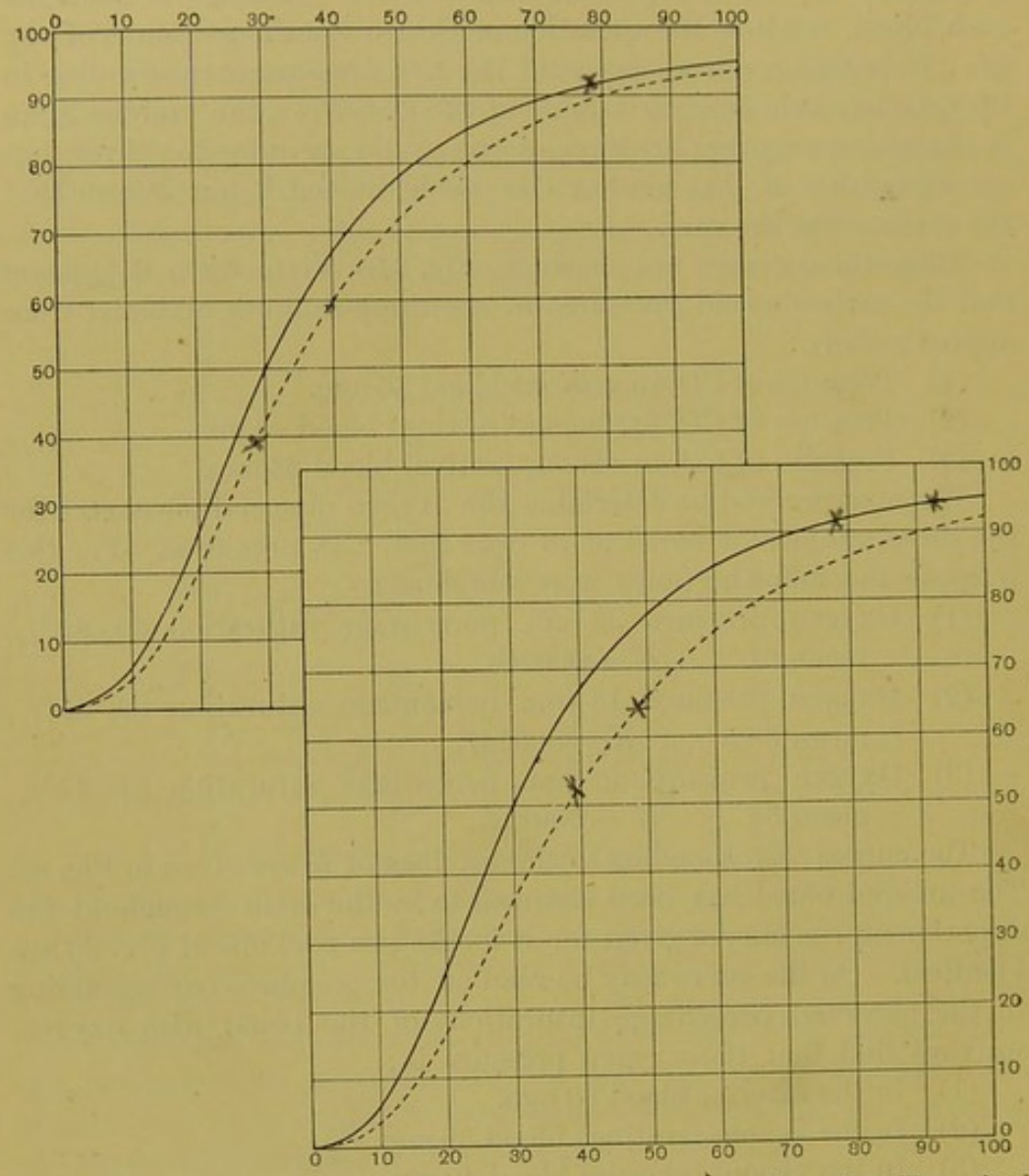


Fig. 88.—Dissociation curves of blood supplying and leaving the submaxillary gland, determined at the pressure of CO_2 which was found in the blood in each case. Upper series = resting gland. Lower series = gland during stimulation of chorda tympani. —— = Arterial blood. ------ = Venous blood.

figures being read off from the arterial curves), whilst the local effects of secretion of carbonic and other acids have secured an extra 5 mm. pressure to the blood from the resting gland and 10 mm. to the blood

from the active gland. So much then for the pressure of oxygen in the capillaries.

The amount of oxygen which will diffuse through the wall depends, other things being equal, on the gradient of pressure across the capillary wall; let us therefore turn to the consideration of the pressure of oxygen in the tissues.

The quantitative measurement of the extra-vascular oxygen pressure in certain organs expressed in mm. of mercury has been attempted by Verzàr[1] during the portion of his Wissenschaftliche Reise which he spent at Cambridge. The principle on which these determinations were based was the following. The quantity of oxygen which diffuses through the wall will depend, other things being equal, upon the difference of oxygen pressure within and without the capillary, and inversely on the distance of diffusion, i.e. altogether on the pressure gradient.

If therefore Q be the quantity of oxygen which passes out per minute, p the intra-capillary pressure and p' the extra-capillary or intra-cellular pressure

$$Q \propto p - p'.$$

Naturally Verzàr's first endeavour was to test the current view of intra-cellular oxygen pressure, which is that the pressure in the tissues is *nil*. Though no quantitative experiments of this character are simple or easy, this fortunately is one of the least difficult, for if

$$p' = 0, \quad Q \propto p.$$

On this hypothesis it is possible to institute a direct experimental test as to whether the amount of oxygen which left the capillary varied directly with the pressure of oxygen in the capillary. The amount of oxygen can of course be directly measured from a knowledge of the oxygen in the blood going to and leaving the organ in question, and of the quantity of blood which goes through it in a given time. The measurement of p offers a much more difficult problem. Theoretically Verzàr should in each experiment have gone through the whole gamut of determinations we have set forth in the example given above. In practice we had to approximate. A first approximation to the capillary oxygen pressure will be arrived at by an application of the percentage saturation of the arterial and venous blood to the dissociation curve of the arterial blood of an animal of the same species. The measurement of Q involves the amount of oxygen in the blood: if the oxygen capacity be also known the

percentage saturation is arrived at. We can, then, determine the percentage saturation of the arterial and venous bloods and, by laying them off on the dissociation curve, we can determine the pressure of oxygen in the artery and the vein, and in that way at least ascertain within limits the pressure in the capillary at a given time. We need scarcely interrupt the course of our discussion to point out to the reader the extreme fallibility of the methods in which we are engaged. We are in the position of one navigating a difficult channel in foggy weather. Nevertheless, it may be that the points which we have to observe are sufficiently obvious to stand out even in the fog, that in short there are fixed laws determining the pressure in the tissues which can be appreciated by methods as fallible as the best at present within our reach.

Let us assume then that we can determine Q and p. Can we now vary one or other of these and see whether the relation holds $Q \propto p$? By the following means Verzàr varied p. The cat which was anaesthetised with urethane was made to breathe gas from a gasometer by means of water valves attached to the trachea tube. The gasometer contained air, but as the animal drew upon the supply of air, the gasometer in turn drew upon the contents of another much larger gasometer which was filled with nitrogen, this in turn being displaced by water. Thus the cat was getting gas which constantly and gradually became less and less rich in oxygen, and in time the oxygen pressure in its arterial blood began to fall visibly as indicated by the colour of the blood. When this process had gone far enough, samples of blood were again taken for analysis.

For the following reasons it will be best to consider the experiments made on the salivary gland first. They gave very uniform results which were easy to interpret. They were free from the complications of alteration in the blood flow since it has been shown by Müller and myself[2] that the amount of oxygen acquired from the blood by the resting submaxillary is not appreciably affected even by great changes in the rate of flow. In the third place experiments on the submaxillary follow naturally upon the investigations described above relating to intra-capillary pressure of oxygen in the same organ. What a satisfactory organ the submaxillary gland is—easy of access, easy of control, relatively unaffected by the usual anaesthetics!

The experiments can best be understood by following a single one in detail. Let it be Experiment I.

The data of this experiment are given in graphic form in Fig. 89. There are two periods, the first in which the animal was breathing air, the second in which the quantity of oxygen in the inspired mixture swells to 8·4 per cent. or about 63 mm. The pressure of oxygen in the arterial blood is indicated by the line A_1—A_2, that in the venous blood by V_1—V_2. The arithmetic mean of these pressures is shown as C_1—C_2 and is taken to be the capillary pressure. The question is, Can we draw a line T_1—T_2 representing the extra-capillary or tissue pressure. Let us consider what the inferior limit of the tissue pressure must be. At no time can it be less than zero. Let us suppose

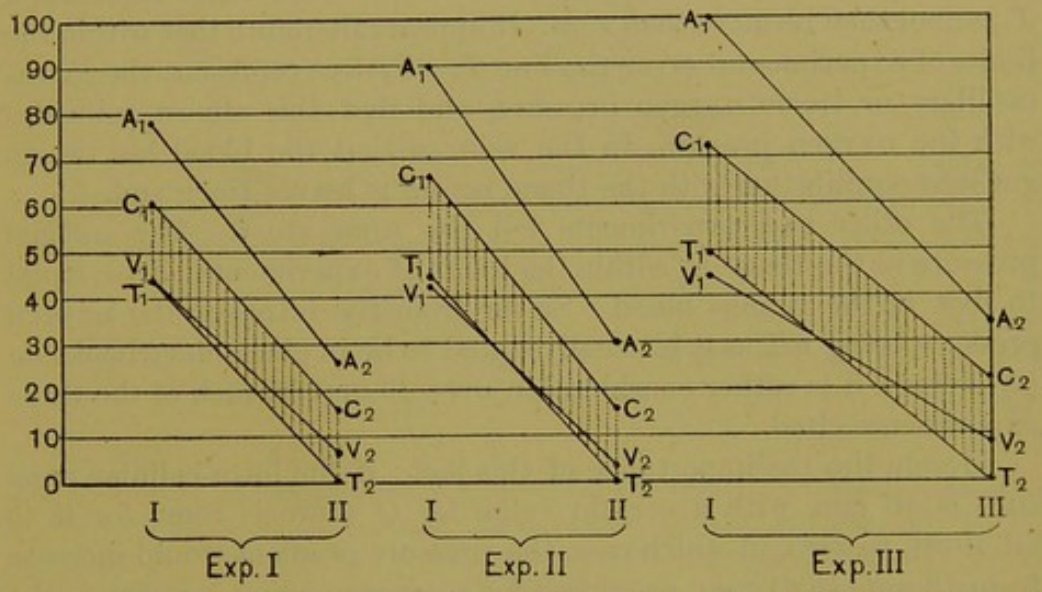


Fig. 89.—A_1—A_2 pressure of oxygen in artery, V_1—V_2 in vein, C_1—C_2 in capillary and T_1—T_2 in tissue, during the periods of each of three experiments on the submaxillary gland. In period I the animal was breathing air, in period II a mixture of oxygen and nitrogen poor in oxygen. Ordinate = mm. pressure. The numbering of the experiments follow that in Verzàr's paper.

therefore that when the intra-capillary pressure p is at its lowest the extra-capillary pressure p' is zero. Then the point C_2 would represent p and T_2 would represent p'. But $C_2 = 16$ mm., $T_2 = 0$. Therefore $p - p' = 16$, or rather $p - p'$ cannot be greater than 16. To find the inferior limit of the extra-capillary pressure in the first period, we need the following additional data. The quantity of oxygen used by the gland Q_1 was ·025 c.c. per g. per min. and in the second period $Q_2 = ·022$ c.c. Q therefore, so far from varying directly with p', remained approximately constant in spite of the fact that p sank from 61 mm. to 16 mm.

We wish to find p' in the first period, in which p is represented by C_1 and p' by T_1.

$$Q_1 : Q_2 :: C_1 - T_1 : C_2 - T_2,$$

$$C_2 - T_2 = 16.$$

$$\therefore \quad \frac{\cdot 025}{\cdot 022} = \frac{C_1 - T_1}{16} = 18 \text{ approx.},$$

but $$C_1 = 61, \quad \therefore \ T_1 = 43.$$

On the assumption that $T_2 = 0$, $T_1 = 43$, the extra-capillary pressure cannot be less than 43 mm. But also T_1 cannot be more than 43 for this is the venous pressure, and by a reversal of the above calculation T_2 cannot then be more than zero. It appears therefore that within the limits of experimental error the line T_1—T_2 does represent the extra-capillary or tissue oxygen pressure, and that this almost coincides with the oxygen pressure in the vein, so that the blood has nearly got into equilibrium with the tissue before it leaves the gland.

The other two experiments tell the same story. The oxygen pressure in the tissue is, within the limits of experimental error, equal to that in the venous blood. So far from the intra-cellular oxygen pressure being *nil*, as it is usually stated to be in elementary books on physiology, it is rather considerable, over 40 mm. in each of the three experiments cited.

Herein lies the importance of this fact: if the intra-cellular pressure is 40 mm. with a certain value for Q, there is room for it to fall down to zero, in which case the pressure gradient would increase from 18 mm. to 61 mm., providing for a corresponding increase in the value of Q should the cells demand it.

We have no corresponding data unfortunately with respect to the active submaxillary gland; so the submaxillary story must end here.

We pass to skeletal muscle; the experiments are not so uniform, nevertheless they seem to justify certain positive conclusions. I will take the two extreme (Exps. V and VII in Verzàr's paper) cases as examples because they are satisfactory inasmuch as the blood flow in each remained fairly constant. The first one for consideration is Exp. V (Fig. 90). In this the values of C_1 and C_2 were 43 and 19 mm. respectively. Let us endeavour again to ascertain the value of T_1 by assuming that T_2 is nothing.

$$Q_1 = \cdot 043, \quad Q_2 = \cdot 016 \text{ c.c. per min.}, \quad C_2 - T_2 = 19 \text{ mm.},$$

$$\therefore \ C_1 - T_1 = 19 \times \frac{\cdot 043}{\cdot 016} = 50 \text{ mm.}$$

But C_1 is 43 mm., therefore T_1 would work out at -7 mm. As the experimental error was not much more than 7 mm. the true value for T_2 cannot have been much above zero.

This is not the point I wish especially to emphasise. The real contrast between this experiment on muscle and those on the salivary gland lies in the fact that in Period I the intra-cellular pressure was 24 mm. below the venous oxygen pressure.

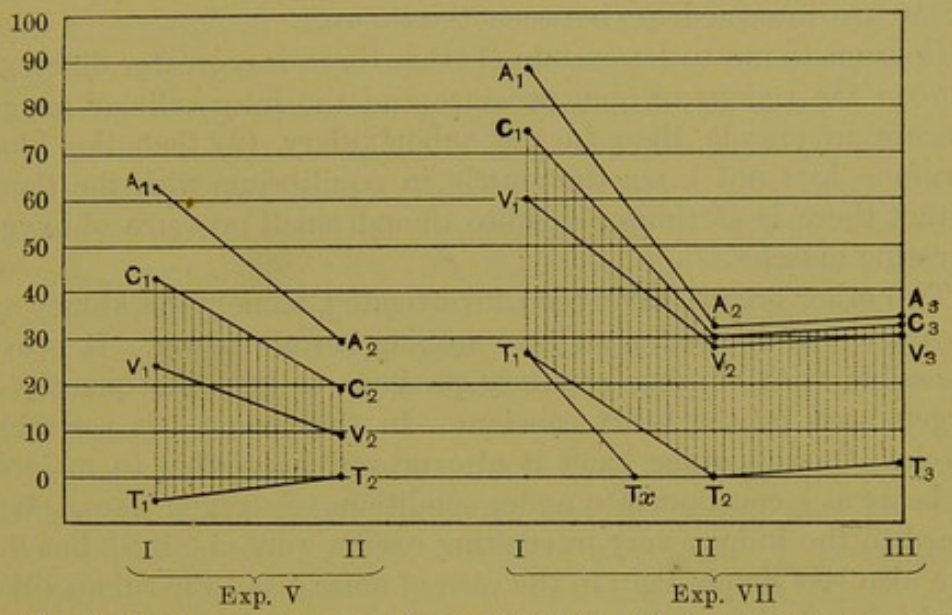


FIG. 90.—A_1, A_2, A_3, oxygen pressure in arterial blood. V_1, V_2, V_3, oxygen pressure in venous blood. C_1, C_2, C_3, mean oxygen pressure in capillary blood. T_1, T_2, T_3, oxygen pressure in tissue, in two experiments on the gastrocnemius muscles. In the first period the animal is breathing air, subsequently a mixture of oxygen and nitrogen poor in oxygen. Ordinate = mm. pressure. The numbering of the experiments follows that of Verzàr's paper.

To consider Exp. VII next. There are three periods in the experiment, the following are the data tabulated on the basis of $T_2 = 0$:—

Period	I	II	III
C	74 mm.	30 mm.	32 mm.
Q	·0061 c.c.	·0039 c.c.	·0035 c.c.
T	26 mm.	0 mm.	4 mm.

The figure four in the last column has, of course, no significance, not differing materially from zero. If T_3 be assumed to be zero instead of T_2, T_1 would work out at about 20 mm., a figure which agrees with that given by the independent method of calculation employed by Verzàr[1]. The highest computation for muscle is 27 mm.*

* Value used on p. 178.

It is clear then that in this experiment there was a positive pressure oxygen in the tissues during Period I. I picture the course of the experiment thus: as the value of C fell along the line C_1—C_2, that of T fell along a parallel line T_1—T_x until it reached zero, after which the intra-cellular pressure would remain at zero and the quantity of oxygen diffusing out of the vessels would vary with the intra-capillary pressure. The other two experiments quoted by Verzàr are intermediate between these two.

It seems to me to be certain (1) that there is a greater difference between the venous oxygen pressure and the intra-cellular oxygen pressure in muscle than in the submaxillary, (2) that the blood therefore does not leave the muscle in equilibrium with the tissue, (3) that there is at times a definite though small pressure of oxygen in resting muscles.

Two other organs have been investigated, namely the kidney and the heart. Of the latter we have nothing certain to say. It proved impossible with varying capillary pressure to keep the quantity of oxygen used by the heart constant. In that respect it resembled muscle. On the other hand it also proved impossible to maintain the beats at a constant rate under conditions of oxygen want. With regard to the kidney very interesting results were obtained, but they were also less simple than in the case of muscle and the submaxillary gland. When the capillary pressure of oxygen falls, the amount used by the kidney (Q) rises provided that the amount of oxygen brought to the kidney is at least sufficient to allow it to rise.

For the moment I will pass over the interest of this as a problem in metabolism and consider it solely as a problem in physics. It is clear that whatever be the need of the cell for oxygen there cannot be less than no oxygen pressure in it. Therefore let us consider the limiting case where $p' = 0$.

$$Q = \propto (p - p').$$

At this point Q is observed experimentally to have, and therefore $(p - p')$ must have, its maximum value. With higher values of the capillary pressure p, Q is observed experimentally to be less, and therefore $(p - p')$ must be less. Thus p', the tissue pressure, can no longer be zero, but must be even nearer to p the average capillary pressure than it was before. But p' can in any case not be greater than the venous pressure of oxygen, for otherwise the blood could never by diffusion have got reduced to its venous condition. But in

point of fact the venous O_2-pressure was very small, the venous blood was almost reduced, in the case when the animal was given only 4% O_2 to breathe; and assuming the tissue O_2-pressure p' in this case to have had its minimum value of zero, it was even so not so very far from the venous O_2-pressure. Thus when p is greater, and $p - p'$ less, p' must be even closer to the venous O_2-pressure than it was when p was small. We see therefore that in the kidney the O_2-pressure in the tissue is determined very largely by the O_2-pressure of the venous blood, and is only definitely less than it when the venous pressure is small owing to incomplete saturation of the arterial blood.

It therefore seems that the kidney like the submaxillary is an organ in which the oxygen pressure in the tissue approaches that in the venous blood, which, in the renal veins of animals breathing normally, is remarkably high. Further than this we cannot make any statement about the quantitative values of oxygen pressure in the tissues except that in such organs as the parotid and the sublingual the conditions are likely to be similar to those prevailing in the submaxillary.

We have then two distinct types: in the first class the difference between the final capillary pressure and the tissue pressure is so small that the tissue oxygen pressure approximates to the pressure of oxygen in the venous blood; in this class are the glands which have been studied. In the other class, that of skeletal muscle, the difference between the capillary pressure and tissue pressure is so large that the latter is 25 mm. or less in the cases measured. In the former organs the blood flow can be considerably diminished without diminution of the oxygen taken in by the organ, in the latter it cannot.

REFERENCES

(1) Verzàr, *Journal of Physiol.* XLV, p. 39, 1912.
(2) Barcroft and Müller, *Ibid.* XLIV, p. 259, 1912.

CHAPTER XI

THE RATE OF EXCHANGE OF OXYGEN BETWEEN THE BLOOD AND THE TISSUES

At the risk of being thought to have taken somewhat of a jump I shall discuss a very different aspect of the capillary circulation.

At the end of Chapter V, I gave a short summary of the effect of salts, temperature and carbonic acid upon the form of the dissociation curve especially in relation to its physiological significance. I showed that the form of the curve was such as to give it a double aspect; to favour both oxidation of the blood in the lungs and reduction of the blood in the tissues.

Whilst this is true it must be borne in mind that the dissociation curve is essentially an equilibrium curve. One cannot tell from it, how long will suffice for the oxidation of blood under any one set of circumstances as compared with the time necessary to reduce it under any other.

But the conditions of respiration are not statical ones; in no tissue is there equilibrium between the tissue and the blood running through it; therefore useful as is the knowledge which has been acquired from a study of the effects of salts, temperature and acids on the final equilibrium between haemoglobin and oxygen, the reader cannot have any complete view of the significance of these factors without some knowledge of the way in which they affect the rates of oxidation and of reduction of the haemoglobin.

I will therefore give some account of the experiments which have been performed for the purpose of gaining such knowledge.

The theory of the experimental procedure was very simple and was an expansion of that which Hill and I used in studying the rate of reduction of haemoglobin and which Mathison improved. It was to pass a uniform stream of nitrogen through blood and to observe after successive intervals of time the degree of reduction which had taken place.

The practical difficulties about a method which sounds so simple are considerable. As compared with a haemoglobin solution great trouble is caused in the case of blood by frothing, which may easily become sufficiently serious to vitiate the experiment.

On the whole the best method was arrived at by taking advantage of the tendency to froth. It consisted in allowing nitrogen to bubble at a uniform rate through a tube 9 mm. in bore of the shape shown in the figure. The tube was placed in a bath at a known temperature. The gas entered at *A*. Each bubble, as it arrived at the surface of the blood *B*, formed a film which was pushed up the tube until at last it was broken by a spiral of wire greased with vaseline.

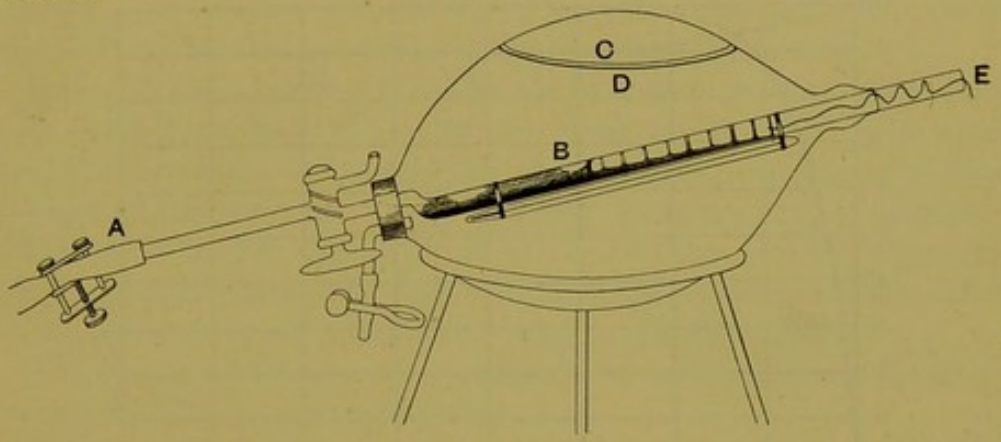


Fig. 91.—*A*, rubber tube leading from gasometer attached to glass tube of capillary bore, the volume of which up to the tap should be ·1—·15 c.c. *B*, bubble of gas about to form a film. *C*, mark on glass bath for purpose of levelling the apparatus. *D*, surface of water in bath at 37° C. *E*, spiral of greased copper wire.

The films were formed and driven up the tube with quite remarkable regularity. It seemed that in spite of a certain degree of empiricism the method did give reliable comparisons between one sample of blood and the next. It is a matter for regret that the method, unlike the dissociation curve, is not one which has any absolute physical significance. The validity of the comparisons made depends upon their all being done in the same tube under the same conditions of inclination, &c. If experiments of this character could be carried out under conditions which could be reproduced at will, they would be the source of the most illuminating information.

At suitable intervals of time the bubbling was stopped, a little blood was abstracted for analysis by taking off the tubing at *A* and using the portion of the tube below the tap as an automatic pipette.

Thus a curve was obtained of the degree of reduction which took place at any given time.

The first experiment which I shall discuss was one in which Nikiferowski helped me; it was on a dialysed solution of haemoglobin, and at the temperature of the room 14—15° C. We endeavoured to reduce the oxyhaemoglobin by passing commercial nitrogen, which contained perhaps a millimetre of oxygen pressure, through the tube at the rate of 30 bubbles in 15 seconds.

This proved to be a hopeless affair; at the end of 150 minutes the haemoglobin was still 90 per cent. saturated with oxygen, nor had it become appreciably reduced in the last hour and three quarters of this

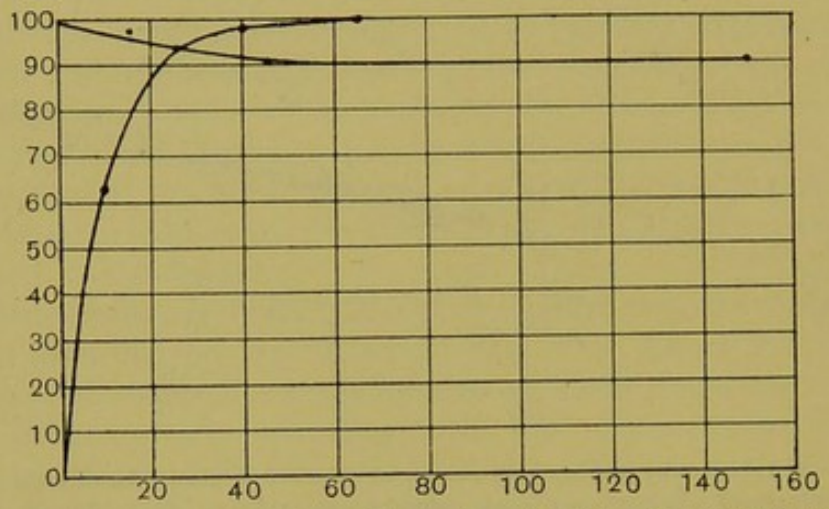


Fig. 92.—Relative rates of oxidation and reduction of a dialysed haemoglobin solution at 16° C. Ordinate = % saturation of the solution with oxygen. Abscissa = minutes of time reducing gas nitrogen. Oxidising gas nitrogen + 100 mm. oxygen.

time. Another experiment was performed on the same haemoglobin solution. The haemoglobin was completely reduced at a higher temperature, cooled down to 15° C. in the tube and then nitrogen containing oxygen to the extent of about 100 mm. pressure was bubbled through the haemoglobin at the standard rate. The haemoglobin oxidised rapidly, in 10 minutes it was 63 per cent. saturated with oxygen and in 15 more it was 93 per cent. saturated.

The course of the experiment may be followed from Fig. 92.

It may occur to the reader to ask why we chose this particular mixture of oxygen and nitrogen for the oxidation process. The reason is that we wished to imitate as nearly as might be the oxygen pressure which is to be found in the lungs during normal respiration.

Through the experiment which I shall describe we have adhered to this pressure of oxygen for the oxidation.

The above experiment lives in my mind as being one of the most instructive with which I have had to do. Consider the task which is set to Nature in providing a medium for respiration—a medium which is at one moment in the lung acquiring oxygen, at another in the tissue imparting the amount it has acquired. Think, too, that the blood must be prepared to yield up its oxygen in a space of time of the same order as that in which it acquires the oxygen. Then turn to Fig. 92 and think of the rapidity with which the haemoglobin acquires its oxygen in the experiment and the tenacity with which it holds to the gas. I believe the haemoglobin would have gone bad before it had become reduced under the conditions of this experiment. Truly Nature has been set a wellnigh hopeless task.

But with the advent of the salts the process of reduction becomes much more easy, as is shown by the work of Oinuma[1], who commenced his research by performing the experiment on blood instead of haemoglobin solution. Here also (Fig. 93 A) the rate of reduction of the blood is slow out of all proportion to the rate of oxidation; still the disparity is not of that apparently hopeless character which it assumes in the case of the dialysed haemoglobin solution.

The results of Oinuma's experiments turned out to be intensely interesting. They were as follows:—

The rate of reduction was increased

(1) by the addition of CO_2 to the reducing gas,

(2) by the elevation of temperature.

The rate of oxidation was retarded

(1) considerably by the addition of CO_2 to the oxidising gas,

(2) to a very slight extent by elevation of temperature. [It should be stated explicitly that this statement does not actually imply a diminution in the velocity constant of the reaction $Hb + O_2 \longrightarrow HbO_2$, for reasons given in Oinuma's paper.]

The effect of these factors will be seen at once from Fig. 93 A, B, C. In the first there is no symmetry in the relation between the rates of oxidation and reduction. The oxidation takes place with much greater rapidity than the reduction. But as the conditions of the experiment approximate more and more closely to those of the body, the rates of oxidation and reduction become more and more nearly equal until at last, when the conditions of the body are imitated as closely as is possible, the curves which represent

the two phases of respiration are almost like an object and its image. These curves are shown in Fig. 93 C.

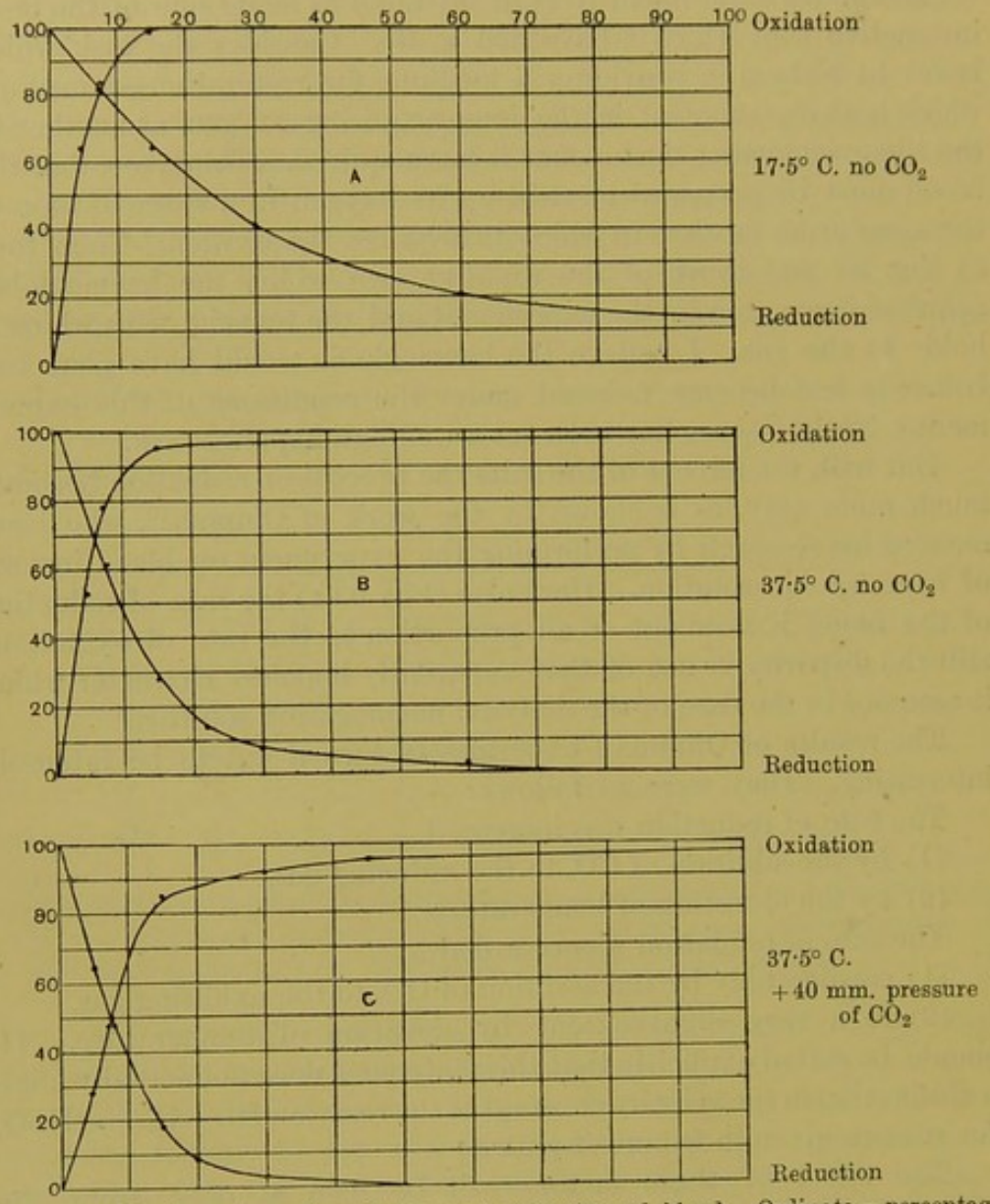


FIG. 93.—Relative rates of oxidation and reduction of blood. Ordinate = percentage saturation. Abscissa = time in minutes. *A*, temp. 17·5° C., reducing gas hydrogen, oxidising gas hydrogen + 100 mm. oxygen. *B*, temp. 37·5° C., gases as in *A*. *C*, 37·5° C., reducing gas hydrogen + 40 mm. CO_2. Oxidising gas hydrogen + 100 mm. oxygen + 40 mm. CO_2.

The contemplation of this figure gave me a great deal of enjoyment till it occurred to me that the process of respiration only involved the upper portion of the curve in each case. Now the

upper portion of the curve of reduction is steep whereas the upper portion of the oxidation curve is not steep but gradual. If these curves give a true picture of the time relations of oxidation and reduction in the body, it would seem that the blood is much more rapidly reduced in the tissues than it is oxidised in the lung.

This check made me anxious to pursue the matter and find out just what portion of each curve was involved in the ebb and flow of respiration. Data were required, firstly as regards the curve of oxidation. It is probably true that the blood in the lung of the resting person is exposed to an oxygen pressure of about 100 mm., and therefore that I was justified in using the curve of oxidation as given. But does the blood become saturated up to the point of being in equilibrium with this pressure? This is a point on which the most diverse views are held by physiologists, who have estimated the percentage saturation of the arterial blood at various figures from 88 per cent. upwards.

The variety of the estimates is no doubt a measure of the difficulty of obtaining an accurate experimental procedure for the determination of the facts. They are based upon various experiments on animals in which the respiration was probably upset to some extent, either in one direction or the other.

The only obvious way of getting at the percentage saturation in the arterial blood was to get some person who would submit to having a cannula put in an artery as he sat in a chair in his usual health. Some of the arterial blood could then be withdrawn without coming in contact with air, and analysed. It chanced that shortly after Oinuma's experiments my path crossed that of such a person. Arthur Cooke [2] decided on the extreme measure of performing the old operation of transfusion. The patient was transfused from the radial artery of his sister and I am indebted both to the surgeon and the lady for allowing me to collect a sample of arterial blood at the end of the operation, which proved to be 94 per cent. saturated with oxygen. In my attempt to discover how much of the oxidation curve was involved in the process of respiration, I took Fig. 93 C. as my starting point. I marked the curve at the point of 94 per cent. saturation and blackened from here downwards. The next question was where to stop. To this question there is no definite answer. In different organs the blood becomes reduced to different degrees. I should like to draw a diagram for each organ in the body. But for the present let me suppose that on the average half of all the oxygen which blood can

hold is taken, i.e that the blood is reduced from 94 per cent. to 44 per cent. saturation. The blackened portion of the curve of oxidation was to represent that portion of the curve which is involved in the actual oxidation of the venous blood.

Fresh difficulties assailed me when I endeavoured to apply the same sort of process to Oinuma's curve of reduction Fig. 93 C. In the first place was it justifiable to make use of this curve? It involves the assumption that the oxygen pressure in the tissue is *nil*. Verzàr's investigations were undertaken for the purpose of testing this assumption. It proved to be incorrect for resting organs generally. The oxygen pressure in the tissue, like the percentage saturation of the blood leaving it, might be anything below a certain maximum.

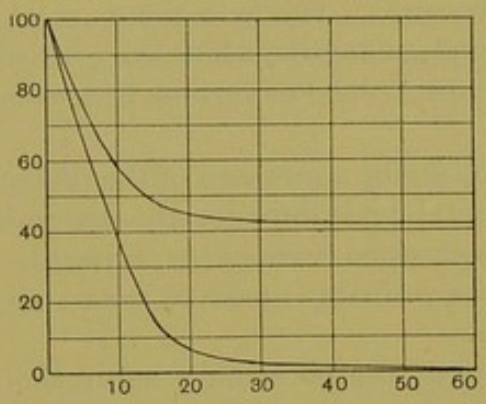


Fig. 94.

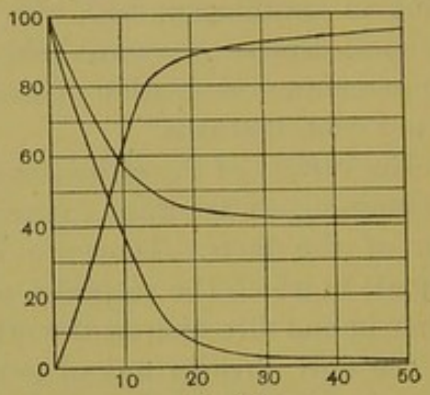


Fig. 95.

Figs. 94 and 95.—Rates of oxidation and reduction of blood. Temp. 37° C. Ordinate = percentage saturation with oxygen. Abscissa = time in minutes. Fig. 94.—Upper curve, reducing gas nitrogen + 27 mm. oxygen + 40 mm. CO_2. Lower curve, nitrogen + 40 mm. CO_2. Fig. 95.—Reduction curves as in Fig. 94, oxidising gas nitrogen + 100 mm. oxygen + 40 mm. CO_2.

We imagined a case in which it is 27 mm.* Fresh determinations were made by Nikiferowski and I for the purpose of relating the curves of reduction of blood in the presence of 0 mm. O_2 and of 27 mm. O_2 respectively. The result is given in Fig. 94. The 27 mm. curve of course came to a different base line from the 0 mm. curve, inasmuch as blood in presence of 27 mm. oxygen comes to an equilibrium at about 40 per cent. saturation.

Here then was my base line for the 27 mm. curve. It is the upper reduction curve in Figs. 94 and 95. It now remained to be blackened between the points of 94 and 44 per cent. saturation to arrive at the portion involved in the reduction.

* See p. 169.

As I had carried my speculation so far, I indulged in the luxury of patching the curves of oxidation and reduction together, or rather

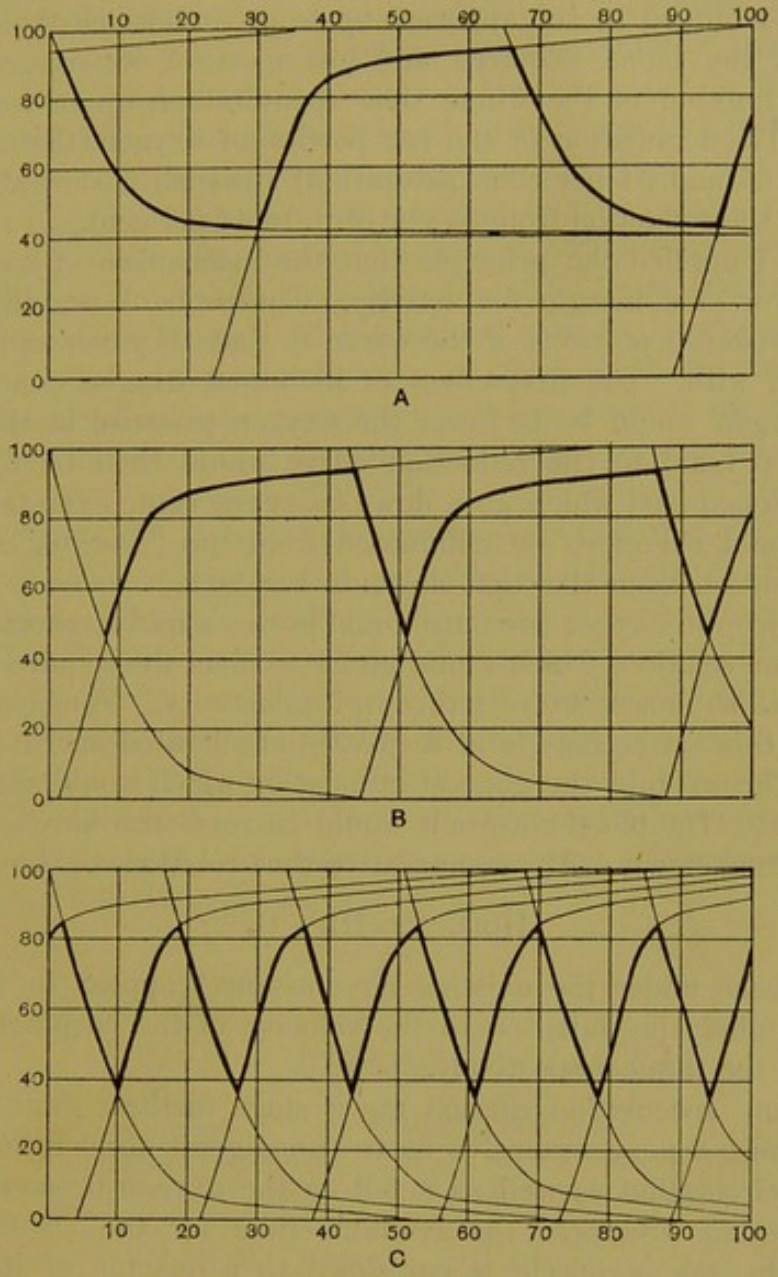


FIG. 96.—Curves of oxidation and reduction of blood corresponding to conditions approximating to those of *A*, oxidation in the lung and reduction in a resting muscle. *B*, oxidation in the lung and reduction in a small active muscle. *C*, oxidation in the lung and reduction in muscle during general muscular exercise. Ordinate = percentage saturation. Abscissa = minutes of time.

the "working" portions of the curves, in order to form a picture of the relative rates at which the tide of oxygen rose and fell in the

blood. Fig. 96 A was the result of this process. Now the symmetry has returned.

I was troubled by the apparent waste of time which these curves revealed, the blood acquired and lost most of its oxygen in so small a fraction of the whole time spent taking up or losing the whole. The acquisition of the last portion of oxygen (that acquired between 85 and 94 per cent. saturation) was such a slow affair, and similarly the reduction from 55 per cent. to 44 per cent.

Then I applied the principle that the mechanism of respiration must allow of a margin for activity. The probable significance of these horizontal portions of the curve is that they can so easily be dispensed with. The first effect of increased oxygen consumption of the organ would be to lower the oxygen pressure in the organ. The sharp bend in the reduction curve would then be far below the blackened part which goes down to 44 per cent. The tail of the curve would therefore be eliminated from the "working" portion which would become like that shown in Fig. 96 B.

This drop in oxygen pressure would have secured a rearrangement of the time relations which would go far to meet the requirements of some smallish muscle were it thrown into activity. For blood would traverse this active muscle at a greatly accelerated speed, it would then be thrown into the general circulation which would scarcely be quickened. The blood corpuscle would traverse the muscle quickly and the lung slowly. The comparative time relations of the reaction

$$HbO_2 \rightleftarrows Hb + O_2$$

taking place under the existing circumstances appear in Fig. 96 B and are admirably adapted to the velocity of the corpuscle in the lung and the tissue respectively.

But my speculation carried me a stage further. Suppose that the exercise was so extensive as to cause great acceleration of the general circulation as well as a fall in the oxygen pressure of the active tissues. Suppose the available time for the acquisition of oxygen by the corpuscle is cut down to a quarter of its former value. It would become about 85 per cent. saturated, with a little time to spare. Of course enormously more oxygen would be leaving the lung under these circumstances. The blood then reaches the tissue 85 per cent. oxidised, the tissue extorts its 50 per cent. of oxygen, and therefore reduces the blood to 35 per cent. saturation. This it can do in approximately the same time as that in

which it had previously reduced the blood from 95 to 45 per cent. saturation. Now the corpuscle goes back to the lung where it has to be oxidised from 35 to 85 per cent. to complete the cycle. This process can take place within the time allotted, namely one quarter of the period which the blood formerly spent in the lung in which the leisurely oxidation from 45 to 95 per cent. had previously taken place. This cycle is represented in Fig. 96 C. The difference then between Figs. 96 A and 96 C are as follows: firstly as regards the assumptions made, in the first case the "high and low water" marks for oxygen are 95 and 45 per cent. and the pressure in the oxygen tissue is 27 mm. In the second case the marks are 85 and 35 per cent. saturation and the oxygen pressure in the tissue is *nil.* These alterations in the assumed conditions provide for a four-fold increase in the rate at which the haemoglobin can acquire and expel a given quantity of oxygen.

There is a certain amount of evidence in favour of the view that the arterial blood is slightly less oxidised during activity than during rest. The experiments most to the point are those of Hill and Nabarro [3]. The average of their normal animals gave 17·44 as being the oxygen in the arterial blood (15 animals). During the tonic phase of fits induced by absinthe the average was 16·11, whilst in the clonic phase 17·21. The subject is one which might suitably be reinvestigated with due precautions to ascertain the percentage saturation in each case and to ensure that there was efficient oxygenation in all phases of the experiment.

REFERENCES

(1) Oinuma, *Journal of Physiol.* XLIII, p. 364, 1911.
(2) Barcroft and Cooke, *Ibid.* XLVII; *Proc. of Physiol. Soc.* Dec. 1913.
(3) Hill and Nabarro, *Journal of Physiol.* XVIII, p. 218, 1895.

CHAPTER XII

THE ACQUISITION OF OXYGEN BY THE BLOOD IN THE LUNG

We have now arrived at the point at which it would be desirable to apply our knowledge of the dissociation curve of blood to the solution of a number of physiological problems, and to give some complete history of the transportation of oxygen from the lung to the tissue. Before doing this however we find that there is a difficulty confronting us in the nature of the transition of gases through the epithelium of the lung itself. The older physiologists would freely have assumed that because the oxygen in the alveolar air of the lung exercises a pressure of about 100 mm. of mercury, the blood would be saturated with oxygen up to the point corresponding with that pressure; in other words, that the pressures of oxygen in the lung and in the arterial blood would be approximately equal. This assumption was based upon the work of the Bonn School of physiologists.

If I am to give a coherent account of the controversy which has taken place between those who believe that oxygen is secreted by the pulmonary epithelium and those who believe that it diffuses from the alveoli into the blood, I must go a little further into the history of the subject than I have been wont to do heretofore.

The aerotonometer methods of the Bonn physiologists seemed to have established the theory of diffusion in the lung on a satisfactory basis when Bohr, using similar but not precisely the same methods, found that there was

(1) A greater pressure of oxygen in the arterial blood than in the alveolar air.

(2) A greater pressure of carbonic acid in the alveolar air than in the arterial blood:

neither of which phenomena could be accounted for by simple

diffusion; though Fredericq performed yet other aerotonometric experiments which supported the diffusion theory, the fact remained that those who studied tensions with aerotonometers were divided.

Whilst Bohr's theory of pulmonary secretion was more complicated than its rival, it had a certain charm about it which has appealed to many minds. Its reasonableness was based upon morphological considerations which the diffusion theory entirely ignored. It started with the swim-bladder of the fishes; this organ in the cod if emptied gradually refills, and the gas on analysis yields the remarkable result that some 80 % of its volume is oxygen. Whatever secretion may be, here is a case of it. Is it strange that the swim-bladder of the cod should have this power? It is an offshoot of the alimentary canal; if the cell of the stomach can secrete hydrochloric acid; if the cells of diverticula, such as the pancreas and the liver, secrete sodium carbonate, is there anything strange about the idea that another diverticulum of the great secreting mass—the alimentary tract—should secrete oxygen? Rather would it be strange if it could not secrete. Moreover the unity in function between the swim-bladder and other diverticula, such as the glands of the stomach, is made more evident when we discern, as Bohr did, that the secretory function of the swim-bladder is governed by the vagus just as is the secretory function of the stomach. Cut the vagi—the swim-bladder will secrete oxygen as little as the stomach, similarly crippled, will secrete pepsin.

Passing from the fishes to the other vertebrate types, the alleged secretion in the lung was studied very carefully by Maar in the tortoise. It proved to be possible, and in fact simple, to study the gaseous exchange in each lung of the tortoise separately. To quote Krogh on the subject: "While the necessary operation is rather difficult in the mammals, and may vitiate the results by injuring the animal, the tortoise appears to be specially adapted for this kind of experiment. The trachea divides just behind the head, and the main bronchi run parallel to one another and loosely connected along the whole length of the neck."

Maar discovered that when the vagus was divided the gaseous exchange of the lung was so altered as to make it clear that the vagus has a very definite action on the pulmonary respiration of the tortoise, but the action is not easy of interpretation. As explained by Maar, however, there was a sort of tonic action, the vagus providing inhibitory nerves. The figures were regarded as being lifted

out of the domain of vasomotor effects in the two lungs by control experiments on atropinised lungs.

The experiments carried out by Maar on the tortoise were, to some extent, supported by those of Spallitta, an Italian physiologist, on the turtle. This author found that whatever mixture of gas was put into the turtle's lung, and enclosed therein, the oxygen was practically all absorbed, whilst the CO_2 was always found to be about 6·5 %.

Nor was the action of the vagus on the respiration of warm-blooded animals overlooked in the Copenhagen laboratory. Henriques studied the effect of brief stimulation of the vagus on the gas exchange of dogs. Practically it resembled that of the tortoise, namely, it inhibited the secretion of oxygen relative to CO_2, and in this way caused the respiratory quotient to approach unity. Here again circulatory changes were supposed to have been excluded since the change in the respiratory quotient was produced in cases in which the circulation was both quickened and slowed.

We are not now criticising the case for secretion in the lung but merely stating it, and from the statement it appears that right up the animal kingdom there is evidence, which sometimes appears good and sometimes bad, for the possibility of active secretion of gas, the activity being influenced, sometimes in one way, sometimes in another, by the nervous system.

The diffusion and the secretory theories were then rivals. The one is simple, inasmuch as it demanded of the lung no greater powers than are possessed by a piece of parchment, but it involved no great biological generalisation; it is a theory which attracts the physicist because it presents a simple and intelligible relation of facts as they are, but which repels the biologist because his training has taught him to regard the different organs of the body as specialised groups of cells which confine their activity to carrying out individual fractions of the functional complex appertaining to living protoplasm. The other theory may be said to be complicated, but at least it demands no greater mystery than is conceded to almost every other cell of the alimentary tract—the mystery of metabolic activity on the part of the cell itself. This may be among the greatest of all mysteries, but it may fairly be claimed that an even greater would be a cell whose purpose was in no way a reflection of its own metabolic activity.

Nor must the reader in judging the theory of pulmonary secretion

forget its close analogy to the glomerular function of the kidney. The epithelium covering the latter organ is, in a general way, similar to that covering the lung; how vast is the mass of physiological literature which tacitly assumes that if secretion can be proved in the amphibian capsule of Bowman, it may be taken for granted in the case of the primates. We do not wish to insist unduly upon the inherent reasonableness of Bohr's point of view, but we regard his position as at least more reasonable than that of the lecturer who in one lecture denounces Bohr's view as extravagant, and in the next teaches a doctrine of universal glomerular secretion on the basis of a long series of well-established experiments on frogs. Neither Bohr, nor any of his supporters, have ever gone so far as this; their position has always been that even granting secretion of oxygen in the cod and other low forms, its existence in higher ones remains a matter to be decided by experiment.

About twenty years ago, it was therefore of the utmost importance that some totally new method should be sought, which would decide between the rival aerotonometricians. Such a method was devised by Haldane [1], and in his hands and those of his collaborators, Lorrain Smith and Douglas, has played a very important part in the physiological thought of the last two decades.

The principle of the method may be summed up in a few words. If haemoglobin be exposed to a mixture of oxygen and carbon monoxide, the resulting quantities of oxy- and carboxy-haemoglobin depend upon the relative pressures of oxygen and carbon monoxide. In short there is a balanced action

$$CO + O_2Hb \rightleftarrows COHb + O_2.$$

If therefore any three of the quantities involved are known, the fourth can be calculated. Haldane administered CO in known quantities, i.e. at a known pressure (for the pressure of CO in the air was taken as the index of its concentration in the plasma); he estimated the relative quantities of O_2Hb and COHb present, and from these data calculated the pressure of oxygen in the blood.

Before this could actually be done, however, a great deal of ground had to be cleared; in the first place a method of estimating the relative proportions of oxy- and carboxy-haemoglobin had to be devised; in the second place the necessary data had to be accumulated for determining what was the precise relationship of the four substances in question in all possible cases in which equilibrium existed.

Haldane and Lorrain Smith exposed haemoglobin to mixtures containing varying known concentrations of oxygen and carbon monoxide, and determined the relative amounts of CO and O_2 haemoglobin. In practice the process is somewhat simplified by the fact that if as much as ·1 °/₀ CO—and that is more than would be desirable for the experiment—be mixed with air, there is but a negligible diminution in the partial pressure of oxygen. We may conveniently make up mixtures of CO in air—assuming that the oxygen is 21 °/₀ in each

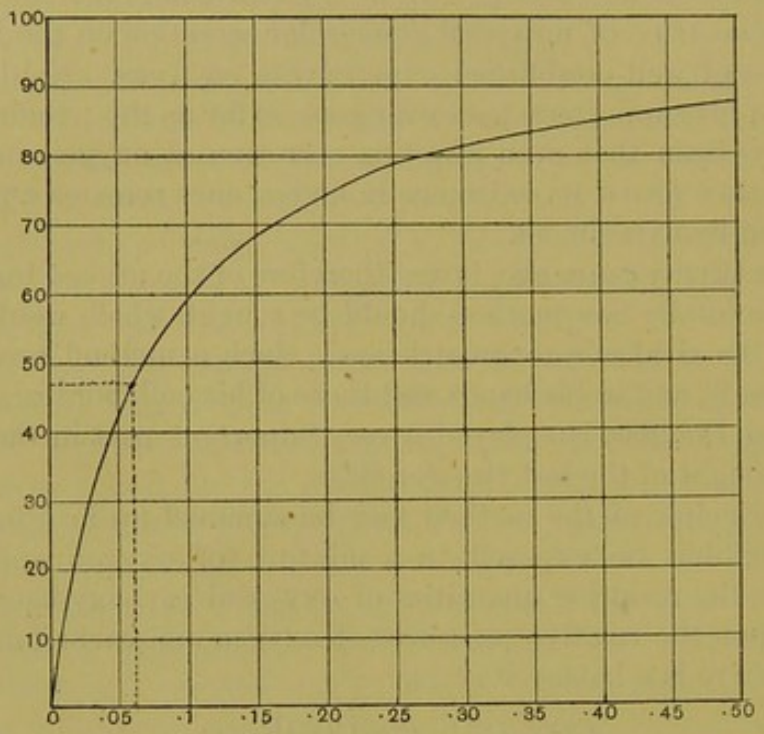


Fig. 97.—Curve of Haldane and Lorrain Smith indicating the partition of haemoglobin between oxygen and CO. Ordinate = percentage of the total haemoglobin present as CO haemoglobin, the remainder being oxyhaemoglobin. Abscissa = percentage of CO in air.

case—shake them with haemoglobin solution, and when equilibrium is established analyse the air for CO and the blood for the relative amounts of the two pigments. Plotting such a result, Haldane and Lorrain Smith obtained the above curve, which relates the relative quantities of COHb and O_2Hb to the relative pressures of CO and oxygen gas.

We have now at our disposal, in theory at all events, the data for carrying out one of Haldane and Lorrain Smith's experiments (we quote their own figures).

Suppose ·08 °/₀ of CO was in the air inspired by the subject; when he had been breathing the mixture long enough for equilibrium to have been established, some of his blood was drawn, and the relative quantities of oxyhaemoglobin and CO haemoglobin estimated, the result being that there was 46 °/₀ of the latter and 54 °/₀ of the former. If we consult their curve (Fig. 97) it informs us that the oxygen and CO must have exerted in the fluid, with which the haemoglobin was in contact, pressures related in the proportion of ·06 of CO to 20·9 of oxygen. But the actual pressure of CO was ·08 °/₀ of an atmosphere, therefore the oxygen pressure in the plasma was $\cdot 08 \times \frac{20 \cdot 9}{\cdot 06} = 27 \cdot 9$ °/₀ of an atmosphere For the sake of simplicity we have omitted a slight correction for aqueous vapour, which does not affect the main point, namely that the oxygen pressure in the plasma exceeds that in the atmospheric air, and still more that in the alveolar air, being in this experiment almost double the latter.

Perhaps there was nothing more convincing about the researches which we have described than the experiments with which they were controlled. Their authors seemed to show not only that there was evidence of secretion in the lung, but that under conditions of impaired vitality that secretion disappeared. Moreover, this aspect of the question transferred the problem from one of merely theoretical importance into one of great practical interest to the clinician, for if the body depends upon the secretory activity of the lung, and this is easily impaired, then the primary object of treatment in the case of lung complaints should be a care for this precious function. It should aim at the restoration of the lung from a condition analogous to that of dyspepsia.

Of mice for instance the authors say "After exposure to a cold atmosphere for a short time their body temperature begins to fall, particularly if they are exposed to a somewhat high percentage of carbon monoxide, and they become torpid." It was easy to cool down the surroundings of the mouse by placing the bottle containing it in cold water. The result invariably was that the oxygen tension went down to about 15 °/₀ of an atmosphere.

In the light of more recent work I am not now greatly concerned to quote the results of Haldane and Lorrain Smith at great length. I am concerned to state the problem in such a way that the reader shall be in a position to criticise it intelligently.

Indeed for fifteen years it divided the physiological world in a

very interesting way; practically the critics were on one side and the experimenters on the other; those who taught and wrote for the most part refused to accept the secretory theory, whilst with the exception of a few senior physiologists, such as Fredericq and Zuntz, the workers at respiration inclined more and more to the secretory theory. Indeed matters seemed to have reached a deadlock. The diffusion theory was supported entirely by aerotonometer experiments, which were held by the supporters of the secretory theory to be fundamentally vicious, on the ground that the blood reduces itself when removed from the vessels; this charge seemed to be unanswerable—at all events it was unanswered. Its weight lay in the fact that, at high oxygen pressures, a very slight amount of reduction would produce a very big drop in oxygen pressure in the blood; therefore, if any appreciable reduction took place in the tonometer, the blood in the vessels might easily have had twice the oxygen pressure which was observed in the tonometer. This criticism remained unmet; moreover those who refused to accept Haldane's results proved quite unequal to the task of showing where he had gone wrong.

Experiments on the effects of fall of temperature on the oxygen tension of arterial blood.

Animal	% of CO in air	Temp. of bath	Duration of exp.	Saturation of Hb with CO %	O_2 tension in arterial blood % of an atmosphere
Mouse	·200	47·5°	45 min.	79·5	15
,,	·078	5·0°	30 ,,	61·6	14·2
,,	·103	5·5°	45 ,,	65·5	15·7
,,	·101	6·0°	52 ,,	65·8	15·6

Indeed at the time of which I am writing (six or seven years ago) the supporters of the physical theory had, in their endeavour to throw the burden of proof upon their opponents, frankly taken refuge themselves in metaphysics. They began their arguments with the general statement that we must always believe the most simple of two rival theories unless it can be disproved, because it is the most simple. This statement, like that freely used by the opposite faction, namely, that we must believe the theory which offers the greatest possibility of benefit to the organism because evolution demands it, contains an element of truth. To my mind they break down at

the same point; they assume a knowledge on the one hand of what is simple, on the other of what is advantageous, whilst as yet the facts and principles at stake are admittedly unknown.

Let me illustrate my meaning by an analogy. It is difficult to conceive of a more simple form of column than a cylinder. Yet I have before my eye a hexagonal column—a much more complicated figure—in order to be sure what it is I have to go round it, to measure its angles and count its sides. This column has come from that magnificent natural pile, the Giant's Causeway, in the north of Ireland, where sea over sea of molten rock has shrunk and fractured into a structure composed entirely of columns just similar to that at which I am looking. The man who would urge the rightness of the simple view may retort that his point of view is unshaken, that one would be right to hold that Nature was more likely to produce a cylinder than a hexagonal prism until evidence had been adduced to the contrary, and that only now that I have the hexagonal prism in view am I warranted in changing my mind.

My meaning has escaped such an one. It is that the simplicity of the two stones has been considered apart from their setting, apart from the forces which brought them into being, and especially with regard to the simplicity of those forces. Behind the complicated hexagon there is a simple law of contraction, behind the simple cylinder there is no simple natural process, in fact there is no natural process at all—it has been produced like the dialysing membrane by the hand of man—it might conceivably have been produced from the prism by some natural process of wear and tear, as a dead membrane might have been produced from a living one, which would probably like a diphtheritic membrane be sloughed off by the body. Taken in its setting, the column of basalt from the Giant's Causeway is the simple figure. The truth of the proposition that the simple process was the more reasonable remains, but whilst the processes are themselves unascertained it is impossible to be sure that the process which seems the simpler does not involve consequences far more complex.

There were not wanting those who said that they could not get results at all by the method pursued by Haldane and Lorrain Smith. A confession of one's own weakness is not a convincing proof of some one else's.

But the cup of the diffusion theory was not yet full. Quite another line of argument was adduced which, if correct, showed that

even on their own ground the supporters of the diffusion theory had gone far to prepare their own downfall.

Those who opposed the secretory had thrown upon their antagonists the whole onus of proving that there was a difference of oxygen pressure between the arterial blood and the alveolar air. They said in effect, "Unless you can show that the oxygen pressure in the arterial blood is greater than that in the alveolar air, diffusion will suffice to explain the passage of a gas from one to the other." Their own aerotonometer results showed no measurable margin between the two tensions; they were therefore complacent enough. It was at this point that Bohr replied, "Diffusion from one side of the alveolar epithelium to the other must involve some difference of pressure; this may be immeasurably small, or it may not: the physical theory at least presents the advantage that it offers the opportunity of calculating the difference of pressure necessary to produce the flow of gas."

He attacked the matter as follows. The first operation which confronts a molecule of oxygen, which would pass from the alveolar air through the epithelium, is the physical one of passing from the gas into the fluid surface covering the lung. He therefore proceeded to investigate the laws which governed the passage of gases into fluid surfaces with the following result.

The argument was as follows:

(*a*) To calculate g, the number of cubic centimetres of gas which enters a surface in a minute, let s be the area of the surface, p the pressure of the gas, and γ the *invasion coefficient* which is defined as the amount of gas which enters 1 sq. cm. of the surface in one minute at the atmospheric pressure.

$$g = \frac{s\gamma p}{760}.$$

(*b*) To calculate b, the quantity of gas which leaves the surface of a fluid charged with the gas in a minute, where s the area of the surface, ξ the quantity of gas dissolved in 1 c.c. of the fluid, and β the *evasion coefficient* which is defined as the quantity of gas that leaves 1 sq. cm. of the surface in one minute when 1 c.c. of the fluid holds 1 c.c. of the gas.

$$b = s\beta\xi.$$

(*c*) Consider the special case of a fluid which is in equilibrium with a gas, the pressure of the gas being 760 mm. Since the equilibrium exists, the number of molecules of the gas which enter and leave the surface must be equal, and therefore

$$g = b,$$

in other words

$$s\gamma \frac{p}{760} = s\beta\xi,$$

but in this particular case $p = 760$ mm. and since the fluid is saturated with the gas at 760 mm. the amount of gas which is dissolved in 1 c.c. of the fluid is a, the coefficient of solubility, or the absorption coefficient of the gas in the fluid. Substituting 760 for p, and a for ξ, our equation therefore becomes

$$\gamma = a\beta.$$

Now since a, β and γ are all coefficients, that is to say constant quantities, we may, in any further consideration of the subject, replace β by γ/a, thus getting rid of the evasion coefficient in favour of a which is easily determined experimentally.

(*d*) To calculate the volume M of a stream of gas passing per minute into a fluid in which the concentration of the gas is ξ, the pressure of the gas in the fluid is p', and without the fluid p.

$$M = g - b,$$

i.e. the difference between the amount of gas which passes into the surface and that which passes out.

$$\therefore \quad M = \frac{s\gamma p}{760} - s\beta\xi$$

$$= \frac{s\gamma p}{760} - \frac{s\xi\gamma}{a},$$

in the present case $\xi = ap'/760$; substituting this value we get

$$M = \frac{s\gamma}{760}(p - p').$$

(*e*) For the human lung, M for a person not actively employed may be taken as 400 c.c. a minute, s as 90×10^4 sq. c. γ was calculated by Bohr as being ·012, p as 105 mm., and therefore the only unknown factor in the equation is p'. Now taking these figures, the difference between p and p' is about 25 mm., and therefore p' is about 80 mm.

According to this calculation which contains nothing but mechanical reasoning, the tension of oxygen in the fluid with which the epithelial cells of the lungs have to deal cannot be more than 80 mm. or about 11 °/₀ of an atmosphere, i.e. is lower than all computations of the tension of oxygen in the arterial blood as made by the aerotonometer; and the calculation was therefore regarded by its author as the final and convincing proof of the secretion of gas by the epithelial cells from the place of lower to the place of higher tension.

We may summarise the results obtained by the use of the invasion and evasion coefficients, as follows:

(1) According to Bohr's statement of the diffusion theory, the pressure of oxygen in the capillary would, apart from secretion, be less than that in the alveolar air by at least the amount which the invasion coefficient demands and which is measurable.

(2) The difference between the two becomes greater in direct proportion to the oxygen used by the organism.

(3) The evasion coefficient of CO_2 is such that the difference between CO_2 pressure in the lung, and in the blood of the pulmonary capillaries, is immeasurably small. Figure 98, given by Krogh,

represents the actual measurement of the tensions of oxygen and carbonic acid which he obtained, and which are not inconsistent with the facts as stated above. So far, therefore, as our knowledge of the difference of pressure necessary to maintain an adequate current of oxygen through the lung is concerned, we may assume that the oxygen pressure in the blood cannot exceed about 1 mm. less than that in the alveolar air for every 100 c.c. of oxygen absorbed by the organism, but we are at present somewhat in the dark as to how much it is less than that amount.

The invasion coefficient applies only to the one portion of the physical process of the passage of a gas through the lung, namely the entrance of the oxygen into the moist surface. Bohr[3] made some experiments shortly before the close of his life upon the physical constants involved in the whole process—a subject which had previously been investigated by Hüfner[4], and Zuntz and Loewy[5]. What pressure head is necessary to force the amount of oxygen which can be taken up by the human lung through the epithelium in the time? The method of experiment is as follows: Carbon monoxide is assumed to pass through the lung by diffusion, it is then taken up by the haemoglobin. The amount that is taken up in a given time can be measured. Within certain limits the pressure of CO produced in the blood may be neglected. The amount which diffuses through will then depend simply upon the external pressure of CO. Now the pressure of CO in the alveolar air and the volume of CO which passes through the epithelium in a given time are both measurable and may be related directly to one another. This relation is called the diffusion coefficient for the lung. When it is once determined for CO it is assumed to have been determined for oxygen from the known relation of the diffusion constants of the two gases in physical experiments outside the body.

According to Bohr the diffusion coefficient for the lungs was of such an order as to allow of the actual quantity of oxygen used at rest to pass through the epithelium at the usual alveolar oxygen pressure, but if his determination of the diffusion coefficient were correct it would be impossible for the amount of gas which traverses the epithelium during exercise to pass by diffusion, as also the quantity of oxygen which goes through at rest, when the person is exposed to low pressures of oxygen.

To sum up the situation as it was three or four years ago. The diffusion theory rested upon aerotonometer experiments, which for

the most part satisfied the teachers of the subject, but which more and more failed to satisfy those who were actually at work upon it, and there were not wanting signs that even in the "Landwirtschaftliche Hochschule" in Berlin some could be found who were prepared to coquet with the views which emanated from Copenhagen and were reflected from Oxford.

These views were supported by each piece of experimental evidence that appeared. On the one hand they rested upon a biological conception which could be illustrated from many parts of the animal kingdom, on the other hand they leant upon data which were derived from experiments upon man, not under operation, not under anaesthetics but in whom the normal functions appeared to have scope to exercise their normal activity. Finally the physical theory seemed to be disproved by the pushing of its own requirements to their conclusion.

REFERENCES

(1) Nagel's *Handbuch*, I, pp. 54 et seq.
(2) Haldane and Lorrain Smith, XXII, p. 231, 1907.
(3) Bohr, *Skand. Archiv*, XXII, p. 221, 1909.
(4) Hüfner, *Annal d. Physik. u. Chem.* Bd. LX, p. 141, 1897.
(5) Zuntz and Loewy, *Arch. f. Physiol.* p. 166, 1904.

CHAPTER XIII

THE ACQUISITION OF OXYGEN BY THE BLOOD IN THE LUNG (CONTINUED)

No apology is put forward for the point at which I have drawn the dividing line between the present and the last chapter. It has been drawn at the position in which the subject stood when Hartridge commenced his work. The purely personal point of view from which this volume has been written demands the treatment of the subjects in a personal way. But for Hartridge's work and a trifling contribution made by Cooke and myself the subject of pulmonary secretion would probably have found no place in the book. The last chapter then forms a statement of the problem as it appeared when Hartridge commenced to work at it.

Whilst he was working, two researches of great importance appeared, which quite altered the aspect of the problem. The first was the work of Dr and Mrs Krogh[1], the second the work of Haldane and Douglas[2]. The first clearly proved that in animals under operation all the facts involved in the acquisition of oxygen could be explained by diffusion. The second consisted in a withdrawal of the secretory theory except during conditions of stress, i.e. either of considerable exercise in which the demand for oxygen was greatly increased or of reduced barometric pressure under which the supply is diminished.

It will be desirable to treat of these two researches in some detail.

The change which has taken place within the last three years has largely been caused by an altered point of view with regard to the requirements and possibilities of gas analysis as adapted to physiological purposes. The generation whose hairs are grey in general held to a sound chemical principle, namely that the greater the quantity of the substance which could be obtained for analysis the more certain the

analysis. Yet there are many limitations to this principle, especially on the biological side. Many are the fortunes which have been lost in commerce because the reaction which will take place in the test tube will not take place in the works. The reasons are in many cases apparent. I have been told that in a brewery of great repute the chemical staff found it impossible to reproduce in the laboratory the reactions which took place in the vat because the vat unlike the beaker is practically an adiabatic system, its ratio of cooling surface to volume as compared with that of the beaker is relatively infinitesimal—its substantial walls are poor conductors of heat. With the advent of an adiabatic beaker in the shape of a Dewar's vessel, the conditions of the vat can be reproduced, and with the conditions the reactions.

In the case of physiological requirements the younger generation has grasped the principle that everything depends upon working with the minimal possible quantity of gas.

This is so from whatever view you take it. Biological conditions change so rapidly that the technique of the biologist must be one which can be operated with great rapidity. Moreover they become changed and abnormal by the mere abstraction of the quantities of fluid required for the older methods. In the viscid fluids of the body the process of abstraction of the gases is a much more difficult one than it is in the case of the limpid liquids with which the chemist deals, and is greatly facilitated by increasing the ratio of surface to volume ; i.e. in general by decreasing the volume used. Then again gas analysis is a very special sort of analysis inasmuch as the measure of the gas is usually its volume, and this is extremely sensitive to temperature and pressure. How great would be the anxiety of the analytical chemist to reduce the amount of matter which he weighed to the smallest possible bulk, if before he weighed it he had to convince himself that it was of uniform temperature throughout and that its temperature was known to a hundredth of a degree. Nor have I stated the case as urgently as I might. I have said that the measure of a gas is its volume. In practice this is not the case, the measure of the gas is usually a measurement of length, it is the distance between two points on a cylindrical burette. If there is a temperature error, that will be three times as apparent as it would be if the gas were free to expand or contract along all three of its linear dimensions. It has therefore been the object of the younger school of physiological gas analysts to cut down the quantity

of gas measured to the minimum quantity consistent with the preservation of the measurement being a simple measurement of length. This of course is accomplished by narrowing the bore of the burette, and as it appears to be as easy to draw a capillary tube of uniform bore as a more capacious cylinder, the real limit of the process is arrived at where serious errors are introduced by unavoidable inequalities in the surface of the glass. Moreover, gas in capillary tubes is in many ways easy to manipulate; if enclosed between two fluid surfaces there is not the same tendency for the gas to escape. You can lay the tube down, set it on its end, expel the bubble of gas, withdraw it, manipulate it in all sorts of simple ways, and still you have it, and have it under conditions in which it is, so to speak, all surface.

Whilst in this country Brodie and I were pushing these principles to what we regarded as considerable lengths for the purpose of determining the gaseous exchange of the frog's kidney and found to our own surprise that we could analyse 1/10 c.c. of gas with sufficient accuracy, Krogh was working out a technique along the same general lines, by the side of which ours appears crude to the last degree. Nothing could be more beautiful or fascinating than his method of analysing almost microscopic bubbles of gas, that is to say, bubbles of about 1/100 c.c. This technique reformed aerotonometric methods. A bubble of such small dimensions rapidly attains equilibrium with any liquid with which it is in contact. If placed in a stream of circulating blood, it is tossed to and fro, its surface and that of the blood in contact with it are constantly changing, and the stream in which it is may be so small that each corpuscle is out of the animal but a very short time. Therefore there is no opportunity for self-reduction of the blood, and moreover there is no amount of haemorrhage which signifies. Krogh's method at once received the acclamation of physiologists, partly because of its own beauty and partly on account of the consciousness of its limitations which its inventor betrayed. In a series of papers which are at once a model of certainty of touch and modesty in presentation, he has revised the whole field of the relation of oxygen pressures in the blood and the alveolar air, in so far as it can be revised by methods of this kind.

Krogh investigated the criticism levelled against aerotonometric experiments generally, that their results yield too low an oxygen tension owing to self-reduction of the blood. He found that self-

reduction does take place, corresponding at most to ·0007 c.c. of oxygen per c.c. of blood per minute (or about half a per cent. of the oxygen contained in the blood) and at least ·0002 c.c. It is in some respects fortunate, in others unfortunate, that Krogh chose the rabbit as the object of this research. The blood of the rabbit was found by Douglas to reduce itself with much greater violence than that of higher mammals. If, therefore, the self-reduction does not matter in the case of the rabbit—and it would seem that it must be very slight in the infinitesimal time taken by blood to traverse a tube joining two ends of the carotid artery—it clearly does not matter in the higher types. On the other hand it is probable that if Krogh had used the cat he would have obtained figures probably in the next decimal place and therefore placed self-reduction in that animal far out of the field.

As regards the actual tensions observed and the comparison of them with the alveolar air, Krogh was at the disadvantage under which all workers in this subject who depend upon animal experiments find themselves, that of getting an actual sample of alveolar air to analyse. Calculating it from the air of the bifurcation of the bronchi, he found (1) that the CO_2 pressure in the alveolar air and the blood were the same and kept the same whatever changes took place in the former, (2) that the oxygen pressure in the blood was always below that of the alveolar air by a certain amount, 15—20 mm., and that this margin remained even with considerable variations of oxygen pressure in the alveolar air.

For the moment I wish to focus the attention of the reader upon this margin of 10—20 mm., and to recall the fact that according to Bohr the diffusion theory demanded for the human lung an excess of about 25 mm. of pressure in the alveolar air over that present in the film of moisture on the exposed surface of the lung cells. As well as this a further difference of pressure was required to drive the oxygen through the cells. Owing to the extreme solubility and diffusibility of carbonic acid no measurable margin is demanded between the carbonic acid pressure in the lung and in the alveolar air. Krogh's result then agrees with Bohr's expansion of the diffusion theory inasmuch as he shows a margin in the case of the oxygen and no margin in the case of the carbonic acid.

The margin in the case of the rabbit was less than what Bohr had postulated in the case of man, but that might easily be so. The question, however, could not be left at this point because it would

have meant shirking a difficulty that would surely have asserted itself sooner or later.

To recall the statement made on p. 191

$$M = s\gamma\,(p - p')/760.$$

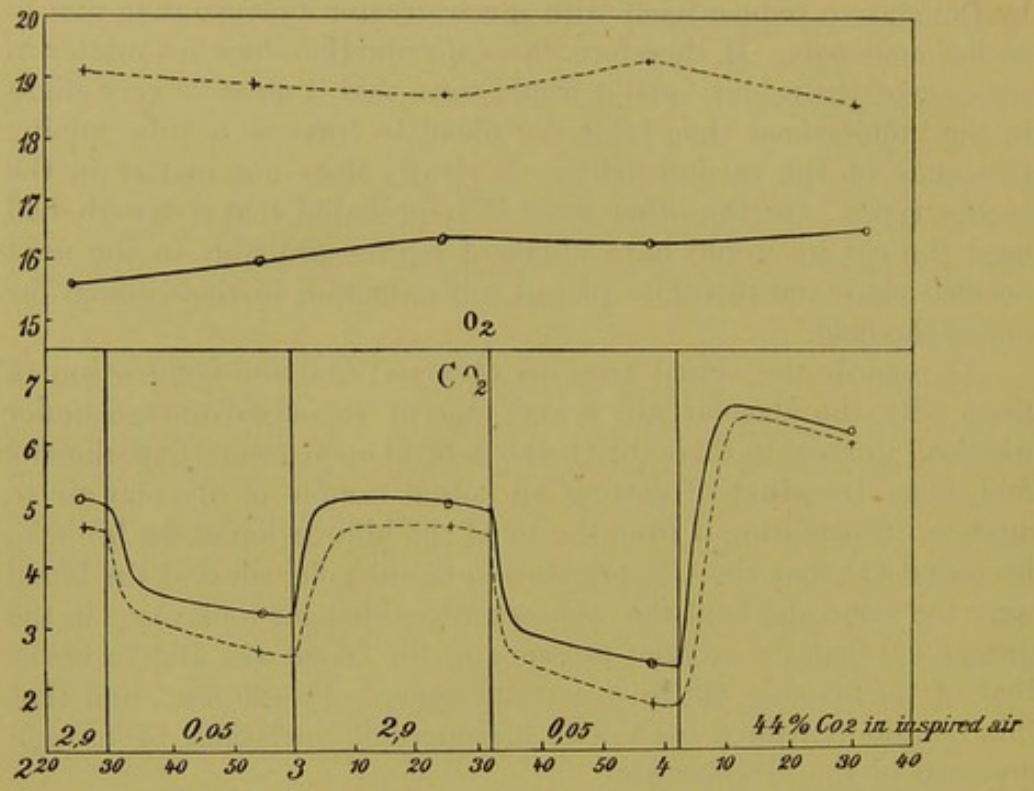


FIG. 98.—Oxygen and CO_2 pressures ..... in the air at the bifurcation of the bronchi, —— in the blood. Ordinate pressure of oxygen expressed in percentages of an atmosphere. Abscissa = time. (Krogh.)

The margin referred to above is of course $(p - p')$, its magnitude is not less than $\frac{M \times 760}{s\gamma}$ and it varies directly with the quantity of gas going through the epithelium. In the case to which I have referred M is taken in man as 400 c.c. of oxygen per minute. It is known, however, that 2000 c.c. of oxygen per minute is not an excessive amount in cases of hard exercise in which case $(p - p')$ would have to be increased 5-fold, that is, to at least 125 mm., in other words p' would disappear altogether, seeing that even with forced respiration 125 mm. probably exceeds the oxygen pressure in the alveolar air.

Therefore it became necessary to reinvestigate the value of the invasion coefficient.

The bubble proved an excellent medium for this purpose.

"The method adopted is easily understood by reference to the figure. About 250 c.c. of boiled distilled water contained in the vessel (1) were saturated at 37° C. and at atmospheric pressure with the gas in question which bubbled through (2), and left the vessel through (3). The saturation was in each case continued for at least 24 hours in order to drive out every trace of any other gas which might be present. The oxygen was prepared by electrolysis of water. When the saturation was completed mercury was allowed to flow into the vessel until the water was above the tap (4) which was then closed.

In the experiments a current of water was conducted past a bubble of gas identical with that in the microtonometer. When the gas pressure in the bubble is equal to the tension of the dissolved gas, the volume of the bubble must remain unaltered, but when a definite difference is set up by raising or lowering the mouth through which the water flows out, diffusion will take place through the surface of the bubble, and the volume will become altered. The mean surface being known and the alteration of the volume during one minute being measured, the invasion may be calculated by the formula given by Bohr

$$p - p' = \frac{M \,.\, 760}{\gamma s}.$$

...A uniform current of water was produced by the pressure from the raised mercury vessel (5) which was suspended by the spring (6) adjusted to maintain a nearly constant pressure in spite of the displacement of the mercury. The flow was regulated by means of the screw clip (7). When the ordinary clip (8) was opened water flowed on to the microtonometer (9) and set the air bubble (10) in revolving motion. The water flowed off through (11) and was collected and measured in a vessel (12) which could be raised or lowered. The water pressure was indicated by the level in the tube (13), placed on a scale graduated in mm.

Before each determination the bubble was measured in the graduated tube of the microtonometer. It was then carried down into the funnel, the clip (8) was opened, and the pressure-gauges (13) read off. After exactly one minute the clip was again closed, the air bubble drawn up into the tube and measured anew, and the volume

of water which passed through the tonometer read off on (12) and noted."

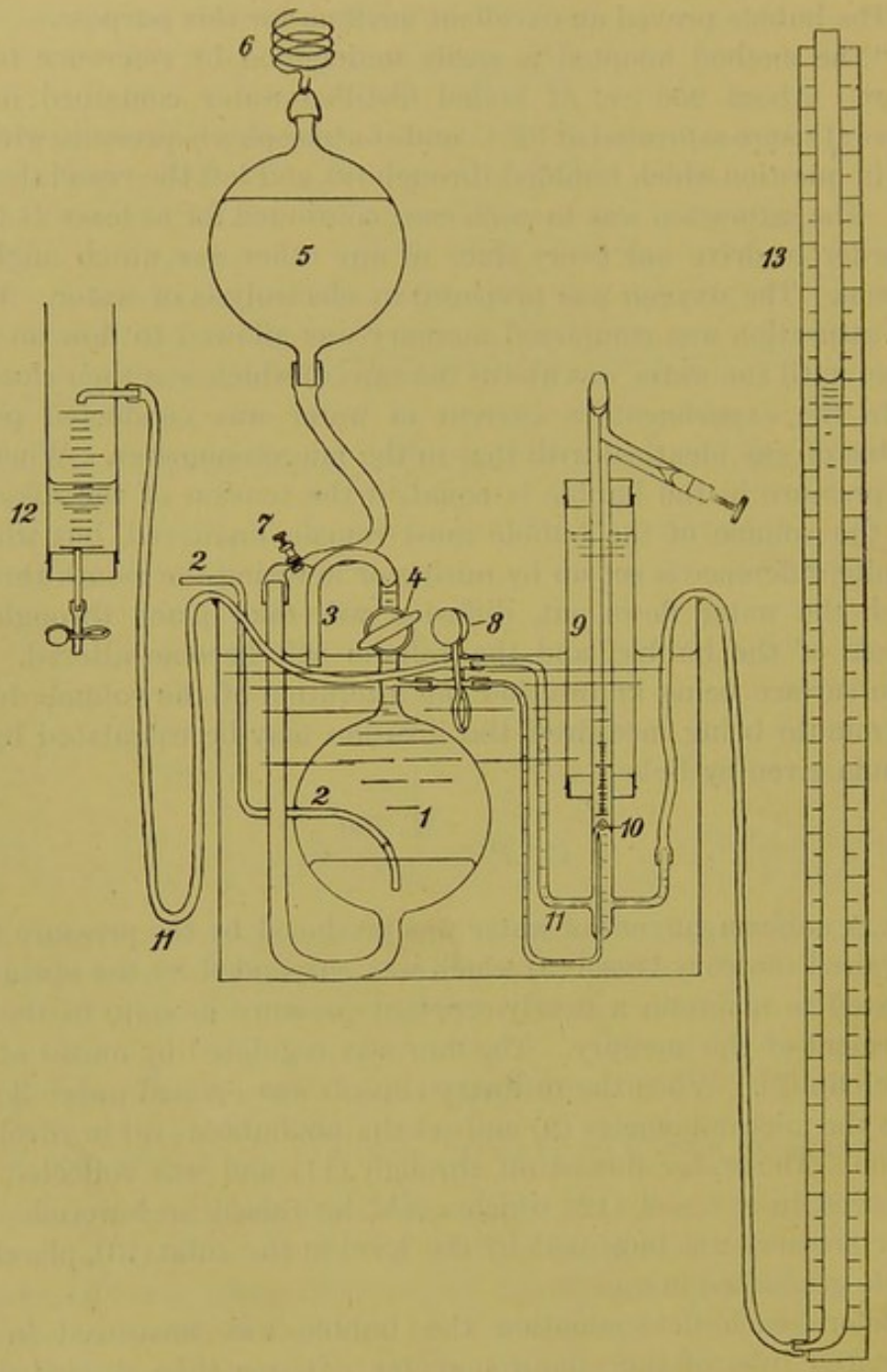


Fig. 99.—Apparatus for the measurement of the invasion coefficient. (Krogh.)

As the result of 38 experiments tabulated, Krogh concludes that the invasion coefficient for oxygen is 0·0776 ± 0·0024 as a minimum

value. This value is nearly seven times that given by Bohr. If therefore the calculation of $p - p'$ for the human lung was repeated with the new coefficient, it would seem to be not 25 mm. but about 3—4 mm. As a very rough calculation of the invasion coefficient shows that it demands a difference of pressure on the two sides of the lung surface of 1 mm. for every hundred c.c. absorbed by the lung per min., it may seem to the reader that I have gone at undue length into the invasion coefficient and that a matter which has been proved by Krogh to be so trifling does not demand the extensive discussion which I have given to it. This is so, perhaps, under the ordinary conditions of life, but let him remember that at high altitudes millimetres of oxygen pressure become precious. They are at best all too few. If they are whittled away in passing through the lung and if this process is magnified by the exact proportion of the amount of work which is expended in climbing, then the reader will agree with me that we do well to keep account of them one by one.

When the oxygen has entered the surface of the lung a further pressure gradient must exist in order to drive it through the cells of the epithelium. About this we have few reliable theoretical data.

In rabbits we have seen that the whole pressure head, as measured experimentally by Krogh, amounts to 15—20 mm. (Fig. 98). How far this reflects the condition of affairs in man we do not know. Rabbits like all small animals use up a proportionately large amount of oxygen; other things being equal, man would use up less, but we have no certain knowledge what the relative surfaces of the lung in man may be as compared with the rabbit.

If we imagine a man situated at an altitude of 15,000 feet, his alveolar oxygen pressure will be about 50 mm. of mercury. If now we suppose him to be at rest and deduct 10 mm. from this for the pressure head necessary to maintain the diffusion, the pressure of oxygen in his blood will be 40 mm., and if further we suppose that he does an increased amount of work, requiring twice the amount of oxygen, and turn this 10 mm. into twenty we leave only a possible 30 mm. as the oxygen pressure in the arterial blood. This seems an impossibly low figure. However, as we have said, these values which we have been using are speculative and 40 mm. oxygen pressure would place the organism in a very different position from 30 mm. But the calculation illustrates the desirability of at once doing experiments upon man himself and finding out under extreme circumstances which, though abnormal, can exist, what the oxygen

pressure in the blood really is—after all, our theory must extend to these facts. Beautiful, therefore, as Krogh's work is, its application to man is beset with many difficulties.

Whilst Krogh emerged complete master of the field which he had so successfully circumvented, his victory was to some extent a Pyrrhic one. For whilst he had been laying his plans the enemy had left the position which he was about to capture and they had retired to another, which they disclosed almost simultaneously with the news of Krogh's conquest. Their position as put forward by Haldane and Douglas was this: Oxygen secretion is a function of oxygen want. Under normal circumstances there is abundant oxygen and therefore there is no oxygen secretion—diffusion is good enough—but when the body demands more oxygen than it can readily obtain, whether by reason of diminished oxygen supply or increased oxygen demand, oxygen secretion in the lung sets in.

Krogh's investigations do not touch this new situation, yet it is one which cannot be neglected, if it is true that it affords one of the most important and most beautiful forms of physiological adaptation. That an essential consequence of exercise is an automatic means of obtaining a forced draught for the necessary combustion is a physiological conception which cannot be treated with anything but respect. Moreover the conception is an extremely reasonable one, for were there any possibility of secretion in the epithelium from which the lung is derived, the process of natural selection would tend to preserve it.

We must give some account of the reasons which led the Oxford school to abandon their former position in favour of their present one. The validity of the experiments of Haldane and Lorrain Smith depends upon the correctness of the following assumptions.

1. That the curve given in Fig. 97 which was determined with ox blood is correct for the blood of all the animals to which they subsequently applied it.
2. That this curve which was determined at the laboratory temperature, correctly represents the facts at the body temperature.
3. That the relative affinities of the blood of different species for oxygen and carbon monoxide are the same.
4. That the relation of oxygen and carbon monoxide to blood is unaffected by dilution.
5. That the same relation is unaffected by the presence of carbonic or other acids.

6. That all the haemoglobin is either oxyhaemoglobin or CO haemoglobin. They really measured the ratio of CO haemoglobin to total haemoglobin. In so far as there was reduced haemoglobin in the blood on which they worked, this would vitiate their result.

We must add one consideration, which, though dealt with by Haldane in another way, was not investigated quantitatively by him—the influence of light.

These points had either been assumed or had been incompletely investigated in the research of Haldane and Lorrain Smith to which I have alluded. Such assumptions if not warranted were at least not in serious conflict with the scientific knowledge of the time (1895). But in the light of the additions to knowledge recounted in the first five chapters of the present volume it will be seen that each of the six propositions was eminently likely to be erroneous.

Probably the fourth and fifth are approximately correct. The rest are certainly wrong in a greater or less degree. In the light of these facts the work of Haldane and Lorrain Smith was done over again by Haldane and Douglas[5] for the purpose of ascertaining whether these errors were serious or negligible.

The Oxford observers concluded that carbonic and non-volatile organic acids did not affect the partition of the haemoglobin between the oxygen and the carbon monoxide, but that the balance was affected to some extent by temperature and that there were individual differences between different persons and different species which could not be overlooked. But although these complications had arisen Haldane and Douglas were able to balance their errors in a way which had not been open to Haldane and Lorrain Smith, for actual samples of alveolar air had become available by the discovery of Haldane and Priestley. Therefore the blood of a person was exposed outside his body to his alveolar air at 37° C., and within it to alveolar air, modified by the pulmonary epithelium, each containing the same concentration of carbon monoxide, and the partition of the haemoglobin between the oxygen and the CO was compared in the two cases. The titrations were both carried out in the same light. If the proportion of CO haemoglobin in the blood from the body exceeded that in the blood exposed to the alveolar air, the pressure of CO being the same in each case, then it would follow that the blood was exposed to a less pressure of oxygen in the lung than in the alveolar air: and if the proportion of CO haemoglobin in the blood from the body was less than in the blood exposed *extra vitam*

to the alveolar air then the blood must have been exposed to a greater pressure of oxygen in the lung than in the alveolar air. The results of some of their experiments are given in the table which faces this page.

In discussing the work of the Oxford School I have intentionally withheld all reference to their method of estimating the relative quantities of oxygen and carbon monoxide associated with the haemoglobin. Clearly the whole value of the results which they obtained depends upon the accuracy of their estimation and no critical account or investigation of the subject can omit to take cognisance of it.

The essence of their method is as follows. Some blood is taken from the object of the research before the CO is administered: this is diluted and set on one side: let us call it A. The carbon monoxide is administered: some more blood B is taken from the patient. B is a different colour from A since it contains CO haemoglobin, but by adding carmine to A the colours of the two may be matched. The amount of carmine solution added is noted down, call this x. B is now saturated with CO, its colour again changes, but again A may be made to match it by the addition of more carmine to the extent of y c.c. From the relation of x to y (which is not a simple linear one), the percentage saturation of B with CO may be calculated.

Apart from the statement made above, that the real estimation is that of the ratio of CO haemoglobin to total haemoglobin, whilst it professes to be an estimation of CO haemoglobin to oxyhaemoglobin, i.e. it assumes that there is no reduced haemoglobin in the blood—an assumption which is probably justifiable when there is no oxygen want and but little CO haemoglobin—the possible sources of error in the above method fall into two general categories which have to do with (1) the possibility in practice of matching the tints, (2) the theoretical soundness of the method. Haldane and Douglas obtained results which closely agree for any particular sample of blood, though they have told me that the quantities of carmine they use in the operation differ. The method is claimed to be accurate to 2 °/₀. This statement must be treated with great consideration. I have no sympathy whatever with persons who attack a method of this kind in a light-hearted way, and having failed to get consistent results after a few titrations or even a few days spent in the endeavour, condemn the method. The method is essentially a subjective one. The accuracy which can be obtained by subjective methods with long and incessant

Experiments on arterial oxygen pressure of men.

Date	Subject	Inspired air. Gases % 5′ after start O_2	5′ after start CO_2	15′ after start O_2	15′ after start CO_2	Just before alveolar sample taken O_2	Just before alveolar sample taken CO_2	Into saturator at finish O_2	Into saturator at finish CO_2	Alveolar air gases % O_2	Alveolar air gases % CO_2	% saturation of haemoglobin with CO In vivo	% saturation of haemoglobin with CO In vitro	O_2 pressure of arterial blood in % of existing atmosphere: not corrected for moisture	O_2 pressure of arterial blood in % of O_2 pressure of alveolar air	
14/12/10	C.G.D.	19·54	0·05	19·75	0·00	—	—	20·55	0·08	[13·6]	[5·6]	23·1	17·2	14·2	[104·4]	Normal oxygen atmosphere. Rest.
16/12/10	,,	22·50	0·04	—	—	—	—	22·07	0·15	15·38	5·63	23·0	16·0	14·1	91·6	
17/12/10	J.S.H.	19·57	0·02	19·72	0·02	—	—	19·75	0·06	12·46	5·51	23·5	16·6	12·8	102·7	
20/12/10	,,	21·70	0·07	21·47	0·07	—	—	21·34	0·16	14·13	5·51	23·2	15·8	13·2	93·4	
21/12/10	,,	25·85	—	25·38	—	—	—	22·39	0·11	14·62	5·63	22·2	17·1	16·2	110·8	
23/ 5/11	C.G.D.	21·78	0·02	21·13	0·10	22·64	0·13	23·19	0·20	[15·7]	[5·6]	23·85	16·35	14·45	[92·0]	
29/ 5/11	,,	19·79	0·29	19·80	0·33	20·22	0·32	20·17	0·35	14·06	5·64	23·7	17·6	13·9	98·9	
														Mean	99·1	
22/12/10	C.G.D.	28·00	—	28·58	0·03	—	—	27·88	0·11	21·65	5·47	23·2	19·0	21·7	100·2	High oxygen atmosphere. Rest.
24/ 5/11	J.S.H.	32·73	0·20	31·86	0·13	31·20	0·16	30·19	0·16	23·46	6·01	23·5	18·5	23·1	98·5	
11/ 1/11	C.G.D.	12·27	0·02	12·56	0·02	—	—	11·79	0·18	5·98	4·93	21·7	16·0	6·9	115·4	Low oxygen atmosphere. Rest.
13/ 1/11	,,	12·82	0·02	13·61	0·03	—	—	13·34	0·11	6·99	4·78	23·0	17·7	8·5	121·6	
16/ 1/11	,,	11·47	0·04	11·27	0·04	10·71	0·12	10·26	0·18	6·40	3·48	22·9	21·6	8·2	128·1	
23/ 1/11	J.S.H.	12·03	0·00	12·00	0·01	11·19	0·04	11·74	0·12	5·53	4·74	23·1	16·4	6·2	112·1	
10/ 2/11	J.S.H.	21·10	0·21	21·00	0·44	18·46	0·57	18·42	0·58[1]	12·82	5·39	23·3	20·9	16·0	124·8	Moderate work with one arm.
11/ 2/11	,,	21·19	0·24	—	—	20·56	0·84	21·43	0·80[2]	15·11	5·33	22·7	21·6	19·9	131·7	Severe work with one arm.
27/ 2/11	C.G.D.	21·88	0·11	21·84	0·11	20·66	1·30	20·53	1·11	15·49	5·69	23·4	24·0	20·9	135·0	,, ,, ,,
26/ 4/11	J.S.H.	17·95	0·33	17·94	0·38	17·68	0·36	18·05	0·36	11·64	5·22	21·1	18·1	14·9	128·0	,, ,, ,,
27/ 4/11	C.G.D.	22·32	0·96	22·88	0·90	23·49	1·07	24·12	0·82	19·38	4·43	24·25	24·75	24·8	128·0	,, ,, ,,
8/ 2/11	C.G.D.	12·50	0·02	—	—	12·87	0·38	14·72	0·24[3]	10·20	3·30	23·5	23·9	15·1	147·6	Moderate work with one arm. Low oxygen.

Normal oxygen.

[1] CO_2 added to saturator to 7·02 %. [2] CO_2 added to saturator to 4·70 %. [3] CO_2 added to saturator to 4·64 %.

The figures in brackets are calculated values.

practice is very great. A farmer by mere inspection will tell you the weight of a bullock with an accuracy which probably exceeds that claimed by Haldane and Douglas for the carmine method of titration.

I have never myself made a carmine titration, and had I made a few I have little doubt that I should have failed to obtain concordant results: but such a failure would have formed no just criticism of the work of those accomplished in the method and would therefore have been a mere waste of my own time.

Anyone who reads the recent work of Haldane and Douglas must be struck by the way in which the method has served them in the determination of the physical relation of oxygen and carbon monoxide to shed blood. Take for instance the curves given in Fig. 35—and these are a sample of many determined since—they are mathematically fair curves with equations, and not only so, but there is independent reason to believe that they are not only fair curves, but that the properties of haemoglobin are such that they could not be of any other shapes than the ones shown. Such curves do not result from unsound experiments.

In spite of the last consideration enough has been said to form a sufficient apology—if apology were necessary—for Hartridge to take the ground that an independent* method of determining the CO haemoglobin present in blood was a desirable preliminary to an independent examination of Haldane's results.

Apology is not necessary however. It is of the essence of sound experiment that the experimental methods should be varied in every possible way. This, at all events, is the school in which I have been brought up. It is the school in which my foretime lecturer, now my friend and colleague Mr C. T. Heycock, F.R.S., untiringly pointed the moral from the work of Stas on the atomic weight of silver.

I should never concede on the one hand that a blood gas worker in the Cambridge laboratory owed an apology to his predecessors for

* Hartridge commenced in 1909 with the idea of revising the method as used by Haldane and Lorrain Smith. Between the summer of that year and the spring of the following, my results on salts, temperature, &c. were published. They materially altered the problem for him and also caused the work to be taken up again by Haldane and Douglas who published their work to some extent as they went. Apart from this I believe neither they nor Hartridge were cognisant of the work of the other until a short time before their final publication. The findings of the two sets of observers, which agree very closely in so far as the physical properties of haemoglobin are concerned, have therefore the merit of being independent.

devising a fresh method of experiment, or on the other hand that his doing so was of the nature of a challenge.

Hartridge's method is based on the fact that the substitution of CO for O_2 in haemoglobin is associated with a movement of the bands towards the violet end of the spectrum.

Hartridge claims that in mixtures of the two the degree of movement of the band is a measure of the relative amount of CO haemoglobin present. Hartridge's spectroscope has two slits; when you look into it you see two spectra, one immediately above the other—but these spectra present this difference, that the blue end of the uppermost one is to the right of the field, the blue end of the

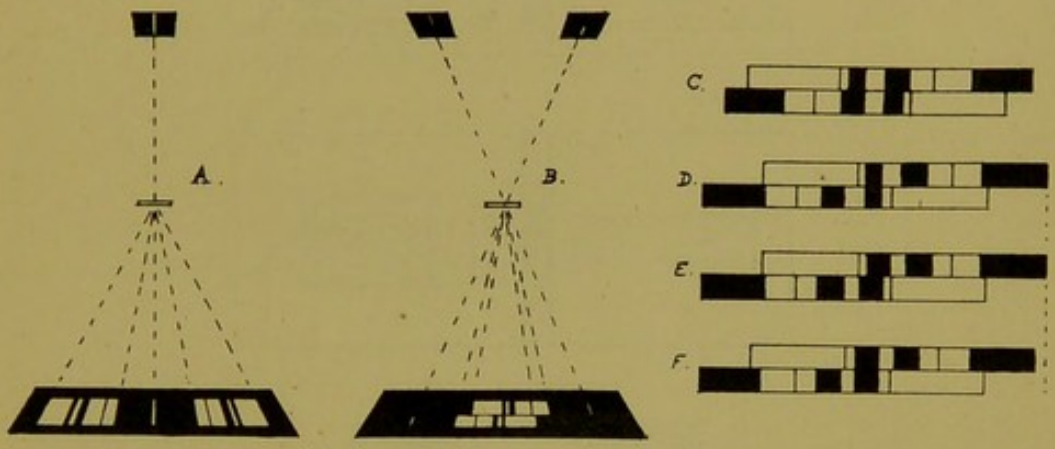


Fig. 100.—The method of formation and adjustment of the reversed spectra. *A* shows the ordinary arrangement with single slit. *B* shows the arrangement with double slit. *C*, the bands of O_2Hb as they would appear in the spectroscope. *D*, the α bands have been adjusted into line. *E*, the oxygen has been replaced by CO, and the α bands have shifted away from one another. *F*, the lower spectrum has been displaced by the micrometer, so that the α bands are again in line.

lower one to the left. One of the spectra is "reversed." Suppose *C* in the figure to be the two spectra. Let us fix our attention on the sharper and narrower of the two haemoglobin bands—the one nearer the red end. By a movement of a lever attached to the instrument the upper spectrum may be shifted in the field of vision till the two α bands are exactly one above the other (*D*). If now the oxyhaemoglobin be replaced by a CO haemoglobin the α bands will no longer appear to be one above the other, but as in *E*. The final operation is to make them coincide once more as in *F*. This is accomplished as I have said by moving a lever on the side of the apparatus—the arc through which it is moved is read off on the

scale, and is the measure of the degree of shifting of the band, which in its turn is a measure of the percentage of CO haemoglobin present.

I do not propose to enter here into a description of the manipulative details, such as screening fluids for blocking out parts of the spectrum, not germane to the inquiry. For these I must refer the reader to the original papers of the author, nor do I propose to discuss the abstract foundation of the method from the point of theoretical optics.

I will confine myself to some criticisms of its practical sufficiency as a piece of laboratory technique.

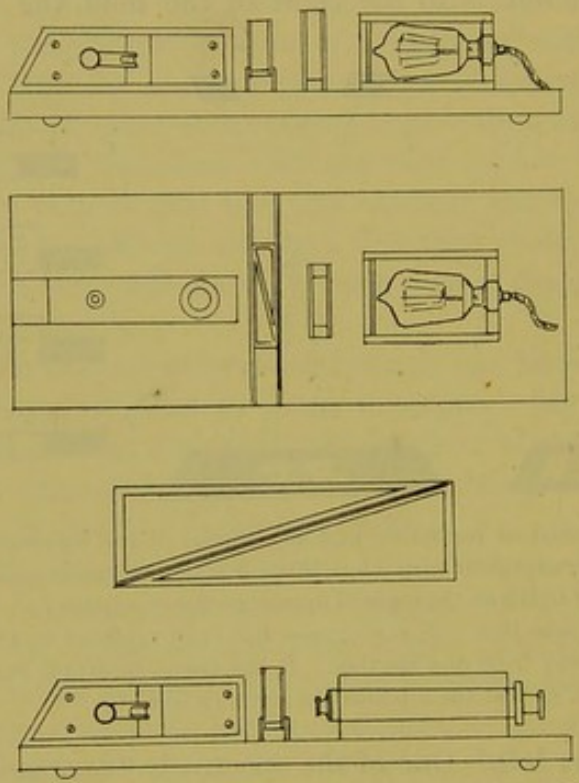


Fig. 101.—The apparatus as employed for the calibration of Hartridge's spectroscope in plan and in elevation.

Like the carmine method it is a subjective method, it depends upon the matching of two things as seen by the eye. Other things being equal it is an easier proposition to put two dark lines into exact juxtaposition than to match two tints to within 2 °/₀. Much depends upon the total range of difference in each case and the errors involved in the two operations are not easy to compare.

Hartridge has been at great pains to define the subjective element. He has spared no labour to satisfy himself that the method gives

readings which are consistent with those given by the blood gas pump. This comparison proved very illuminating. It was of course necessary to calibrate the instrument in some way; there was no antecedent probability that the movement of the band would be a direct linear function of the percentage of CO-haemoglobin present.

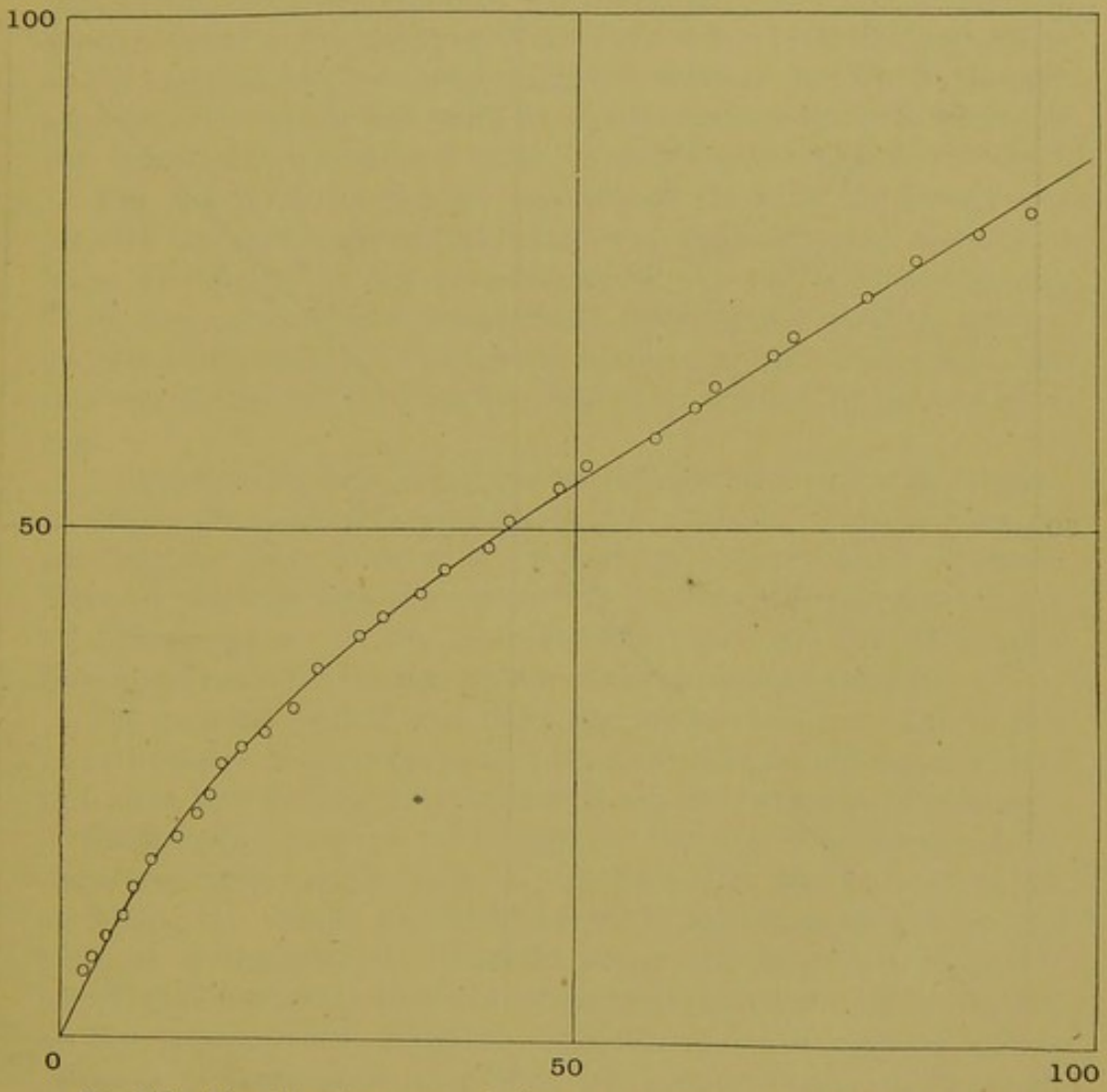


Fig. 102.—The calibration curve. Ordinate = percentage of haemoglobin which is CO haemoglobin. Abscissa = micrometer readings of instrument.

Hartridge began by calibrating the instrument with mixtures of CO- and oxyhaemoglobin in known amounts or amounts at least presumed to be known—i.e. it was presumed that the amounts bore the same relation in the mixture that they did in the aliquot parts. So long as Hartridge continued to use this method his comparisons with the

pump always broke down, especially so at low percentages of CO-haemoglobin. He therefore discarded it in favour of another method of calibration. Oxyhaemoglobin and carboxyhaemoglobin of equal concentrations were placed in wedge-shaped troughs in apposition (Fig. 101): by turning a screw these could be shifted perpendicularly

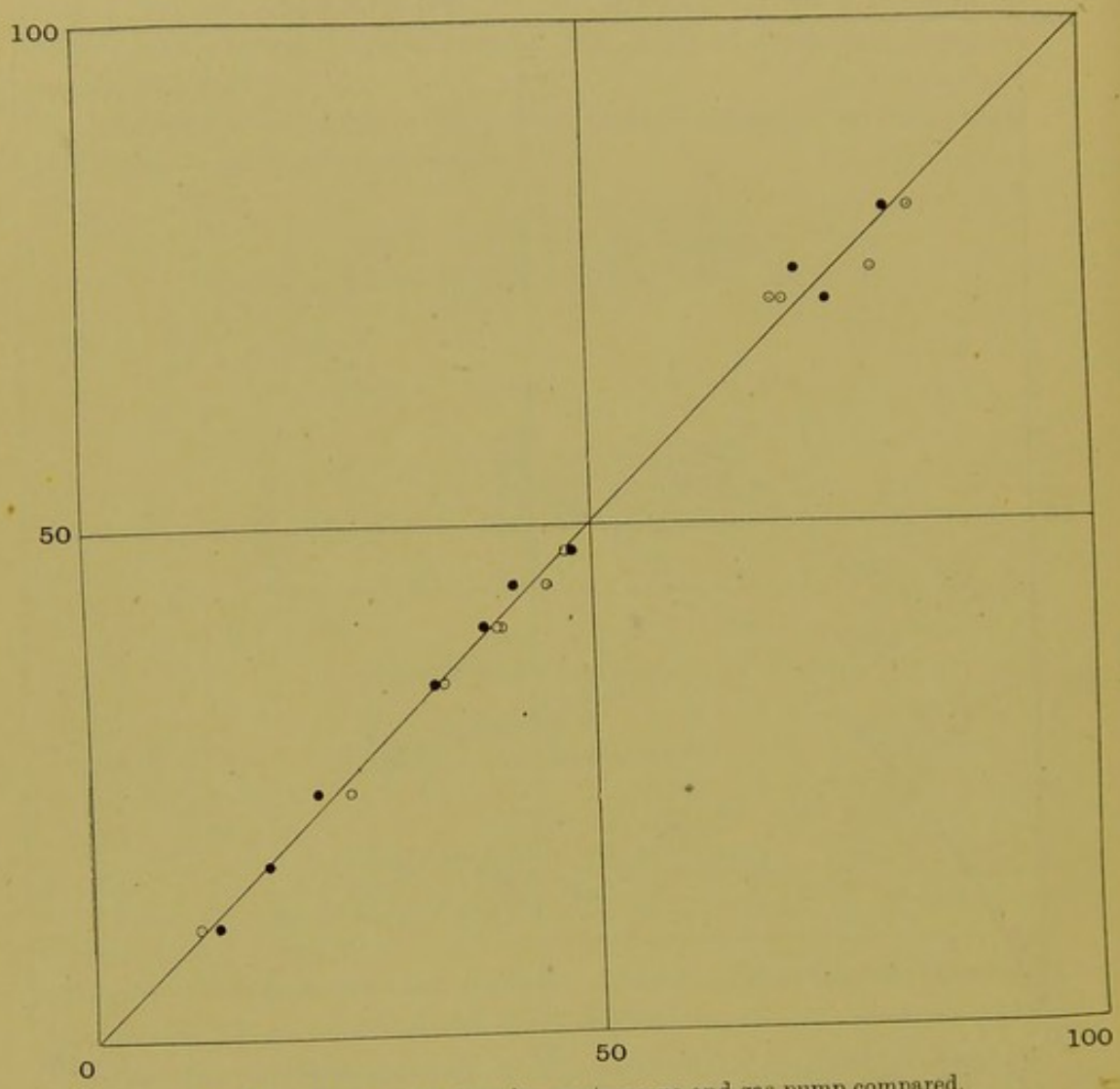


Fig. 103.—The results given by spectroscope and gas pump compared.
Actual °/₀ CO vertical. Observed readings °/₀ CO horizontal. Pump o. Spectroscope •.

to the beam of light so that the light was always shining through oxy- and carboxyhaemoglobin in known proportions to one another before reaching the grating of the spectroscope. After the adoption of this method complete harmony with the pump was obtained by Hartridge and the subjective element to this extent removed. In the case of

the pump the oxygen and CO were pumped from blood boiled in vacuo in the presence of an alkaline solution of potassium ferricyanide. The CO in the gas obtained was estimated by combustion with a glowing platinum wire.

The accompanying figures (102 and 103) will give the reader an idea of the accuracy obtained by Hartridge. The first shows the relation between the linear movement of the band and the percentage of CO-haemoglobin, the latter being the ordinate and the former the abscissa. The important point at the moment is the coincidence of the individual observations with the line representing their mean. In Fig. 103 is shown the correspondence (1) with the pump, and (2) with the spectrometer (calibrated with the wedges) of samples of blood containing known proportions of O_2- and CO-haemoglobin. There are no published comparisons between the carmine method and the pump and so far as I know none have ever been made. In this way Hartridge checked his subjective method by an objective one.

By another method Hartridge has endeavoured, and with success, to define the subjective element in his method. Two persons inspecting the same solution of O_2- and CO-haemoglobin will obtain different readings with the instrument, i.e. they will place the index at different places on the scale because their eyes see differently. This of course is the demonstration of the subjective element.

The carmine method and the spectroscopic one are alike in this respect: there is no presumption that any two people see the same tint when they look at a mixture of blood and carmine. In the case of Hartridge's[5] method the subjective element is demonstrable in units, and tests can therefore be instituted for the purpose of ascertaining the degree to which the subjective element vitiates the accuracy of the method. Winfield, whose eye differed considerably from that of Hartridge, carried out control observations. He calibrated the instrument which Hartridge had in use. The calibration was made as in Hartridge's case with the wedge-troughs of oxy- and CO-haemoglobin and gave the curve which is shown in Fig. 104. This curve was roughly speaking parallel to Hartridge's curve. They then independently made observations upon various samples of blood containing O_2- and CO-haemoglobin in unknown proportions, and each interpreting his readings by his own curve arrived at identical results.

Lastly the method is susceptible of treatment in the way in which

subjective methods should be treated—statistically. Each observation takes but a few seconds and does not affect the composition of the fluid observed. With a given sample of haemoglobin it is but the work of a few minutes—perhaps as long as would be required for a single carmine titration—to take 50 observations from which an average in the strict meaning of the term could be determined.

In comparing the two methods there is one other point on which I must touch. A few pages back I expressed admiration of the

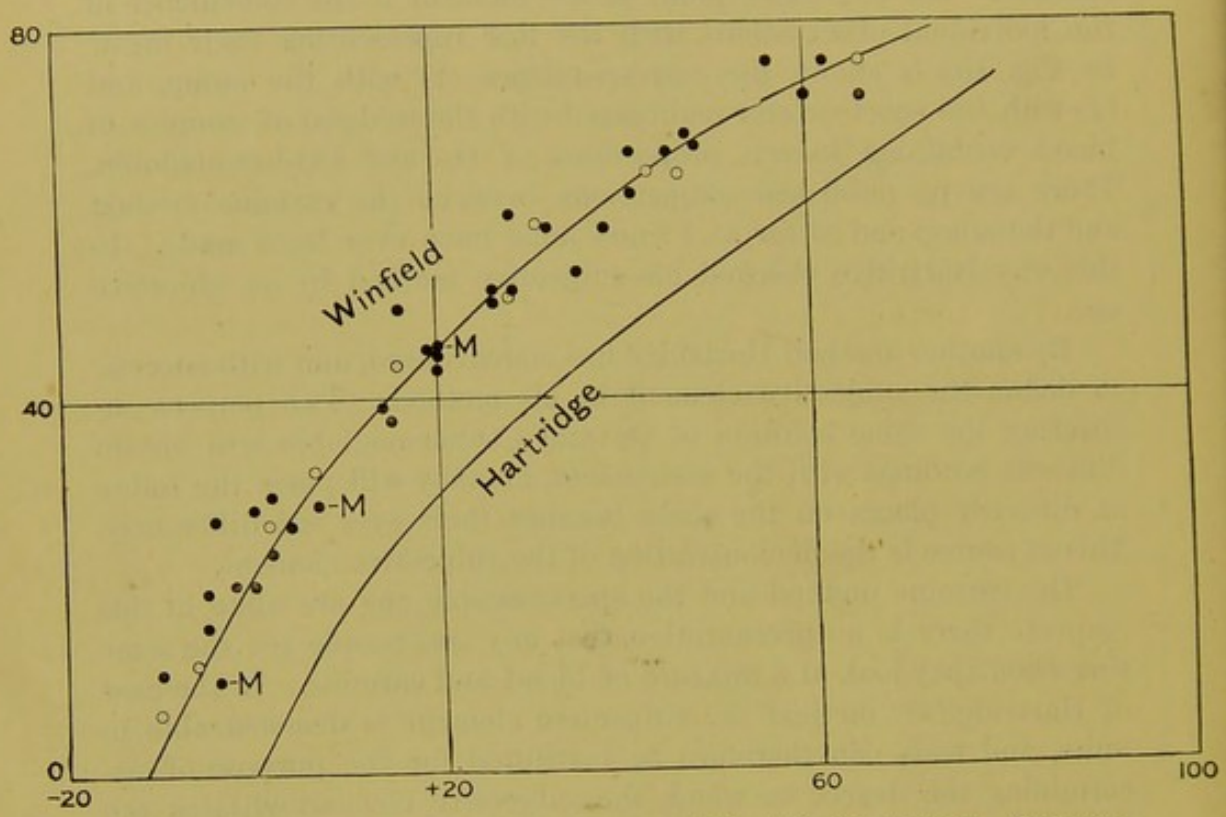


Fig. 104.—Curves showing Winfield's calibration of Hartridge's instrument. Ordinate = percentage saturation of haemoglobin with CO. Abscissa = micrometer readings. • Observations from which curve was drawn. *M* = mean of 4 or 5 observations. o Observations made at a later date to test whether the eye changed from time to time.

carmine method, in that it appeared to yield reliable data concerning the physical laws governing the reactions of haemoglobin; my admiration of the spectroscopic method—and my early bent filled me with nothing but suspicion of spectroscopic methods in general—is not more qualified. It is difficult to suppose the method, were it an essentially bad one, could yield the figures which Hartridge put

before the reader about the effect of temperature on the CO-O_2-haemoglobin equilibrium.

Hartridge[6], like Haldane and Douglas, reinvestigated the whole field of accessory conditions which influence the reaction between haemoglobin and carbon monoxide—the effects* of CO_2, acids, temperature, &c., on the equilibrium. One observation has reference to the effect of temperature on mice†. I give it in his own words: "There are however, oddly enough, experiments on mice in the same paper quoted, which do agree, the experiments being as follows: the mice were placed in the cold so that their rectal temperatures fell in certain cases below 19°, and it was found that then the CO saturation reached was higher than that obtained at normal temperature; that is to say the same effect as similar experiments performed *in vitro*. The mere effect of temperature may therefore explain them." Perhaps the most striking data which he obtained were those of the influence of light on the reaction.

This had not been overlooked by Haldane, nevertheless the following figures show the need of special precautions for excluding the effect of light in a very striking way.

Percentage saturation of a haemoglobin solution with CO under various circumstances of illumination.

Dark 96 °/₀	Sunlight 40 °/₀	Dark 91 °/₀
Sunlight 42 °/₀	Electric light 88 °/₀	Daylight 80 °/₀
Sunlight 35 °/₀	Dark 92 °/₀	6″ Magnesium 64 °/₀

Of these the most interesting is, I think, the daylight. When one considers that the method of carmine titration deals with solutions of only one-tenth of the concentration of that used by Hartridge and for that reason much more accessible throughout its whole mass to the active (ultra violet) rays, one will see that there is at least the possibility of grave errors entering into the carmine method unless special precautions are taken which are defined by quantitative estimations, and if it is necessary to define such a source of error in units in the notoriously gloomy atmosphere of these islands, it is much more necessary to do so in the intensely actinic atmospheric conditions which obtain at higher altitudes and lower latitudes than our own.

* The same results were arrived at independently by Haldane, Haldane and Douglas, *Journal of Physiol.* XLIV, p. 303, 1912.

† These experiments are quoted on p. 187 and tabulated on p. 188 of this volume.

The actual results which Hartridge [7] obtained may now be given. In the first place he agrees with the now universally accepted opinion that under normal circumstances the oxygen pressure in the alveolar air is very close to that in the blood.

The following figures show the percentage saturations with CO (A) of the blood of the body and (B) of blood of the same person previously withdrawn, but exposed at body temperature to the alveolar air, a sample of which was taken from the individual.

Percentage saturation with CO in blood exposed to alveolar air.

Series 1. *Rest.*

		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	Mean
A	In vivo	12	18	21	29	20	36	35	25	43	44	—
B	In vitro.....	10	18·5	22·5	26·5	15	33·5	33·5	24	42·5	40	—
	A/B	1·2	·97	·93	1·09	1·3	1·07	1·05	1·04	1·01	1·1	1·08

These figures show that, if anything, there is a trifling excess of CO-haemoglobin *in vivo*, i.e. a slightly lower oxygen pressure. In two of these experiments Hartridge's blood became 40 °/₀ saturated with CO; up to this point therefore there is no sign of secretion.

More interesting than these results, however, are those which he obtained whilst establishing a greater or less degree of oxygen want.

The first method he adopted for this purpose was to breathe mixtures of oxygen, CO and nitrogen which were much poorer in oxygen than is atmospheric air. The following were the results:

Percentage saturation with CO in blood exposed to alveolar air.

Series 2. *Diminished oxygen supply.*

		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	Mean
A	In vivo	31	30	32	30	29	27	27	29	30	—
B	In vitro	30	22	25	28·5	28	18·4	23·5	28·5	26·5	—
	A/B	1·03	1·4	1·3	1·06	1·04	1·45	1·14	1·02	1·14	1·17
	Oxygen tension in alveolar air mm. ...	55	42	36	49	40					

If a comparison be instituted between these oxygen tensions and those which were actually observed by us at the top of Monte Rosa, at an altitude of 15,000 ft., it will be seen that for the most part they correspond to a considerably higher altitude.

In a third series of experiments Hartridge, whilst breathing from the respiration apparatus, performed a considerable amount of actual physical exercise though not so much as causes dyspnoea to pass into distress; the following were the figures which Hartridge obtained:

Percentage saturation with CO in blood exposed to alveolar air.

Series 3. *Work.*

		(1)	(2)	(3)	(4)	(5)	(6)	Mean
A	In vivo	30	24	24	26	27	25	—
B	In vitro	28	23	20	24	23	25	—
	A/B	1·06	1·04	1·2	1·08	1·24	1·00	1·10

If then we regard the work of Hartridge as that in which the possible errors have been most clearly defined quantitatively, and accept it as being the most mature expression of opinion at the time of our writing, our verdict must be that there is no evidence of oxygen secretion in man as represented by a single individual—Hartridge—either (*a*) at rest, or (*b*) under such restricted conditions of oxygen want as may be induced by diminished oxygen pressure for short periods of time, or (*c*) during moderate exercise of 4 minutes duration.

For what it is worth my own view on the question of oxygen secretion at the present time is this: It is agreed on all hands that there is no oxygen secretion in the resting individual. The issue then may be stated thus. Is the lung capable of oxygen secretion? It is alleged to be so during oxygen want, i.e. during exercise, or at high altitudes or with insufficient functional haemoglobin (as during partial CO-poisoning). The real point to my mind is that of exercise, for it is from its advantage during exercise that such a function in the lung would naturally have been evolved and maintained. Exercise then is the *prima facie* test case—here the experiments of Hartridge and of the Oxford School disagree. Hartridge finds a pressure of oxygen in the blood which is below that in the alveolar air, Haldane and Douglas find the reverse. Yet their methods are similar, the differences consisting in (1) the method of estimation of the CO-haemoglobin in the blood and (2) the nature and duration of the exercise. The issue refines itself largely into the question of which of these methods is the more correct. Reading the accounts of the two methods, I never fail to be greatly influenced by the following facts: (1) Hartridge has tested his so exhaustively against a purely objective one—that of the pump. (2) He found, when testing his apparatus against the pump, the method of actual mixtures unreliable and had to replace it by that of optical mixtures. (3) The carmine method has never been tested in any other way than by that of actual mixtures. (4) Of the rival workers Hartridge has tabulated his errors the more exhaustively.

Therefore, of the two methods I have the greater reliance on the spectroscopic one. So I am forced at present to think that during the exercise taken by Hartridge, with or without partial CO-poisoning, there was no secretion. But perhaps I may take the reader into my confidence so far as to say that I wish it were otherwise.

But while it seems to me fairly certain that there was no secretion in Hartridge's case, there is one possible line along which Hartridge's results might perhaps be brought into harmony with those of Haldane and Douglas. It has been shown that the call for oxygen *follows* upon the activity. The exercise taken by Hartridge was of a violent but spasmodic type, that of Haldane and Douglas was less violent and more sustained. It is not an impossible supposition that the secretion might show some lag after the exercise, and therefore be most evident in the case of sustained work.

Lastly, apart from questions of the detail of individual experiments there are certain general questions that cannot be overlooked by the holder of the diffusion theory. Before this theory can be regarded as proved on the positive side it must be tested under the circumstances which are likely to strain it most. Two questions it must answer. The first is, can it account for the passage of 3500 c.c. of oxygen per minute through the pulmonary epithelium? Perhaps it will be able to do so one day, at present it cannot. At the end of the last chapter I spoke of the diffusion coefficient, here I will only say that the more recent determinations of the diffusion coefficient must be materially changed before they will admit of 3500 c.c. of oxygen traversing the lung along a pressure gradient dropping from 100 mm. of mercury in the lung to 50 mm. in the blood. But if the test of exercise is exacting that of exercise at high altitudes is more exacting. I have granted a 50 mm. gradient and incidentally assumed that the blood in the pulmonary artery is 80 °/₀ saturated with oxygen. But what if the whole alveolar pressure is but 30 mm. (approximately that which must have obtained in the case of the Duke of Abruzzi[8] and his party at 24,000 feet altitude). The work of recent observers on the dissociation curve shows that if the blood were in equilibrium with such a gas it would only be 50 °/₀ saturated* with oxygen. What then are we to allow for a gradient?

* The fact that the dissociation curve of man at rest scarcely changes at high altitudes is treated in Chapter XVII. During activity it becomes meionectic, and that this renders the position even more difficult on the diffusion theory is shown in Chapter XVIII. When Roberts and I arrived at the Capanna Margherita our bloods would have been about 30 °/₀ saturated at 25 mm. O_2 pressure.

If 5 mm. be allowed, the arterial blood would be saturated to the extent of about 40 %. Now if 50 mm. pressure head in the lung will not suffice for the passage of 3500 c.c. of oxygen per minute, 5 mm. would not suffice for the passage of 350 and it is clear that each of the party must have been using three or four times this amount. If the arterial blood during work at high altitudes contained less oxygen than the mean value for normal venous blood at the sea-level, what would the venous blood contain and what possibility would there be of obtaining a pressure gradient sufficient to transfer the oxygen from the blood to the tissues? At present the diffusion theory fails to answer these questions though it may do so one day.

On the secretory theory these considerations are explained, for the members of the Pikes Peak expedition [8] found evidence of oxygen secretion living at an altitude of 14,000 feet. They consider that the secretion increased as their residence continued. Further experiments are necessary in two directions. Hartridge should extend his series to sustained work, both at low and at high altitudes, and should he find no greater pressure gradient than he has already done, fresh determinations of the diffusion coefficient are required in order to prove that the actual quantities of oxygen used can pass by diffusion through the pulmonary epithelium when driven by a difference of pressure of only a few millimetres between the oxygen in the alveolar air and that in the arterial blood.

REFERENCES

(1) Krogh, *Skand. Arch. f. Physiol.* XXIII, pp. 179, 193, 200, 217, 224, 236, 248, 1910.
(2) Douglas, *Journal of Physiol.* XXXIX, p. 453, 1910.
(3) Douglas and Haldane, *Skand. Arch. f. Physiol.* XXV, p. 169, 1910.
(4) Hartridge, *Journal of Physiol.* XLIV, p. 1, 1912.
(5) Hartridge, *Royal Soc. Proc.* Ser. B, Vol. LXXXVI, p. 128.
(6) Hartridge, *Journal of Physiol.* XLIV, p. 22, 1912.
(7) Hartridge, *Ibid.* XLV, p. 170, 1912.
(8) See Douglas, Haldane, Henderson and Schneider, *Phil. Trans.* Ser. B, CCIII, p. 310.

PART III

THE DISSOCIATION CURVE CONSIDERED AS AN "INDICATOR" OF THE "REACTION" OF THE BLOOD

CHAPTER XIV

THE DISSOCIATION CURVE IN MAN

We are now in a position to discuss the dissociation curve in man from what I may call the practical rather than the theoretical side. Firstly, let us consider the constancy of its position in the same person at different times. Secondly, the degree of coincidence in the curves obtained from different persons. Here let me say at the outset that I am only dealing with the individual in his normal condition and not under special conditions such as violent exercise.

The first question which confronts the worker is whether he is to study the dissociation curve in the presence or absence of carbonic acid. Three possible courses are open :

(1) To study the dissociation curve in the absence of CO_2.

(2) To study it at a standard CO_2 pressure.

(3) To study it at the CO_2 pressure of the alveolar air of the person.

Each of these courses has something to be said for it, but it is clear that the first two give slightly different information from the third. The third tells of the blood as it actually circulates in the arteries and is the most interesting and most valuable measurement of the three: it tells most about the condition of the individual. But the first and second methods have a value of their own if you wish to inquire whether the blood of all persons is the same; it is best to study this question under the simplest conditions possible, hence I shall start without the complication of the alveolar air. Nor need the whole curve be studied, let me rather take some convenient pressure of

oxygen, say 17 mm. of mercury, and ask whether the blood of every individual in the absence of carbonic acid is equally saturated with oxygen when exposed at 37° C. to 17 mm. oxygen pressure. Fig. 105 shows that my own blood at this pressure is 75·5 °/₀ saturated with oxygen. This figure was arrived at as the result of some determinations made by Dr Boothby of Boston, U.S.A., and myself. These data will give an idea of the standard of accuracy of the methods employed.

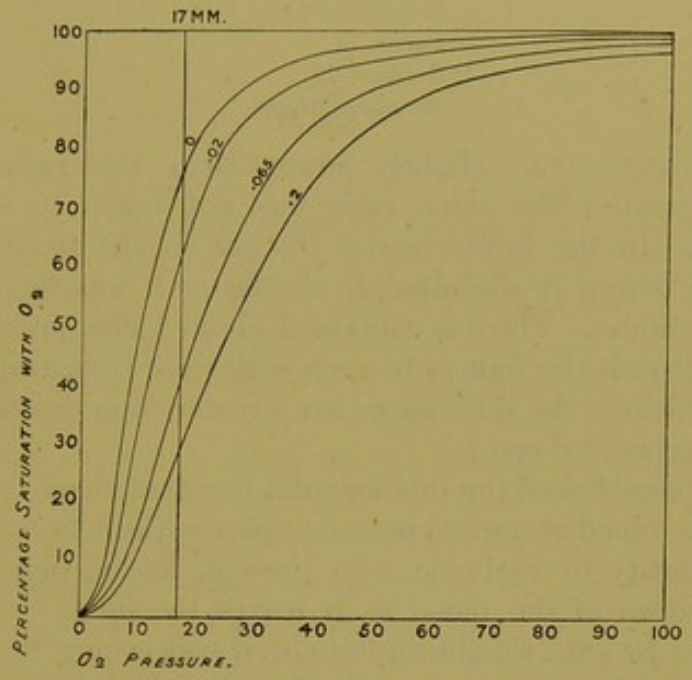


FIG. 105.—Dissociation curve of Barcroft's blood with the addition of 0, ·02, ·065 and ·2 °/₀ lactic acid. The last is only an approximation. Temperature 37° C.

Percentage saturation of blood with oxygen at 17 *mm. O_2 pressure and* 37° *C.*

Barcroft's blood ... 78 77 73 74 75 76 difference between extremes 4 °/₀
Boothby's blood ... 79 77 76 75 77 ,, ,, ,, 5 °/₀
Mean values ... Barcroft's blood 75·5 °/₀
,, Boothby's blood 76·8 °/₀

Corresponding figures of a number of workers are:

Boothby 76·8 °/₀	Hill 74 °/₀
Barcroft 75·5	Miss Chick 73
Winfield 78	Miss Pillmann 76
Burn 76	Miss Martin 79
Higgins 74·3	Dr Klein 68

Were it not for the single case of Dr Klein it might almost be supposed that the whole of the cases given above were sufficiently

close to fall within the six per cent. limit, and the deduction might be made that there was no difference between the blood of healthy persons.

Yet another series of figures obtained by Mathison, at Pisa, on the members of our Monte Rosa party, seems to indicate that the blood of different persons differs.

Ryffel 77 %
Roberts 75·5
Camis 74
Barcroft 72
Mathison 63, 66

These figures run slightly lower than the previous series, probably because the same care was not taken to exclude CO_2 completely. In the former series the gas in the tonometers stood over alkali before it was used, in the latter it was used as it came from the cylinder. The gas contained about 1 mm. pressure of CO_2. The point is that the figures in each series are comparable with one another. Clearly the differences are greater than can be accounted for by experimental error.

Having established the fundamental fact that there is a difference between the blood of normal persons, apart entirely from any question of the quantity of carbonic acid present, let us turn to the dissociation curve of the blood as it leaves the lung.

In order to gain reliable information concerning the blood of a given individual it is necessary that his dissociation curve should be studied over a considerable period of time, running if possible into years. There are now several persons for whom this has been done, Douglas, Roberts, Ryffel, Higgins and myself. The question arises with regard to each of these—Can any difference be made out as between one time and another? This point seems to me to be of such great importance that I propose to set the evidence on the subject in some detail before the reader.

Firstly then as regards Douglas whose normal alveolar carbonic acid pressure is found to be 40—41 mm. of mercury. His dissociation curve is given in Fig. 106. Particular interest attaches to it not only because it has been determined more often probably than that of any other person, but because it has been determined independently by two sets of observers, working by slightly different methods. The points determined by me were each done on 0·1 c.c. of blood in the small differential apparatus, those determined by Haldane

and Douglas were each determined on 1 c.c. of blood in the old Barcroft-Haldane apparatus. Moreover in some of their determinations the Oxford observers used sodium carbonate and in others

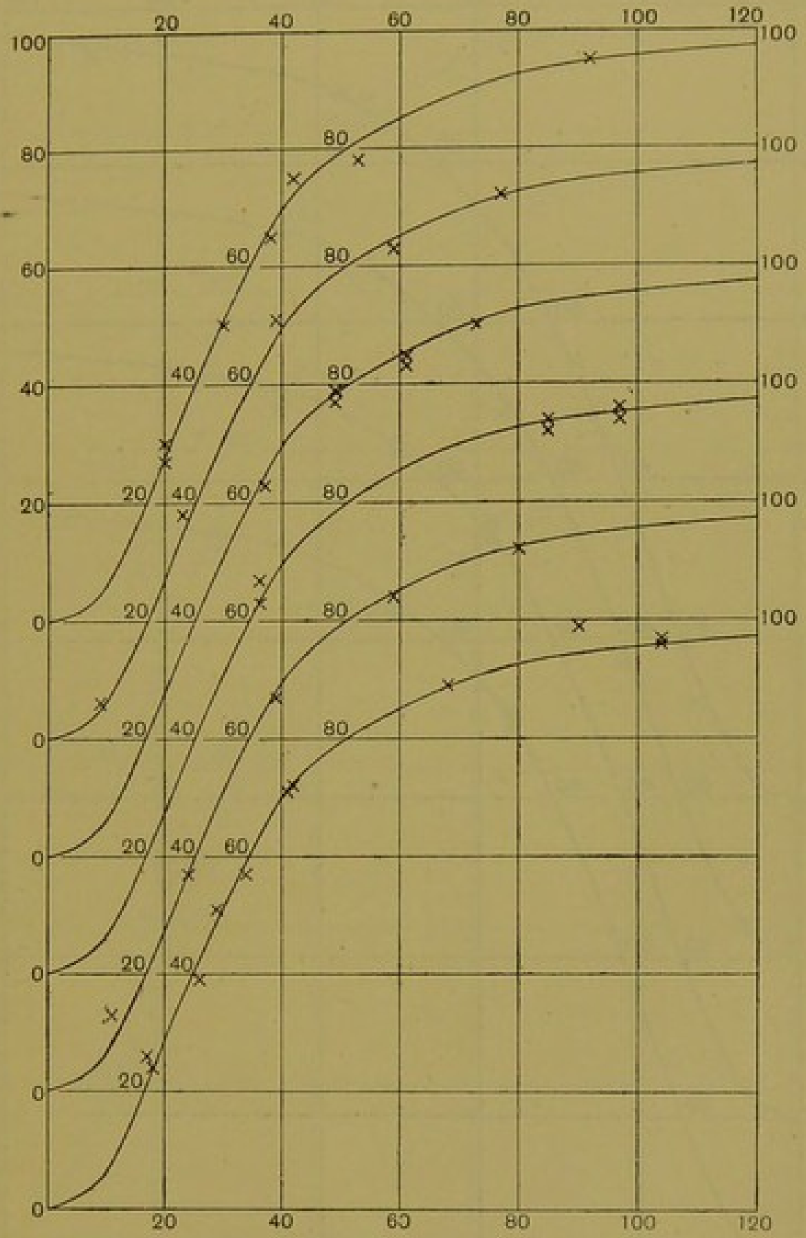


FIG. 106.—Dissociation curve of Douglas' blood as determined on various occasions, extending over about two years. The curve is the same in each case.

ammonia for the purpose of laking their blood. An examination of the figure will show that no difference can be observed in the curve between one time and another. In the figure the curve is the same

in each case. It is repeated six times corresponding to six occasions on which the determinations were made. In all it represents about forty different determinations no one of which is seriously off the line.

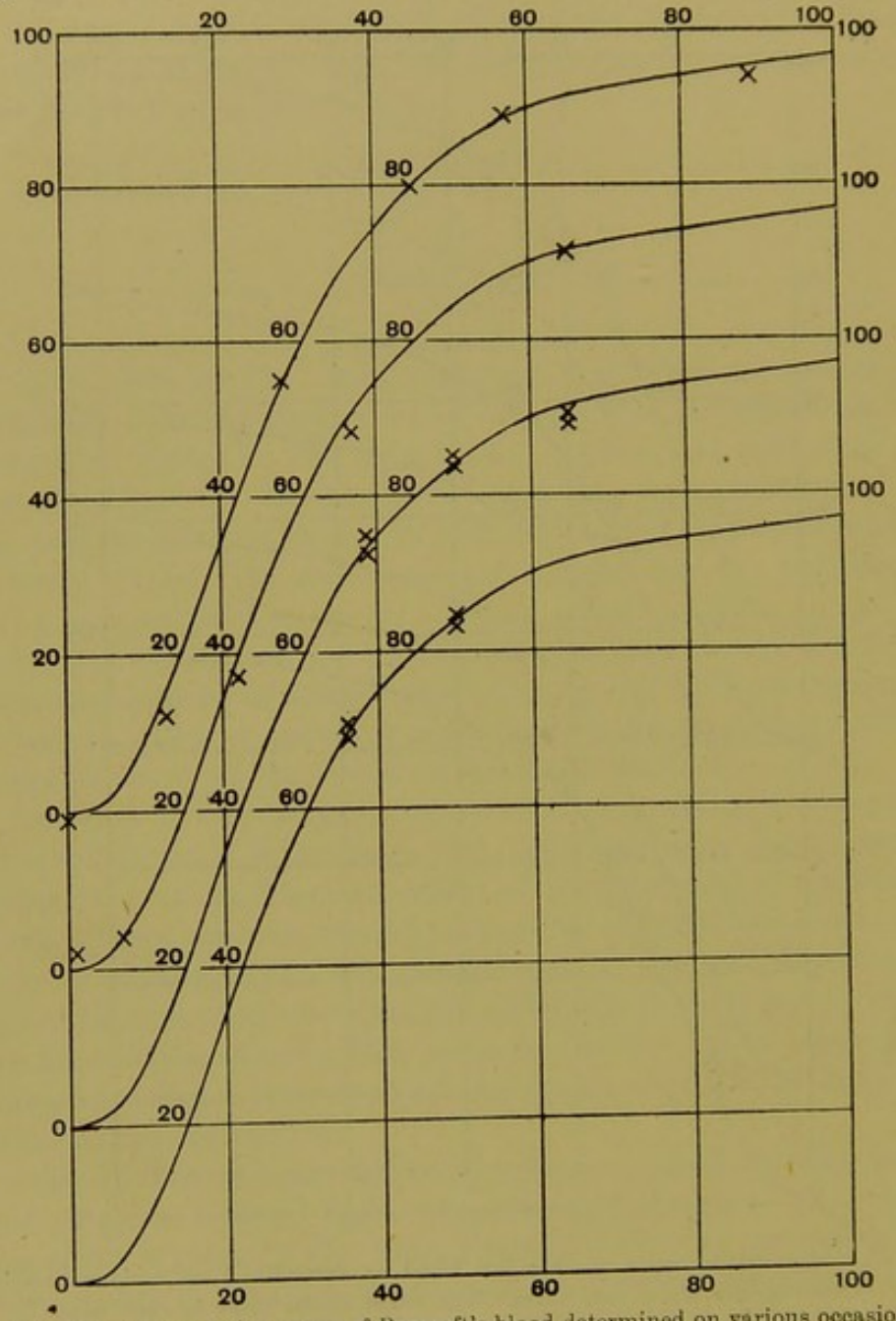


Fig. 107.—Dissociation curve of Barcroft's blood determined on various occasions. The curve is the same in each case.

My own curve has been investigated from time to time—over a period extending from the summer of 1909 to that of 1912. The

following points have been recorded at different times and in different places. In this case also the points have not all been determined by

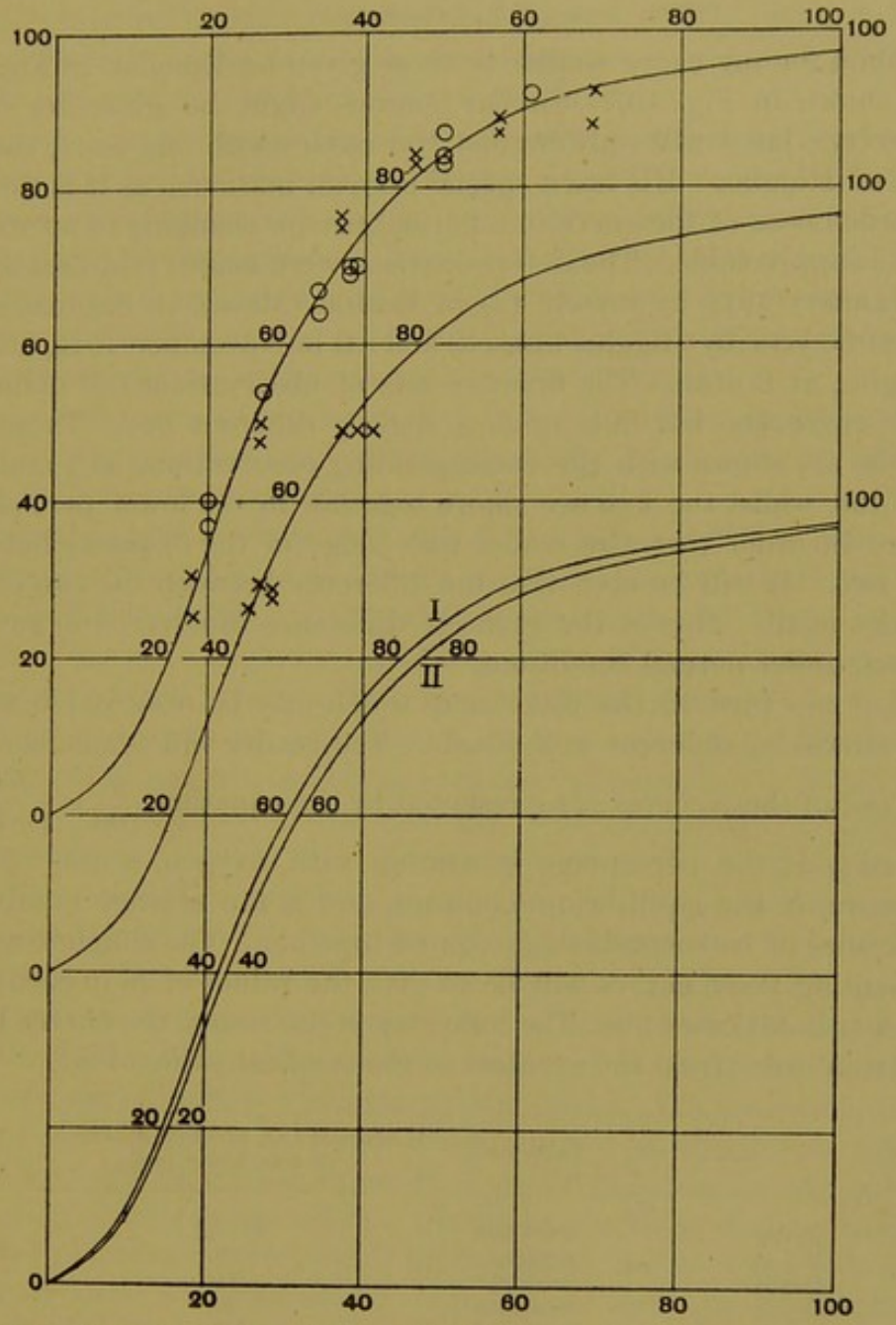


Fig. 108.—Dissociation curve of Higgins' blood, top curve in Jan. 1911 and Sept. 1911, second curve Nov. 1912. I and II show the two upper curves side by side.

the same observer, most were determined by me, but some by Higgins. The places and dates of their determinations were as follows:

Cambridge, Feb. 1910, CO_2 40 mm.
Teneriffe, March 1910, CO_2 40 mm.
Cambridge, May 1910, CO_2 40 mm.
Pisa, August 1911, CO_2 40 mm.

Data for my curve similar to those given for Douglas' in Fig. 106 are shown in Fig. 107. Similar curves might be given for other observers, but I will only burden the reader with one more, namely that of Higgins. His has a special interest inasmuch as it is the one recorded case of the curve in a normal person changing to an extent that is appreciable. The determinations were made (1) in Cambridge in January 1911 by myself, (2) in Boston (Mass.), in September of the same year by Higgins himself, and (3) in November 1912, also by Higgins, at Boston. The first two sets of observations fall upon the same curve, the last falls upon a slightly different one. These two curves are shown with the corresponding observations as 1 and 2 in Fig. 108, whilst the two are shown together in the lower part of the figure in order that the reader may judge of the disparity between the two. It will be seen that the difference between the two curves is very small. This is the greatest difference discovered in any one person under normal conditions.

Let me turn to the differences which may be observed between the curves of different individuals. The reader will remember that any one of these curves is represented by the equation $\frac{y}{100} = \frac{Kx^n}{1+Kx^n}$, where y is the percentage saturation with oxygen, x the oxygen pressure, K the equilibrium constant, and n the average number of molecules of haemoglobin aggregated together. The simplest way of presenting these curves will be to give the values of K in each case, n being in all cases 2·5. The following is the result, the curves being taken in order from the greatest to the smallest value of K.

Name	Value of K	Alveolar CO_2 pressure at which K was determined
Ryffel	0·000363	44—45
Roberts	0·00033	39
Graham	0·000317	41
Camis	0·000315	40
Higgins (1).....	0·000301	38
Barcroft	0·000292	40
Higgins (2).....	0·000264	38
Douglas	0·000212	41
Haldane		40
Mathison		39

These curves all lie within the black area marked in Fig. 109, the upper boundary of this area being Ryffel's curve and the lower boundary that of Douglas, Haldane and Mathison. This area, therefore, represents the limits within which the curves of human beings may be regarded as normal at the present time.

Having determined the limits between which any single individual may be regarded as possessing a normal dissociation curve and the degree of variability which may be regarded as normal in any single person, we may now proceed to discuss some special cases of variation when the body is subjected to abnormal conditions. Before doing so, however, it is necessary to explain the meaning of certain words used to denote the changes which we are about to describe.

If the curve of a particular person moves from its normal position it may move in either one of two directions, i.e. it may be either above or below the normal curve of that person. In other words, at a given oxygen pressure the blood may take up either more or less oxygen than it is wont to do. To express these facts Mr Harrison, Fellow of Trinity College, Cambridge, at the instance of Dr Fletcher, suggested the following nomenclature; when the dissociation curve is above its normal situation, i.e. when at a given pressure of oxygen the blood takes up an abnormally great percentage of its total possible load of oxygen, the curve is called "pleonectic*." When on the other hand it takes up less than the usual percentage of oxygen it is "meionectic"; when it becomes saturated to the normal extent under any specified conditions it is "mesectic." If the curve becomes meionectic the value of K will diminish, if pleonectic it will increase, as compared with the normal or mesectic value. There is one point which must be made perfectly clear to the reader; normal blood in any individual must yield a mesectic curve—but blood which yields a mesectic curve need not necessarily be normal. Let me give a case in point. In Chapter V it was shown that if carbonic acid were withdrawn from blood, the value of K would increase, i.e. the curve would rise; if this happened in a person and nothing else, he would acquire a pleonectic curve, but if at the same time that the CO_2 was withdrawn lactic acid was added to the blood the value of K would diminish, and the curve would tend to return to its former position, and if enough lactic acid were added the curve would become as it was to start with. Again, if this process took place in a human being

* πλεονεκτικός, disposed to take more than one's share. From πλεονεξία, a disposition to take more than one's share. (Liddell and Scott.)

his curve would at the end be mesectic, because the value for K would be what it normally was, but the blood would be abnormal because it would contain more lactic acid and less carbonic acid than usual. The position of the curve, therefore, whether pleonectic,

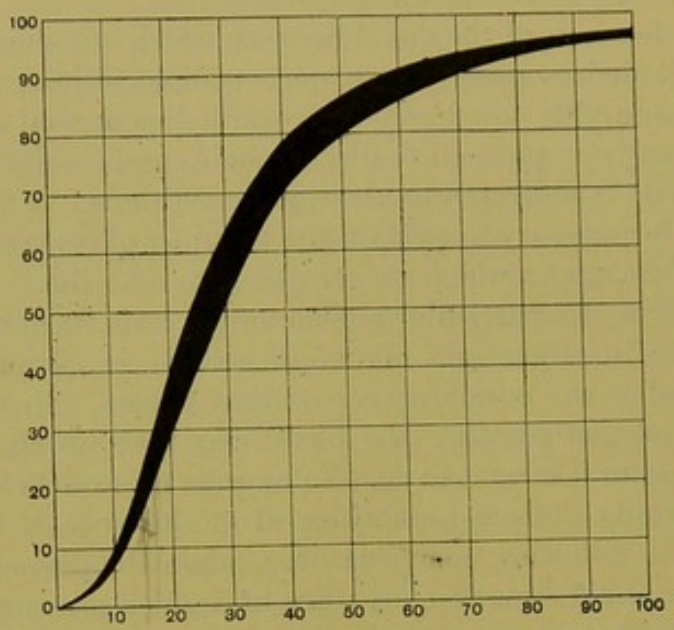


Fig. 109.—Limits within which the dissociation curves of normal persons fall.

mesectic, or meionectic, depends not upon the actual quantity of any one electrolyte in the blood, but upon the balance which is maintained between them.

CHAPTER XV

THE EFFECT OF DIET ON THE DISSOCIATION CURVE OF BLOOD

In company with my colleagues I have made efforts, in several directions, to move the dissociation curve in individuals away from its mesectic position. Naturally such attempts would take the line of subjecting the patients to some form of treatment which is known to alter the acid radicles in the blood either in quantity or in kind. The first of these attempts which I will chronicle was made by Higgins and myself. It is perhaps the simplest, and as it illustrates a good many of the principles involved in the more complicated researches which follow, I will describe it in some detail. My colleague on this occasion was a specialist not only in gas analysis, but in the physiology of dietaries, coming as he did on a visit to Cambridge from the Nutrition Laboratory at Boston, U.S.A., presided over by Professor Benedict. Moreover, since Higgins visited this country he has continued the work at Boston. The *motif* of the research was as follows: all carbohydrate was strictly eliminated from the diet, and this of course led to an acidosis the most obvious evidence of which was the appearance of β-oxybutyric acid, diacetic acid, &c. in the urine. Here let me explain my use of the word acidosis in reference to the blood. In the following pages it will signify the appearance of acids (exclusive of CO_2), abnormal in kind or perhaps only in quantity in the blood or even a decrease in the bases present. By acidosis then I mean an increase of acid relative to basic radicles in the blood, CO_2 not being considered. But by the term acidosis I will signify nothing concerning the final "reaction" of the blood which is largely regulated by the amount of CO_2 present. The question for decision was, Would the dissociation curve depart from its mesectic position owing to these bodies being thrust into the blood by the tissues, i.e. owing to the acidosis? And here we are at once face to face with the distinction which we drew at the close of

the last chapter, between mesexy and normality. It is easy to ascertain that the blood is abnormal, for it is known that such diet causes a lowering of the carbonic acid pressure in the alveolar air which must correspond to a reduced pressure of CO_2 in the blood, but it is also known that organic acids appear in the urine which have come from the blood. To what extent is the loss of carbonic acid in the blood balanced by the gain in other acids so far as the total reaction of the blood is concerned?

Table of diets, &c.

Date	Time	Diet	Table of abnormal constituents in urine for 24 hours, collected about 8 a.m. on the following morning
Sept. 5	12.30	Beef stew and vegetables 2 slices bread and butter No supper	NH_3 1·296 g. NH_3/N_2 °/₀ 6 Acetone 1·348 g.
Sept. 6	8.15	4 eggs 1 cup beef tea	Oxybutyric acid 4·0845 g.*
	12.30	1 tin sardines 6 ozs. cheese	
	7.30	2 pork chops	
Sept. 7	10.00	2 pieces pepsin gum 3 ozs. butter 4 eggs	Vol. 1120 c.c. N_2 18·084 g. NH_3 2·015 g.
	3.30	3 ozs. butter 4 soft boiled eggs	NH_3/N_2 °/₀ 11·2 Acetone 1·725 g.
	7.30	1 piece fried ham and a little butter	β-oxybutyric acid 1·577 g.*
Sept. 8	8.00	A few sips of coffee 1 piece pepsin gum Lemon juice	Vol. 1300 c.c. N_2 18·933 g. NH_3 3·350 g.
	10.30	2 lamb chops	NH_3/N_2 °/₀ 17·6
	3.30	1 box sardines	Acetone 2·791 g.
	8.00	Beef steak	β-oxybutyric acid 8·025 g.
Sept. 9	No food taken till 12.00 when ordinary diet was resumed		

* Later work shows that these are minimal values.

Here is an account of a complete experiment, with all the data of urine, respiration, &c. set forth (pp. 228 and 230), and from it we see that the alveolar carbonic acid pressures fell from 37 to 29·5 mm. of mercury, the latter value being that observed on the fourth day of the diet. On this day blood was taken for analysis and compared with that found on the day before the special diet was commenced. The following curve is Higgins' (Fig. 110 A) mesectic curve. The points marked by

circles are those determined on the fourth day of the diet with an alveolar pressure of 29·5 mm. So far as can be seen from inspection of Fig. 110 A the curve remains mesectic in spite of the changed

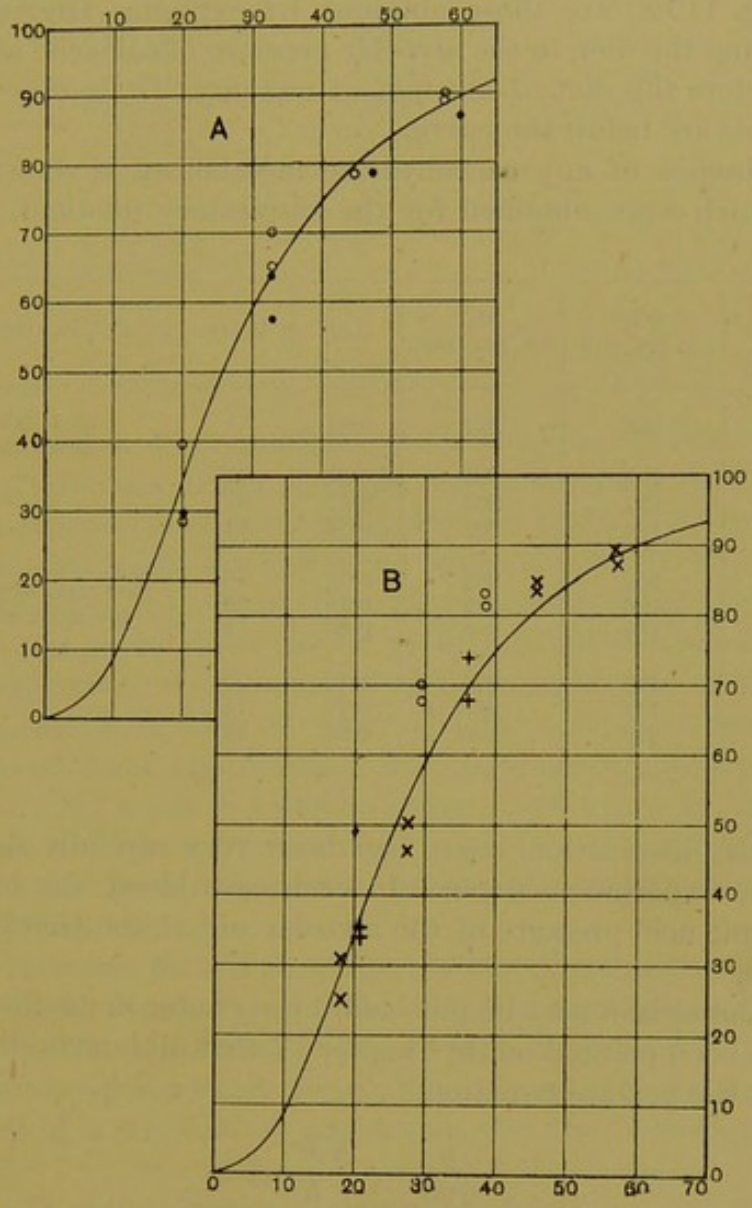


Fig. 110.—Dissociation curves of Higgins' normal blood (normal alveolar CO_2 pressure). *A*, exp. Sept. 8, 1911, o = points determined at existing alveolar CO_2 pressure when on diet; • = the same blood exposed to his usual CO_2 pressure. *B*, exp. Jan. 1911, o = points determined during diet at existing CO_2 pressure; × = points determined previous to, and + subsequent to, diet at normal CO_2 pressure.

condition of the blood caused by the decline in the alveolar pressure of carbonic acid. The most obvious question which will occur to the

mind of the reader is—Were the experimental methods at our command equal to the task of discerning any shifting of the curve, should it exist? The answer to this question is given in the figure. The dark spots (Fig. 110 A) are those obtained by exposing Higgins' blood, taken during the diet, to the alveolar pressure of carbonic acid which existed before the diet 37—38 mm. of mercury. It is clear that all these points are below the curve.

The absence of any carbohydrate metabolism is shown by the figures which were obtained for the respiratory quotient.

	CO_2 c.c. per min.	O_2 c.c. per min.	R.Q.	Pulse	CO_2 in alveolar air
Normal	198	237	0·84	—	36·5, 37 mm.
Sept. 7					
8.50 a.m.	206	305	0·68	85	—
9.14	197	292	0·67	83	—
9.40	198	301	0·66	84	—
Sept. 8					
9.25 a.m.	209	281	0·75	76	29·5 mm. when blood was taken for analysis
9.51	196	279	0·70	75	
10.15	189	280	0·68	74	
Sept. 9					
9.53 a.m.	198	276	0·72	70	—
10.16	191	268	0·71	74	—
Sept. 11	210	243	0·86	69	38 mm.

Higgins' dissociation curve had been very carefully determined before the experiment began. In each case blood was exposed to the carbonic acid pressure of the alveolar air at the time the blood was taken.

The comparison may be put before the reader in another way.

It has been pointed out in Chapter IV that all human dissociation curves fit the general equation

$$\frac{y}{100} = \frac{Kx^n}{1 + Kx^n},$$

in which n may be taken as 2·5. The only variable then is K. If the values of K be calculated for the observed points (1) of the normal blood exposed to normal alveolar CO_2 pressure of 39 mm., (2) blood drawn on the fourth day of the carbohydrate-free diet exposed to the alveolar CO_2 pressure of the person at that time, (3) the blood drawn on the fourth day of the carbohydrate-free diet

exposed to 39 mm. CO_2 pressure, the following values for K are obtained:

	(1)	(2)	(3)
	·000319	·000373	·000227
	·000361	·000216	·000280
	·000307	·000307	·000201
	·000299	·000352	·000236
	·000311	·000261	·000221
	·000242	·000350	
	·000257	·000305	
	·000258		
	·000400		
	·000283		
	·000273		
	·000281		
Mean	·000299	·000309	·000233

Of the twelve determinations given in column (1) five are above the mean value, six below it, while one corresponds with it, all the values in column (3) however are below the mean in column (1), and all but one are below any individual figure in column (1). Since the blood referred to in columns (1) and (3) was analysed at the same carbonic acid pressure there can be no doubt that the blood changed. But a comparison of columns (1) and (2) shows that the values of K as calculated in the two sets of figures are not really to be distinguished from one another, five determinations in column (1) being above the mean of column (2) and seven below it. Indeed the mean value of K given in columns (1) and (2) would not yield curves distinguishable from one another in diagrams of the scale and style of those in this volume.

The conclusion then is that Higgins' blood in this experiment was mesectic on the fourth day, but that whilst mesectic it was abnormal inasmuch as it contained less carbonic acid and an equivalent excess of other acids—presumably those which appeared in the urine.

A point of some subtlety arises here—let the following points be accepted:

(1) That the alveolar carbonic acid falls.

(2) That the dissociation curve remains constant in position.

(3) That the respiratory centre is regulated chemically by the same factors as affect the dissociation curve, of which the most important is probably the hydrogen ion concentration of the blood.

The immediate deduction from considerations (2) and (3) would be that the respiratory centre worked at the same rate on the days

of the diet as on normal days since the curve remained mesectic. How then was the carbonic acid got rid of? If the same quantity of CO_2 was produced on the days of diet as on the days of rest, and if also the CO_2 in the alveolar air was less than normal, it would appear that the CO_2 produced was diluted with a greater quantity of atmospheric air than usual, which would be another way of saying that the ventilation was increased, a deduction which seems to be inconsistent with our original statement, for it would involve stimulation of the respiratory centre and meionexy. The other possibility would be that the actual CO_2 production went down.

In a later experiment which yielded similar data as regards the dissociation curve, the rate of respiration and the total ventilation were measured. There was no very obvious increase on the 8th and 9th of November, the days of diet on which the dissociation curve data were obtained, as compared with the 4th of November.

	Date Nov.	CO_2 per min. c.c.	O_2 per min. c.c.	R.Q.	Average rate of respiration	Ventilation per min. litres
Normal	4	229	254	·90	15·0	6·31
	5	204	242	·84	14·1	5·61
	6	191	244	·78	13·4	?
Diet ...	7	210	302	·70	16·2	6·93
	8	198	250	·79	14·2	6·33
	9	190	272	·70	14·9	6·44
	10	180	264	·68	14·9	6·28

At the end of the period of diet there is an evident tendency for the total CO_2 output to fall. The point is not one of great importance in the present connection, but with a greater degree of acidosis it is clear that dyspnoea, such as takes place in diabetes, must supervene—a definite dyspnoea prompted by the effects of oxybutyric acid &c. on the respiratory centre. Here, however, I wish merely to indicate to the reader the nature of the problems in which he is likely to involve himself if he uses the dissociation curve as an "indicator."

The last point which must be considered is the degree of acidosis which took place. Here let me repeat that I mean by this phrase the degree to which acids other than CO_2 were added to the blood, assuming the bases to remain constant. This point was tested in another experiment; in it also the blood remained mesectic throughout the dietetic period, i.e. it gave the same dissociation curve when

exposed to the CO_2 pressure of the alveolar air at the time the blood was drawn. Before the diet the alveolar CO_2 was 37 mm., on the diet 31 mm. What degree of acidosis compensated for these 6 mm. of CO_2? This could be directly found by using the dissociation curve of the blood without any CO_2 present as an indicator. The curve was the same as that of normal blood to which was added 0·03 °/₀ lactic acid, but no doubt the actual acids present were β-oxybutyric and others found in the urine.

In getting a clear understanding of the distinction between blood-acidosis and meionexy, as I use the phrases, the reader may find the following figure useful, though it pretends to no quantitative accuracy

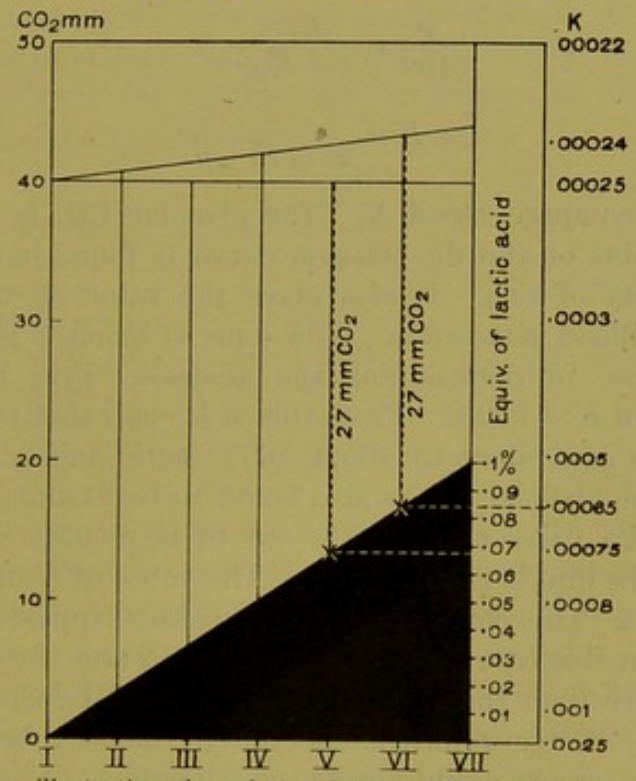


FIG. 111.—Scheme illustrating the relation between CO_2 pressure in alveolar air and blood, acidosis and *K*. The figures in this are purely hypothetical, they do not correspond to the blood of any actual person.

and it takes many things for granted, such as the constancy of the bases present. He may get a conception of the acids which rise and fall in blood as the "floating" acids—they consist of CO_2 and organic acids, lactic, β-oxybutyric, diacetic, &c.

Consider samples of blood I, III, V and VII. The alveolar CO_2 decreases progressively from I—VII. The added organic acids, represented by the black area, increase progressively from I—VII, but the

value of K remains unchanged at ·00025. This represents the reaction of the blood. Such were the samples of Higgins' blood which have been considered so far.

Now take two specific cases V and VI. Let me compare them with (I), the normal blood.

The normal blood I, exposed to no CO_2 pressure, gives a value for K of ·0025 and exposed to the CO_2 of the normal alveolar air of the patient (40 mm.) gives a value of ·00025 for K. Either of these may be found by a single determination of the percentage saturation y at an oxygen pressure of x. Then, taking n, the number of molecules y aggregated together, as 2·5,

$$\frac{y}{100} = \frac{Kx^n}{1 + Kx^n},$$

$$K = \frac{y}{x^n(100 - y)}.$$

With this compare blood V. The alveolar CO_2 is found to be 27 mm. A point on the dissociation curve is found in the presence of this quantity of CO_2. It also gives the value of ·00025 for K, therefore the blood is mesectic. Now some of blood V is taken again for the purpose of determining the acidosis. Free from CO_2, it gives a value of $K = ·00065$. From this it is clear that the amount of the acidosis is equivalent to about ·07 °/₀ lactic acid. Now pass to blood VI. The alveolar CO_2 is also found to be 27 mm. Exposed to 27 mm. CO_2 the value of K works out to be ·00024. The blood is therefore off the line, it is meionectic. The value of K in the absence of CO_2 is ·00075. This corresponds to an acidosis equivalent to ·085 °/₀ lactic acid. In this case therefore the acidosis and the CO_2 together amount to more than the original CO_2. Blood VI being meionectic the respiratory centre would be stimulated; blood V being mesectic the respiratory centre would not be stimulated.

In all the cases I, III, V and VII, not only would the blood be mesectic, since K remained normal, but the rhythm of the respiratory centre and no doubt a hundred other things would remain normal also.

Now consider another series, I, II, IV, VI. In this as in the first the alveolar CO_2 decreases progressively, but the value of K decreases also, since the aggregate of floating acids increases. In this case the blood would become increasingly more meionectic and the respiratory centre would become increasingly active.

In all three experiments made upon Higgins the curve remained mesectic ; one experiment of the same character was performed upon Graham.

Two other experiments have been performed with a different result. In each the blood became pleonectic. One of these was performed upon Higgins, another upon myself, and as the contrast in Higgins' case between this experiment and the one given above is striking I will give the results in the same form, i.e. (1) I will give the curves of the experiment, and (2) I will tabulate the values of K. (They are plotted in Fig. 110 B.)

In the column (1) are the values of K before the experiment began, in column (2) the values a week after it finished ; between these there is no clear difference. In column (3) will be found the values of K on the third day of the diet; in every case the curves were taken at the existing alveolar CO_2 pressures.

	(1)	(2)	(3)
	K before diet	K a week after diet	K on third day of diet
	·000243	·000256	·000477
	·000303	·000260	·000444
	·000218	·000326	·000434
	·000252	·000280	·000538
	·000302	·000362	
	·000374		
Mean	·00026	·00030	·00047

The difference which was noticed between the two experiments in which the subject became pleonectic on the carbohydrate-free diet and some subsequent ones, in which he remained mesectic, was that in the pleonectic experiments the subject became upset, whilst in the mesectic experiments he remained in what appeared to be his normal health. In the most recent experiment which was carried out, Higgins, however, was as much upset as in those just cited and his blood remained mesectic. It is not possible, therefore, to accept this explanation. In the one experiment in which Higgins became pleonectic, he also became anaemic; his haemoglobin value dropped to 80 %. Whether the two things are connected or not I do not know.

CHAPTER XVI

THE EFFECT OF EXERCISE ON THE DISSOCIATION CURVE OF BLOOD

So far the effect of exercise upon the dissociation curve of blood has been but slightly studied. No doubt there is a great field for work in this direction. What information there is, however, bears directly upon the relation of the reaction of the blood to the activity of the respiratory centre.

Investigations have been made upon the dissociation curve of blood before and after a climb of 1000 feet from the sea level, or nearly so. For this purpose Carlingford Mountain, in the North of Ireland, was chosen. On that mountain a climb of 1000 feet was marked out. The first experiments were made by myself. I chose a rate of climbing which did not entail any sort of "distress." Roughly speaking this meant going up at the fastest rate at which respiration could comfortably be performed through the nose. As the results of these tests it seemed that 30 minutes for the climb was a suitable speed; 25 minutes was to me a definite effort whilst anything slower than 30 minutes gave the impression of loitering. I am of course fully conscious that any such subjective index is of the roughest possible character, that probably one changes from day to day and that what appears to be an effort one day is not so on another. It is difficult, however, to arrive at any standard for mountain climbing; the staircases of some of the tube stations in London would form a very good standard course for ascents of varying degrees of rapidity. We however desired something which could be compared more definitely with an Alpine climb, to which I shall refer presently. The equipment which I took on the actual ascent consisted in a couple of Haldane's vacuous tubes for the collection of alveolar air, a long rubber tube for the same, some clean needles with which to prick my finger, a crucible in which to whip the blood and

some feathers for the whipping, and a small stoppered test tube in which to put the whipped blood. At the top of the ascent I obtained a sample of alveolar air which proved to have a partial pressure of 35 mm. of CO_2 as opposed to 40 mm. at the sea level, also a sample of my blood for analysis. The analysis was two-fold—firstly the blood was exposed to 17 mm. oxygen pressure in the absence of CO_2 for the purpose of determining by Mathison's method* the degree of acidosis, if any. The blood at 17 mm. was, as the result of two determinations, 54 °/₀ and 57 °/₀ saturated with oxygen, whilst before the climb it was

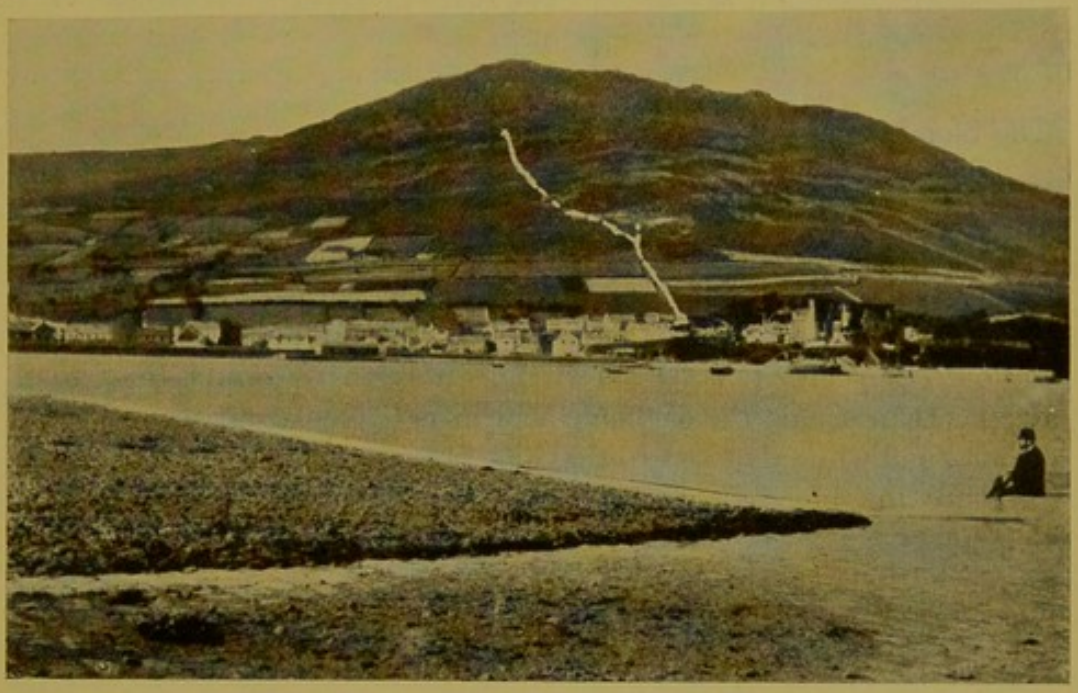


Fig. 112.—Carlingford, showing the climb of 1000 feet.

75 °/₀. The difference corresponds to an added amount of acid which is equivalent to ·023 °/₀ lactic acid.

Against this however the amount of CO_2 in the alveolar air and presumably in arterial blood went down from 40 mm. to 35 mm. The question at once arose, did this fall in CO_2 compensate for the increase in other acids? In other words, was the actual blood in the body pleonectic, mesectic, or meionectic? The answer of course could only be found out by experiment. This experiment formed the second part of the analysis. The blood taken at the end of the ascent was exposed to oxygen pressures in the presence of CO_2

* See p. 257.

at 35 mm. pressure, its dissociation curve was thus determined and compared with that of my normal blood at 40 mm. CO_2 pressure.

The data for my normal curve have already been given. The data at the end of the ascent compared with those of my normal blood were

	CO_2 pressure	O_2 pressure	Percentage saturation	K^*
After ascent ...	35 mm.	27·5 mm.	$\left.\begin{matrix}43\\37\end{matrix}\right\}$ 40 %	·000168
Normal	40 mm.	27·5 mm.	53	·000292

The blood proved to be meionectic.

This experiment was controlled by one in which the ascent was made much more slowly—in three-quarters of an hour. This slower speed is much more nearly the mountaineer's rate of climbing. There was scarcely any departure from my normal condition when I reached the summit of the 1000 feet, my respirations were 18 per minute (they are 17 as I sit writing), though no doubt they were deeper than usual; the carbonic acid in my alveolar air proved to be 38 mm. and while exposed to that CO_2 pressure and to 31 mm. oxygen pressure my blood became 56 and 55 % saturated in a couple of determinations. As compared with my normal blood, the following were the data:

	CO_2 pressure in alveolar air	O_2 pressure of analysis	Percentage saturation	K^*
After ascent ...	38	31	56	·00023
Normal	40	31	61	·000292

The slower ascent showed all the features of the faster one but to a less marked degree, i.e. the drop in alveolar CO_2 pressure and the meionexy: the departure from the normal was just, but only just, appreciable. The first of the two experiments was also controlled by another of which Roberts was the subject. He made the ascent more rapidly than I did, going up the 1000 feet in 20 minutes. The data which he yielded were as follows:

* See Appendix III.

	CO_2 pressure in alveolar air	O_2 pressure of analysis	Percentage saturation	K
After ascent ...	35—36 mm.	33 mm.	53	·00018
Normal	41—42 mm.	33 mm.	71	·00033

Moreover, as in the first of the two experiments upon myself which I cited, Roberts' blood was tested by Mathison's method for the degree of acidosis. At 17 mm.* oxygen pressure his blood when freed from CO_2 was 55 % saturated, which, according to his curve, corresponds to an added amount of acid equal to ·029 % of lactic acid. Now in this experiment the actual amount of lactic acid in Roberts' blood was analysed by Ryffel before and after the ascent. The blood used was a portion of the same sample used for the gas analysis. The method depends upon the conversion of the lactic acid into acetaldehyde, which is distilled off and estimated colorimetrically with Schiff's reagent. Before the climb commenced Roberts' blood contained ·014 % of lactic acid, at the end of the ascent it contained ·046 % of lactic, the difference ·032 % corresponded within the limits of experimental error to the acid added to the blood as indicated by Mathison's method.

Here then is a complete story. During the ascent, at the rate at which Roberts made it, lactic and carbonic acids, and these only, are added to the blood. The blood becomes meionectic, therefore the respiratory centre is stimulated, the increased respirations cause the excessive carbonic acid to be expired and not only the excess of carbonic acid but somewhat more than this. The carbonic acid pressure in the alveolar air therefore falls. The lactic acid, however, is not got rid of so quickly as the carbonic acid, and is retained in sufficient quantities to make the blood meionectic so long as the exercise is taking place. We have no data which enable us to judge of the subsequent duration of the meionexy.

So much for the facts: let us turn to their physiological significance, that is to say to the consideration of the extent to which the changes are beneficial to the organism or otherwise.

First as regards the blood itself. The change in the curve suggests a decrease in the value of K. (1) In the tissues each corpuscle will lose its oxygen more quickly, other things being equal. (2) For a given degree of reduction the pressure of oxygen in the capillaries

* See Figs. 105 and 114.

will be higher. These factors form the necessary chemical and physical complement to vascular dilatation and more rapid flow of blood through the capillaries. Our picture of the capillary circulation through the active tissues is that each corpuscle spends less

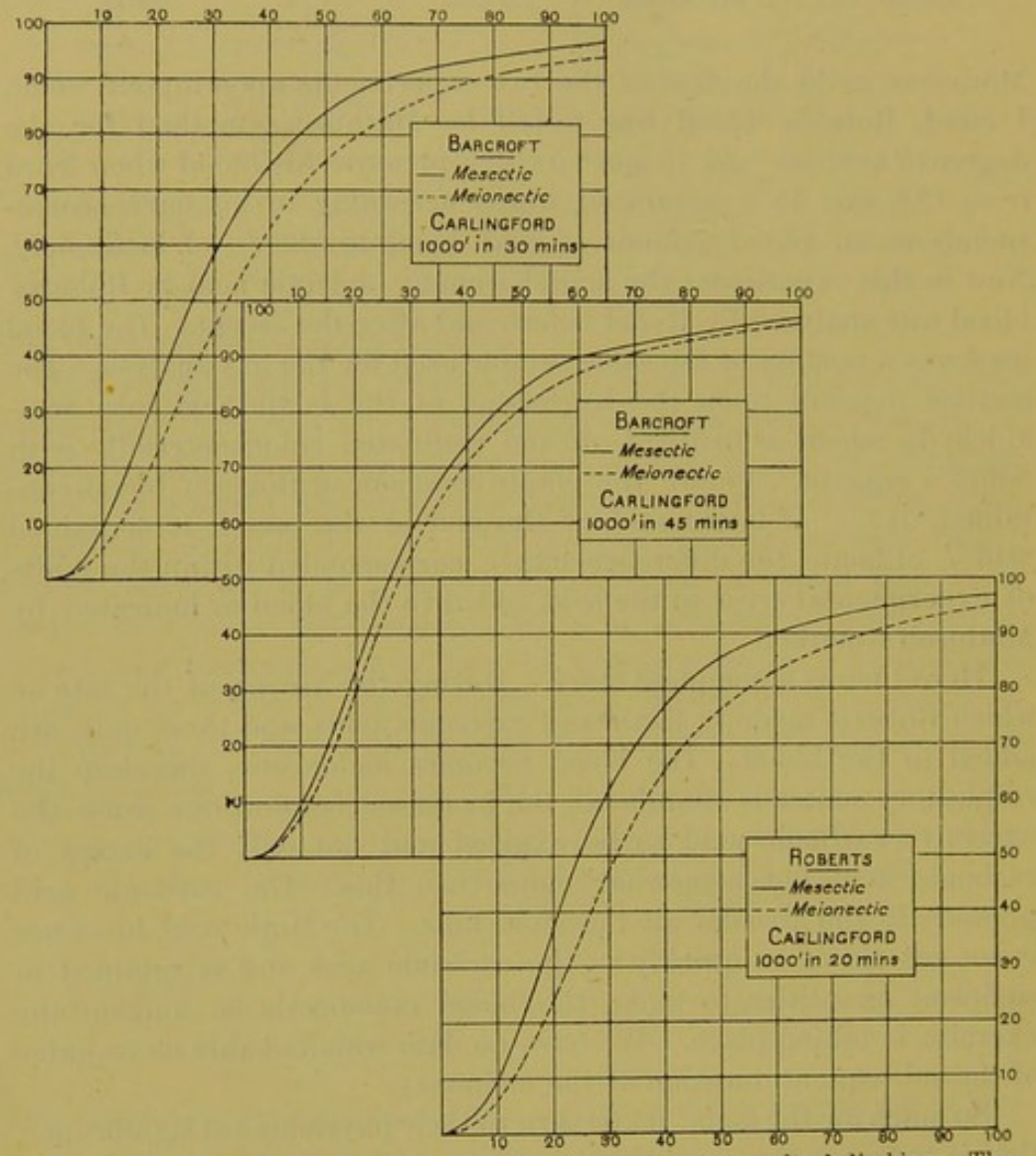


FIG. 113.—Showing changes in the dissociation curve as the result of climbing. The dotted curve is in each case the one which results from exercise.

time in the capillaries; each corpuscle is reduced to about the same extent as during rest; this is made possible by the diminished value of *K*. More oxygen leaves the blood, and this again can only diffuse out by a rise in the intra-capillary oxygen pressure combined with a fall in the extra-capillary oxygen pressure. It must be borne in

mind that the extra-capillary oxygen pressure is, in the case of muscle, never high, being perhaps 20 mm. or less and at most 27 mm.

In the capillaries we have then a most beautiful mechanism for rapid unloading of the red corpuscles during their speedy transit through the active tissues.

It might at first sight appear that what is gained in the tissue is lost in the lung. The decreased value of K tends to make each corpuscle take up oxygen less quickly in the lung, other things being equal; but so far as the lung is concerned other things are not equal.

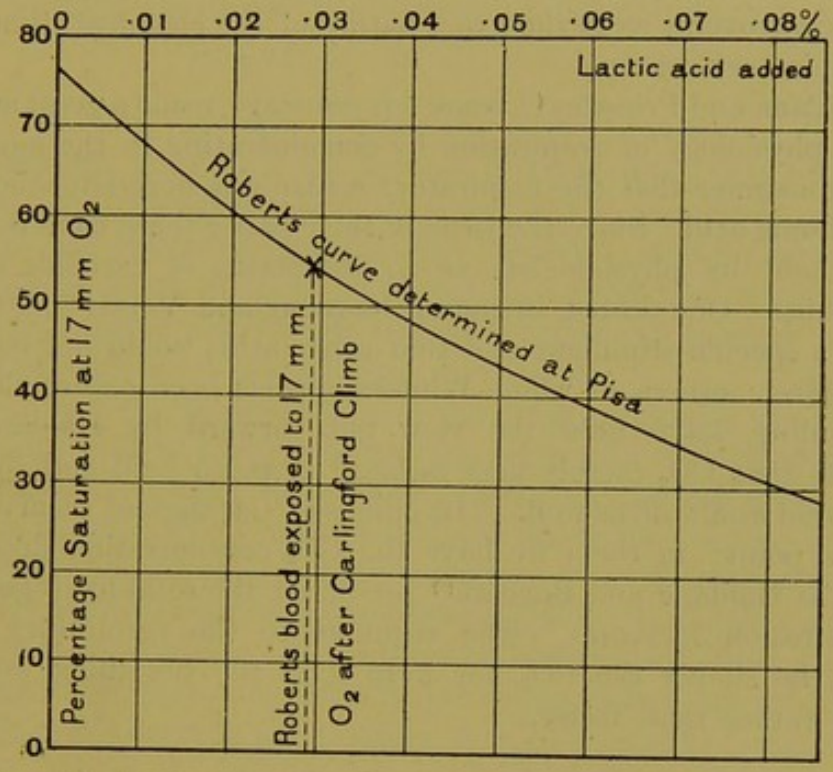


Fig. 114.—Showing relation of percentage saturation of Roberts' blood at 17 mm. oxygen pressure to acid added.

For the meionectic condition of the blood stimulates the respiratory centre and causes increased ventilation of the lung. We have seen that there is a fall in the carbonic acid in the alveolar air. The counterpart of this is that there is a rise in the pressure of oxygen in the alveoli. The rate at which the oxygen is taken up, other things again being equal, depends upon the pressure of oxygen.

Apart from the fact that nature can more than compensate for the diminished value of K by increasing the respirations, it must be

borne in mind that it is more important that the blood should become more rapidly reduced in the active tissue than more rapidly oxidised in the lung of the active person. This is most evident if a very small muscle be considered. Let it be in full activity: the blood will go through it with ten-fold rapidity, hence the necessity for rapid reduction of the haemoglobin: but the muscle being small the quantity of blood involved would not be so large as greatly to quicken the circulation through the lung—the blood would have its usual time for the acquisition of oxygen.

It seems impossible to close this chapter without some reference to the controversy as to the exact nature of the chemical stimulus to the respiratory centre.

Haldane and Priestley [1], some ten years ago, made a great advance in the physiology of respiration by demonstrating in the most convincing manner that the respiratory centre was normally stimulated by carbonic acid. Since the issue of their paper there have been two views held by physiologists as to the status of carbonic acid as a stimulus. One school, including Laqueur and Verzàr [2], hold that CO_2 is a specific stimulant and that other acids would not have the same effect: others, of whom Winterstein has been one of the most outstanding, have held the view put forward by Haldane and Boycott that CO_2 merely acts because it is an acid and that any other acid would do as well. The climbs at Carlingford seem decisive on this point; in them we have the CO_2 concentration decreasing while, as Haldane and Boycott [3] predicted, the total hydrogen ion [4] concentration increases. The stimulus to the respiratory centre cannot be simply the CO_2, for were it so the breathing would be slower rather than faster.

REFERENCES

(1) *Journal of Physiology*, XXXII, p. 225, 1905.
(2) Laqueur and Verzàr, *Arch. f. d. ges. Physiol.* CXLIII, p. 395, 1911.
(3) Haldane and Boycott, *Journal of Physiology*, XXXVII, p. 355, 1908.
(4) Appendix IV.

CHAPTER XVII

THE EFFECT OF HIGH ALTITUDES UPON THE DISSOCIATION CURVE OF AN INDIVIDUAL

This problem has now formed the subject-matter of a couple of expeditions, the first of which went to Teneriffe in 1910 under the auspices of the International Commission for the Study of High Altitudes and Solar Radiation. The President of the Commission, Professor Pannwitz, is much to be thanked for the completeness of the arrangements, which made it possible to carry through a great amount of scientific work in a very short time.

The island of Teneriffe is in some ways especially suitable for such work, owing to the fact of its very temperate climate, temperate in the sense of being neither too hot, too cold, nor too windy. Our object in working there was the study of the human subject when at rest. In Teneriffe this object is particularly easy of attainment. No one in the island, so far as my experience goes, either takes or wants to take violent exercise. In the Alps no one has any other object in view than exercise in some form or other, but to walk up the Peak of Teneriffe would be only less peculiar than to ascend to Col d'Olen on a mule.

Our sea-level station in Teneriffe was at Puerto Orotava, where we stayed at the Grand Hotel Humbert, and every facility was given us for carrying out our work. The hotel was perhaps 300 feet above the sea, the climate when we were there, which was in March, was much like that of the English summer and was cool compared to that which we subsequently encountered at the sea-level station of our Italian expedition—Pisa.

The work performed at Orotava consisted in getting a base line, so to speak, for our subsequent observations. The two persons studied most completely were Mr C. G. Douglas, Fellow of St John's College, Oxford, and myself.

Our dissociation curves had been mapped out in England before we left, they have been re-determined on several subsequent occasions in this country. We attached great importance to the accurate determination of them at the time and still greater importance now. At that time they were important merely as objects of comparison for our other curves. Now they are more important, for it is known that if one curve is accurately ascertained, any other curves for the same individual can be found by the accurate determination of a single point.

Fig. 115.—The station in the Cañadas (7000 feet). The peak in the background. (Douglas.)

We gave in Chapter XIV the dissociation curve of Douglas, showing the points on it which have been determined by us, together with those which have been determined by Haldane and Douglas for the same carbonic acid pressure, namely 40 mm.

The second station in Teneriffe was at Las Cañadas. From the point of view of the meteorologist this station is of especial interest. It is about 7000 feet above the sea-level and therefore not much higher than many centres of population. Johannesburg, for instance, is close upon 6000 feet—putting aside therefore such places as the mining towns in the Andes, the Cañadas are a fair example of the

higher altitudes at which the working life of man is carried out on a normal scale. As a station for the study of climate the Cañadas offer, among other advantages, a relative immunity from wind. This in view of the investigations published by Lyth [1] is a factor worthy of consideration. The island of Teneriffe consists roughly of a huge crater about 8000—9000 feet in height. The diameter from lip to

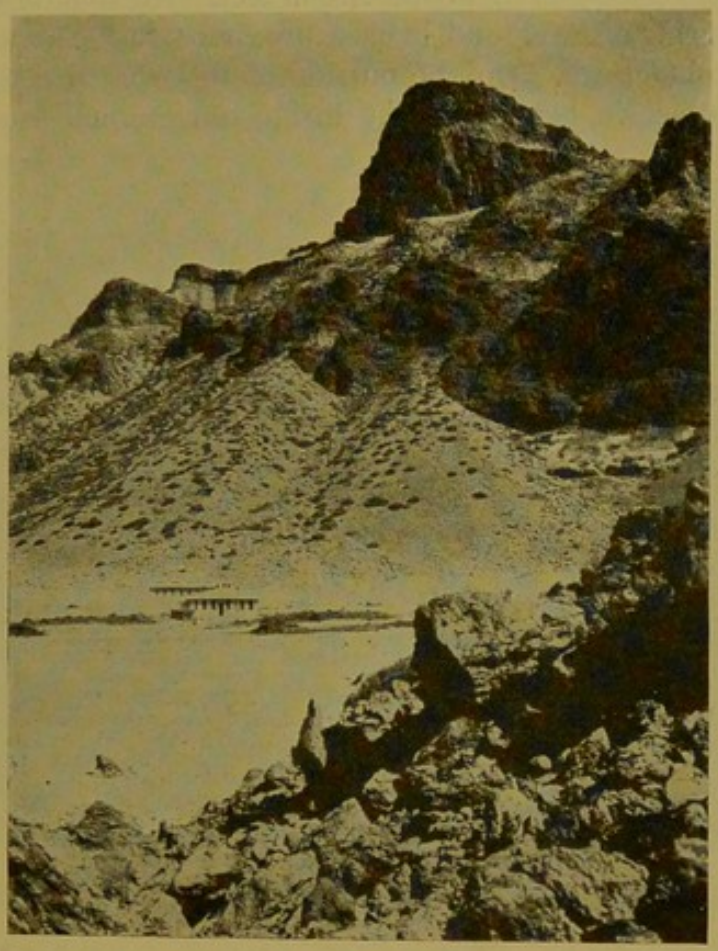


Fig. 116.—The Cañadas showing the living house and the laboratory. Espigone, one of the summits, on the lip of the "old crater" in the background. (Douglas.)

lip is eight miles. On the south side of the island the lip is incomplete. The inside of the tip is a steep, not quite precipitous, cliff, down which you must climb for a thousand or two feet, unless you enter the crater as we did by a gap in the cliffs we called the Portillio. We were then inside the old crater; our back was to the cliff, which in places rises in named summits, Guajara and Espigone for instance. Our faces were towards a level plateau of sand.

From the description I have given so far it might be supposed that a sandy plain now stretched before our eyes, and that in the distance six or seven miles away we saw the opposite lip of the old crater before our eyes. But this is not so, for the new crater arose out of this plateau; this was in front of us; it appeared as a majestic peak, bursting suddenly upon us as we emerged from the Portillio, and rising to a height of about 12,000 feet. All that is left of the plateau is a ring of level sand, in close proximity to the almost vertical lip of the old crater. On the outside of this ring rise the cliffs to about 1000 feet in height, on the inside the gradual ascent of the

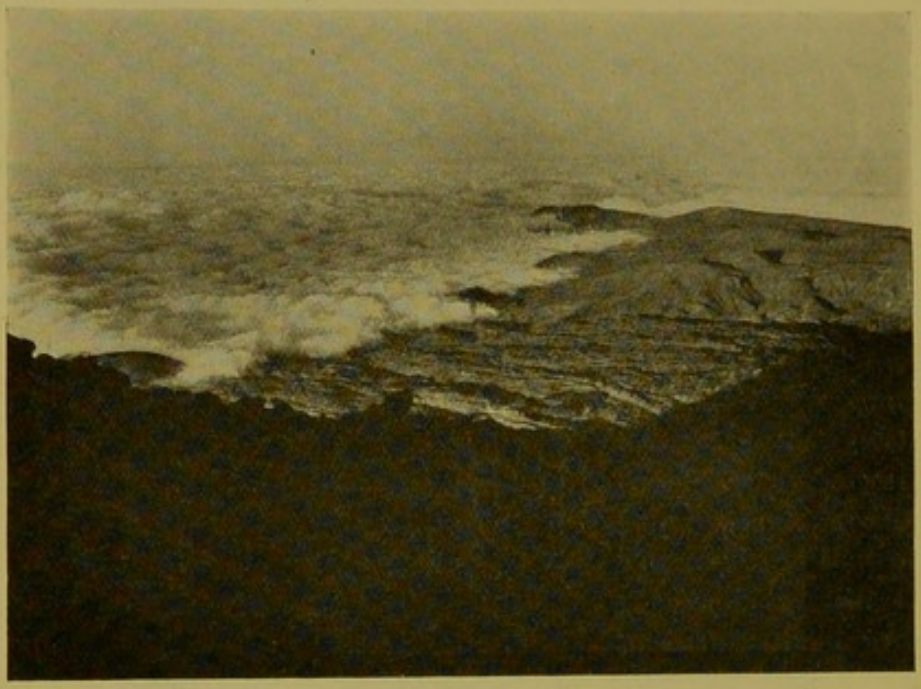


Fig. 117.—View from the Alta Vista hut, showing the Cañadas and the Portillio. (Douglas.)

peak. It was on this sand that our station was placed. No place at this altitude could have been more sheltered by natural barriers.

It was quite unlike any place to be seen in Europe. Compare it with much higher altitudes in the Alps, and the comparison is a very remarkable one. The complete dryness of the atmosphere at the Cañadas spells the lack of the beautiful vegetation which makes the Alpine snow line so attractive. Go out of the laboratory at Col d'Olen, everything is moist underneath your feet, the cracks in the rock are filled with saxifrages and gentians. Not so at the Cañadas although the vegetation at lower altitudes in Teneriffe is no less

beautiful than in the Alps. To get to the Portillio you must ride through woods of heath which rises seven or eight feet in the air and bursts into blossom above your head. But this has all been left behind. The moisture condenses into clouds, which hang over the island in a sheet at an altitude of 4000—5000 feet; through these clouds you penetrate. Once you arrive within the old crater you have reached a new climate. Between you and the clouds there is an impassable rampart. No vestige of mist was seen either above us or around us during our sojourn in Las Cañadas, the occasional appearance of a cloud top above the lip of the crater was the only reminder that such a thing as a cloud existed.

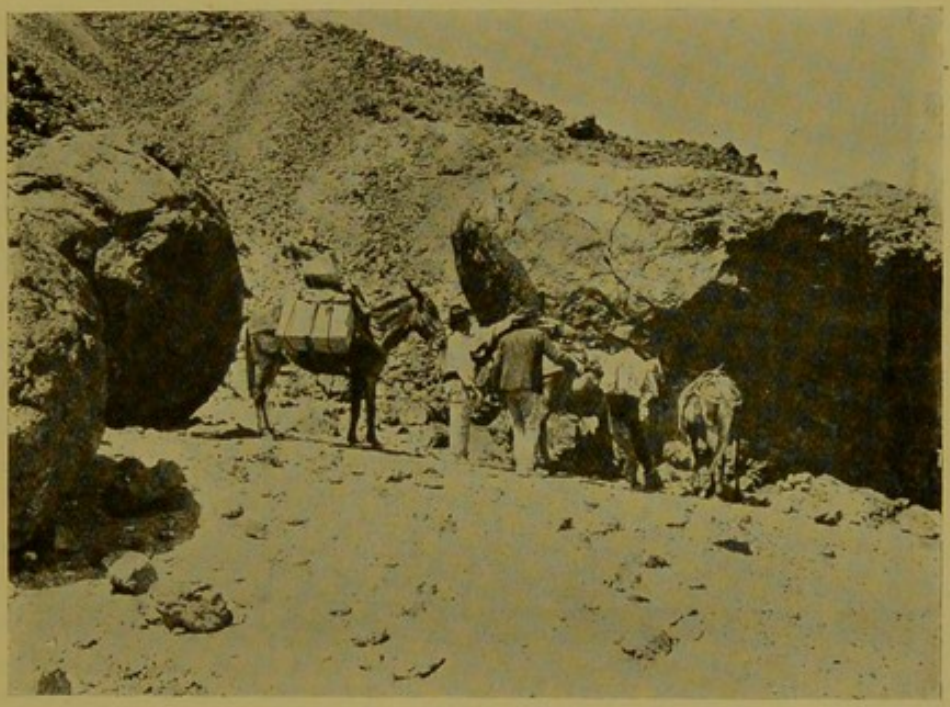


Fig. 118.—Transport of apparatus to the Alta Vista. (Douglas.)

With the absence of moisture there was a corresponding absence of vegetation—scarcely anything green was to be seen. There is a broom, called retama, that grows in considerable quantities, there is a viola which seems to survive, but, beyond these, I noticed no plant. Everything was arid. Imagine a mountain of coke from the gas-works rising 5000 feet above you and you have pictured to yourself the peak of Teneriffe as seen from the Cañadas.

In some places, the "coke" is replaced by pummice, in others the

pummice is reduced to sand, as in the Montana Blanca or the extreme summit of the Peak ; otherwise all is black, shading into brown or perhaps red, all is crumbling and broken. How different is the ascent of the peak from the Cañadas to the ascent of Monte Rosa from the Col d'Olen Laboratory. There is no element of exhilaration about the former ; you start in the afternoon, you sit on a mule, you wonder at its skill in putting its fore feet on the appropriate blocks of broken lava, you think perhaps it sees where it puts them ; but when it comes to finding an explanation of how your mule places its hind feet with equal certainty you "give it up."

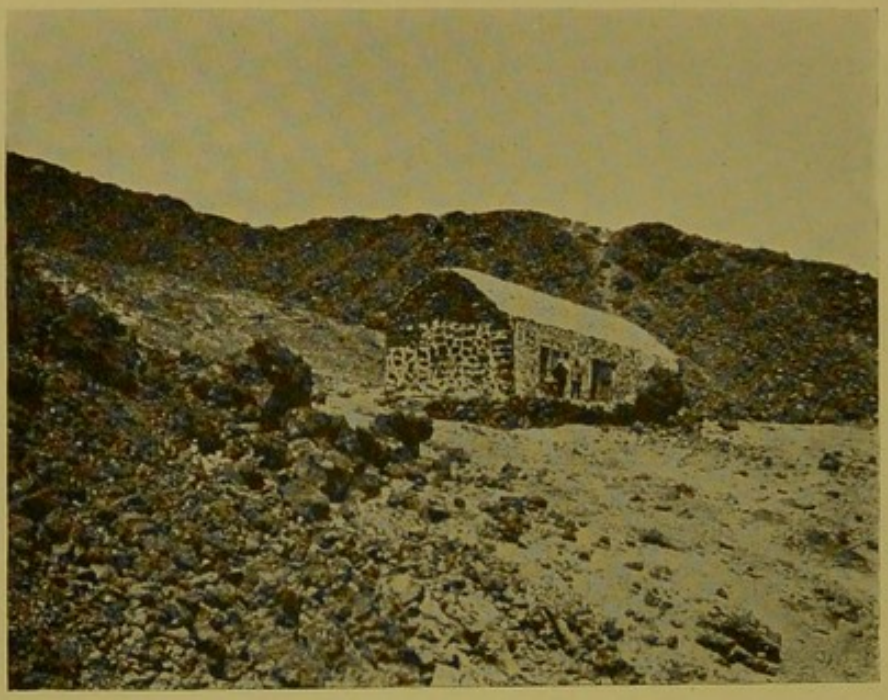


Fig. 119.—The Alta Vista Hut (11,000 feet). Standing near door dressed in black is Geheimrat. Professor Zuntz. (Douglas.)

How different from the Alps, from the bustle of guides and porters and ropes before sunrise, from the hope and the beauty of new things that comes with the rising of the sun as you stop for the party to be roped before it ventures among the crevasses, from the sense of 2000 more feet behind you as you rest and have some refreshment at the Capanna Gnifetti. Then, as you tramp across the Lysjock Glacier, you make up your mind for the last 500 metres which is to bring you to the Margherita hut on the summit. How white and exhilarating it all is and how far from the mind is any thought

of "giving it up" on Monte Rosa. Surely if Excelsior is the motto of the Alps, Mañana (to-morrow) is that of Teneriffe.

What fields of research lie in front of the physiologist before he can explain how the subtleties of climatic conditions affect the human mind, that entity of which all human activity is a manifestation.

How gross seem the avenues at present open to such investigations. You are one person in one place, another in another. At the Alta Vista, I became as one incapable of arithmetic—I vow that I would have been at the bottom of the class with the dunce's cap on and that I could not have helped it. At Col d'Olen I have heard two clever and distinguished physiologists pause to discuss whether or no four times eight made thirty-two. At Johannesburg I have been told that a cricket team representing England so lost their nerve that they laughed like children with quite trivial turns in the course of the game and fell an absurdly easy prey to their South African opponents. At the Margherita hut I have seen one of the pleasantest and most considerate of companions behave as though he were suffering from alcoholic excess in a mild degree. What of the surprise that comes to us when we hear of cautious and skilful climbers losing their lives doing extravagantly reckless things. Such incidents are caused by the little recked of cerebral changes which appear from time to time as the incidents of life at high altitudes. They are doubtless the effects of acid intoxication—but of this later.

The climber depends for the most part on his cerebellum, his cerebrum takes its chance and is little considered. One day these psychological changes, which, in my opinion, appear much earlier than cerebellar ones, such as defective coordination and giddiness, or medullary ones, such as vomiting, will be studied for their own sake.

In the meantime we have got the pioneer work to do, we have got to make a road into this forest wherever we can, we have got to find out the changes which take place in the blood at such altitudes, and in truth this is enough to tax our powers.

It may seem that I have depicted Teneriffe, as compared to Monte Rosa, in a light unfavourable to the former. Let me disabuse the reader's mind of this idea. Teneriffe has many advantages as a place for the study of climate. Truly it takes longer to get there, more truly it takes longer to get back, and most truly it costs more money. If, however, you can secure the time and the money, there is much to be said in favour of Teneriffe. From England, at all events,

the problem of getting your equipment there is much more simple. You put it on the steamer, with due leisure, in Liverpool, London or Southampton, and you take it off at Santa Cruz. If you do not mind travelling in a steamer of 2000 tons, you may disembark under the very windows of the Hotel Humbert and have your things carried up by the hotel porter. We were fortunate enough at Teneriffe to be passed through the customs—the same consideration, indeed, was shown to the Monte Rosa expedition, for which I should like, here and now, to record my thanks to the Ambassadors of France and Italy: yet, even taking these acts of encouragement to scientific workers into consideration, the difficulty of getting your apparatus intact to Col d'Olen is very great. Think of the embarking and the disembarking on the channel steamer, think of the terrors of the custom house, even if the luggage is unopened, of the justifiable resentment of your fellow-passengers if you take it in bulk in the railway carriage and of the impossibility of putting it in the van ; delicate as my apparatus was, I brought all the important pieces back intact from Teneriffe: little but broken glass arrived in London from Col d'Olen.

But, perhaps, the greatest advantage of Teneriffe is that you can start your work at the sea-level. On our Monte Rosa expedition we made Pisa our base of operations. This, of course, is a far cry from Col d'Olen as compared with the mule ride from Orotava to the Cañadas—that perhaps is a minor consideration and would not be a consideration at all if Turin were made the base—but there seemed to me a much greater difference between the climate of the plains of Tuscany and that of the high Alps than exists between Orotava and the Cañadas. In the latter case there was the difference in the moisture, upon which we have already touched, but the difference in the temperature nothing near so great.

In spite, however, of these differences, altitude clearly had an effect which was the same in both cases. There were two obvious changes, both of them well known from the work of previous authors, which must, in some way, affect the amount of oxygen in the arterial blood—both of these are evident from a study of the alveolar air. The first is the diminished oxygen pressure in the inspired air, the second the diminished carbonic acid pressure in the expired air. Of these two, the latter shall claim our interest first.

Great stress was laid by Mosso upon the diminished CO_2 in the breath, not because its diminution is of any importance in the breath, but because this is but the reflection of lowered CO_2 pressure in the

body generally. To this absence Mosso attributed the symptoms of mountain sickness, as Henderson[2] has more recently attributed the effects of surgical shock ; this is, in fact, the "Acapnia theory."

That the want of carbonic acid would, other things being equal, affect the affinity of the blood for oxygen is, of course, clear from what has been said in the earlier chapters of this book. This had not escaped Bohr, who pointed out that the increased affinity of the blood for oxygen in the presence of a diminished carbonic acid pressure in the blood, would form a very beautiful adaptation on the part of the organism to the rarefaction of the air.

Here then was an obvious line of work.

The results were so different from the predictions made by Bohr that we have been at great pains to verify them. Inasmuch, however, as we have obtained them with a number of different individuals both in Teneriffe and on Monte Rosa, and as these results have been confirmed by Haldane on Pikes Peak, it seems to be established that so far from the blood gaining in affinity for oxygen by its loss of CO_2, the affinity of the blood for oxygen remains as a first approximation unaltered in spite of the lowered CO_2 tension.

The following are the carbonic acid tensions in the alveolar air of various workers at various altitudes expressed in mm.

	Sea-level	7000 feet	10,000 feet	12,000 feet	15,000 feet
Douglas.....	40—41	36	—	32	—
Barcroft	40	40	33—32	38	29
Camis	39	—	37	—	29
Roberts.....	39	—	33—34	—	26
Mathison	39	—	32	—	28
Ryffel	45	—	35	—	30
Zuntz	35—36	29	—	27	—

The accompanying Figures 120 and 121 show the affinity of the blood for oxygen at these different places and at different carbonic acid tensions. The result is sufficiently astonishing; whatever be the place, the blood exposed to the carbonic acid pressure in the blood at that place always possesses the same affinity for oxygen. It is different in different people ; in Douglas's and Mathison's blood, for instance, it is less than in that of Roberts, Camis and myself ; this does not matter, the blood of each person has a certain affinity for oxygen, that affinity remains almost unaltered. It was the same in Douglas's case at Orotava with 41 mm. CO_2 pressure, at the Cañadas with 35 mm. and at Alta

Vista with 33. My own blood had the same affinity for oxygen at Orotava with 40, at the Cañadas with 40 mm., at Alta Vista with 38, at Col d'Olen with 30 mm. and so on. This is the principle of the

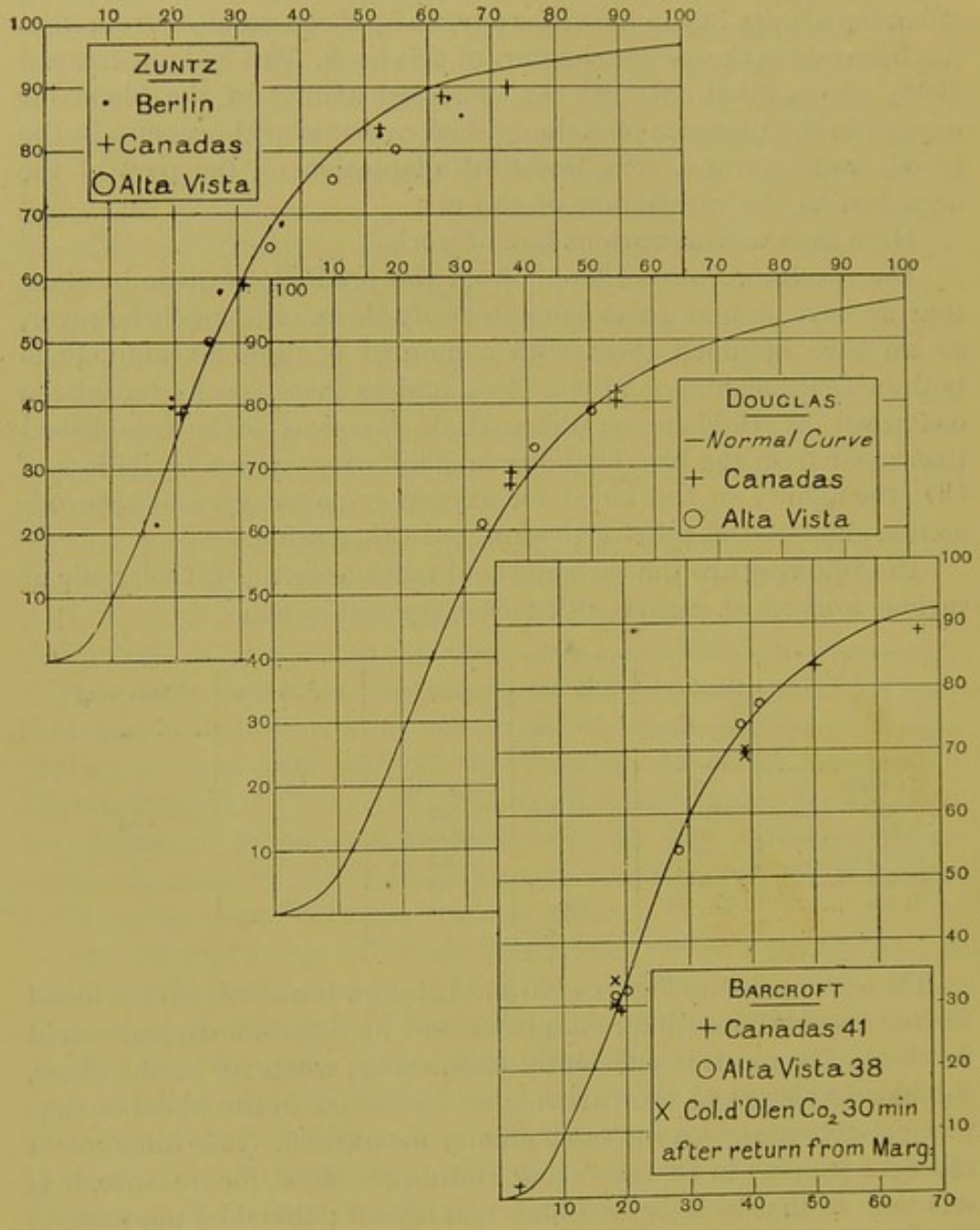


FIG. 120. —Dissociation curves of Zuntz, Douglas and Barcroft. The points in each case were determined with the blood exposed to the existing alveolar CO_2 pressure of the place.

individual constancy of the dissociation curve, which seems to be true as a first approximation.

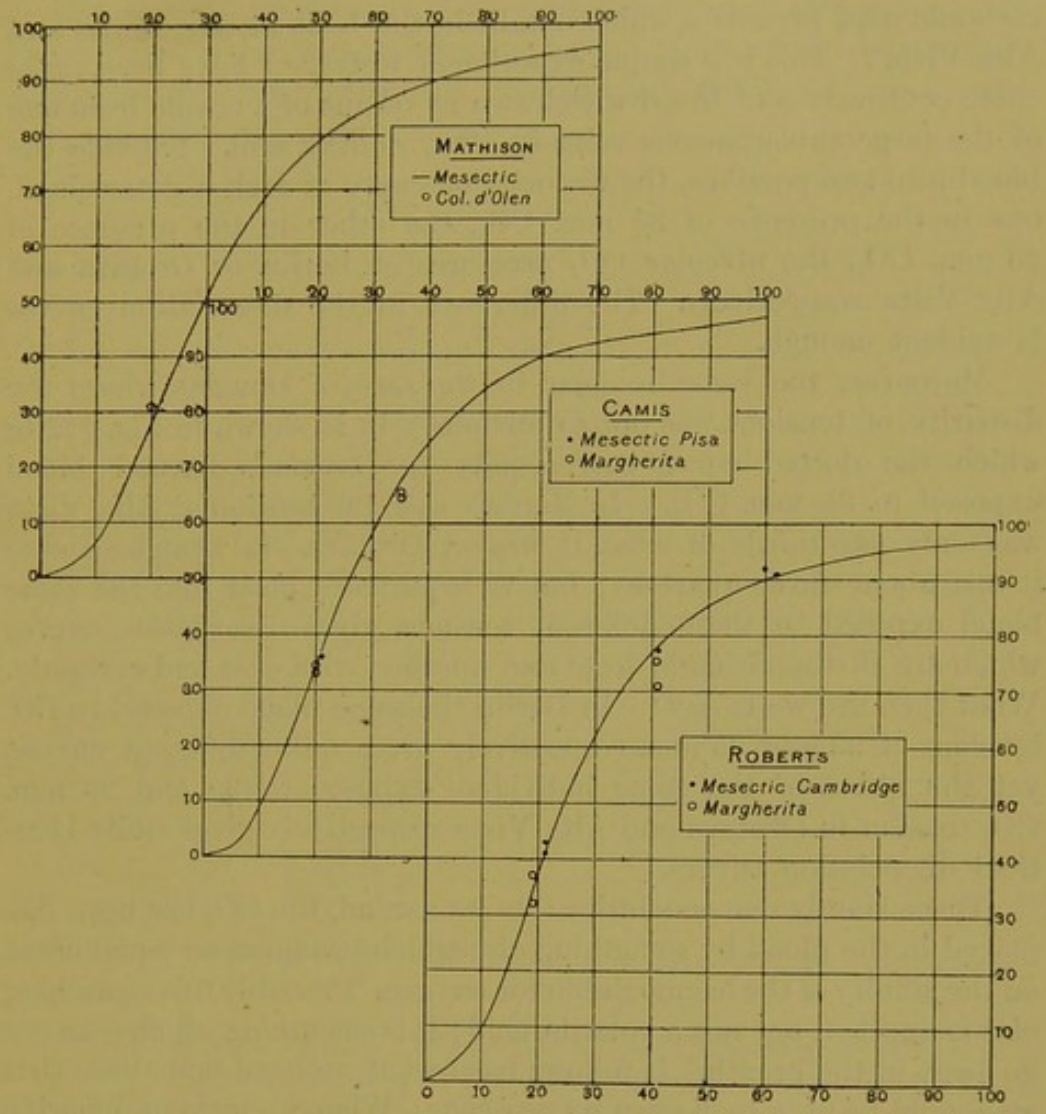


FIG. 121.—Dissociation curves of Mathison, Camis and Roberts, with points determined at high altitudes, the blood being exposed to the existing alveolar CO_2 pressure of the place.

The figures to which we have just referred the reader will convince him, at all events they have convinced us, that the principle is true, within the limits of the method of which we have been treating. But may it not be that these limits are too wide for us to be able to discern the changes due to the decreased carbonic acid pressure? Have we a right to expect that we can observe the

differences which the diminished CO_2 pressure would produce? Could we, in short, by the same methods detect any difference in the dissociation curves of our blood when exposed at sea-level to the two carbonic acid pressures which obtain in the body at, say, Berlin and Alta Vista? This is a simple experiment to try. I have here three cubic centimetres of blood withdrawn by means of a needle from one of the large subcutaneous veins in Prof. Zuntz's arm. I divide the blood into two portions, the dissociation curve of each is determined, one in the presence of 35 mm. CO_2, the other in the presence of 23 mm. CO_2, the alveolar CO_2 pressures at Berlin or Orotava and Alta Vista respectively. The difference in the dissociation curves is evident enough.

Moreover, the same is clear in the case of Douglas where the disparity of tensions was not so obvious. It is shown in Fig. 122 in which the dotted curve corresponds to Douglas's normal blood exposed to 34 mm. CO_2. In Zuntz's case the tension at Alta Vista was only two-thirds of what it was at Orotava—in Douglas's case it was about three-quarters; but it is perfectly clear that the same blood exposed to these different tensions gives dissociation curves which are distinguishable from one another with ease and certainty. What then are we to say? In Berlin the same blood exposed to CO_2 tensions of 23 and 35 mm. respectively gives quite different curves, yet the blood of the same individual exposed to 35 and 23 mm. CO_2 tension in Orotava and Alta Vista respectively gives quite identical dissociation curves.

There is only one conclusion: as we ascend, the CO_2 has been displaced in the blood by something else which produces an equal effect on the affinity of the haemoglobin for oxygen. Probably this something else is another, but not a volatile, acid; it is something which does not go away in the breath. It follows from what we have said above that we should be able to discern its presence. When we were in Teneriffe we did not fail to try. With Orbeli[3] I repeated the experiment when we arrived home by subjecting cats to a diminished oxygen pressure. In the interval between the Teneriffe expedition and the Monte Rosa expeditions Mathison worked out the method for estimating the amount of acid so thrown in the blood. We used this method and made estimations by it and by other methods at Monte Rosa. Up to a certain point the matter is perfectly clear. Let us say what there is to be said about it. We will take up the points in the order in which we have enumerated them:

(1) Firstly then, what evidence have we that the blood changed, apart from the CO_2, as we ascended the Peak? The simplest way of attacking this problem is to expose the blood to equal CO_2 pressures in the various places and determine its dissociation curves. In Fig. 122 are given the dissociation curves of Douglas's blood, withdrawn respectively at Orotava, Cañadas and Alta Vista and, in each case, exposed to 41 mm. CO_2 tension.

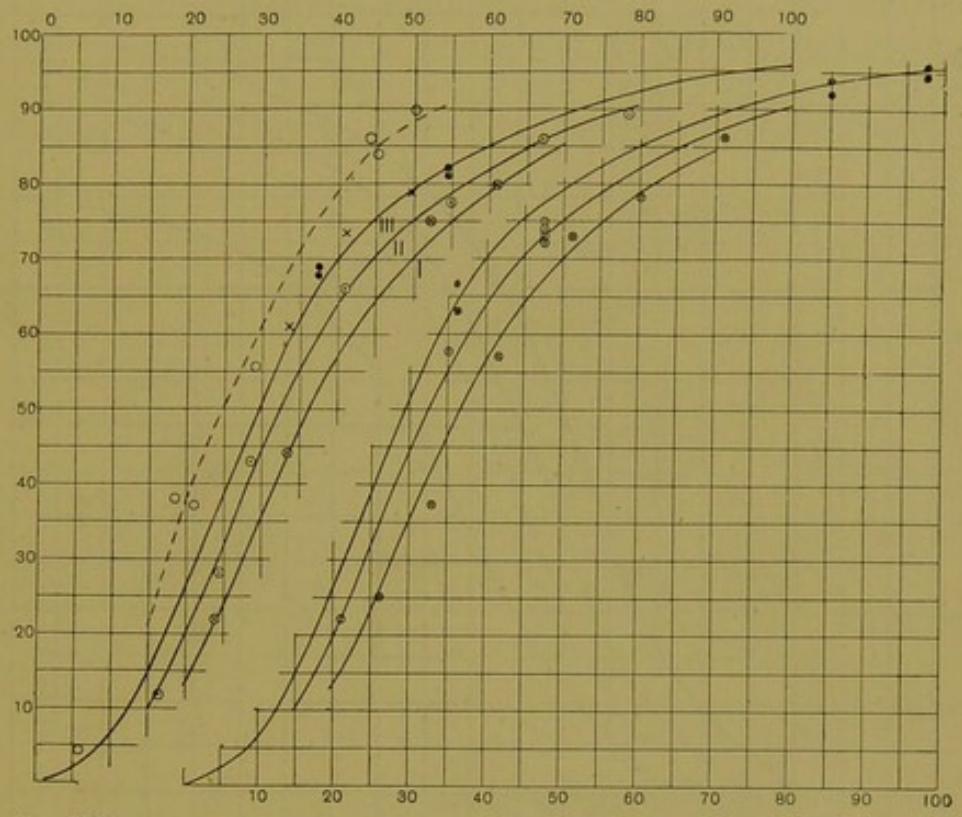


Fig. 122. Fig. 123.

Fig. 122.—Dissociation Curves (I—III) of Douglas's blood exposed to 40—41 mm. CO_2 pressure: III at sea level, II at 7000 feet ⊙, I at 11,000 feet ⊗. • Blood taken at 7000 feet exposed to 36 mm. CO_2, × at 11,000 exposed to 33 mm. CO_2, ○ at sea level exposed to 34 mm. CO_2.

Fig. 123.—Lines as in Fig. 122. Douglas's blood at Oxford. • + no lactic acid, ⊙ + 0·03—0·04 °/₀, and ⊗ + 0·07—0·08 °/₀ lactic acid.

The difference between them is clearly to be discerned; with each rise of altitude the curve is displaced somewhat to the right—enough to be seen. This suggests an increase in the acid radicles, or a decrease in the bases of the blood. It becomes a matter of interest, therefore, to see whether these curves could be imitated by the addition of small quantities of acid to blood. Fig. 123 shows that

this can be done. To some of Douglas's blood lactic acid was added to the extent, in one case of about 0·037 per cent., in another 0·075, the dissociation curves were then determined; their similarity to those shown in Fig. 122 is very striking.

That these changes in the blood were due to simple oxygen want was tested by trying to induce them in animals. In two cases a cat

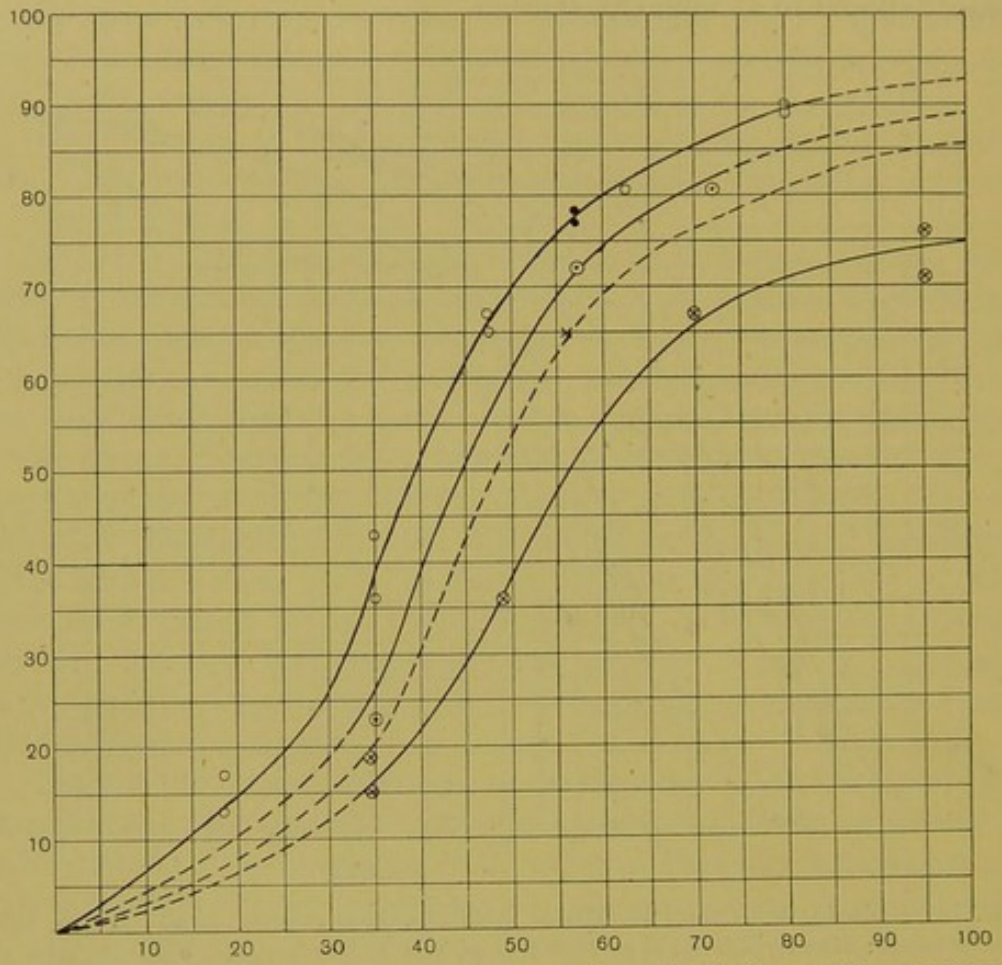


Fig. 124.—○ Defibrinated cats' blood. ⊗ Points from Cat I after partial occlusion of trachea and 15 minutes breathing of gas of increasing poverty in oxygen. • Cat II at beginning of exp. ⊙ after 15 minutes gas-respiration. × after 21 minutes ditto. The gas was becoming continually poorer in oxygen, at the end it was about 4 °/₀ oxygen.

was subjected by Orbeli and myself to an atmosphere in which the oxygen gradually got rarer and rarer as time went on. The curves obtained are shown in Fig. 124.

(2) We have said enough to indicate the possibility of a method for the purpose of estimating the effective strength of the acid or acids thrown into the blood apart from the carbonic acid.

If the blood be thoroughly shaken the CO_2 may be shaken out. With the small quantities of blood which we used (1—2 c.c.) about ten minutes shaking was usually employed. Suppose now, we have some blood which has been so treated. Let it be some of my own for instance, it will give a certain dissociation curve: with another portion of the same blood to which ·01°/₀ lactic acid has been added another dissociation curve will be obtained, and if ·02°/₀ lactic acid be added we get yet a third curve. Now let us take a certain oxygen pressure, say 17 mm. of mercury, it will be seen from an inspection of Fig. 125 that at 17 mm. oxygen pressure we get percentage saturations corresponding to the stated quantities of

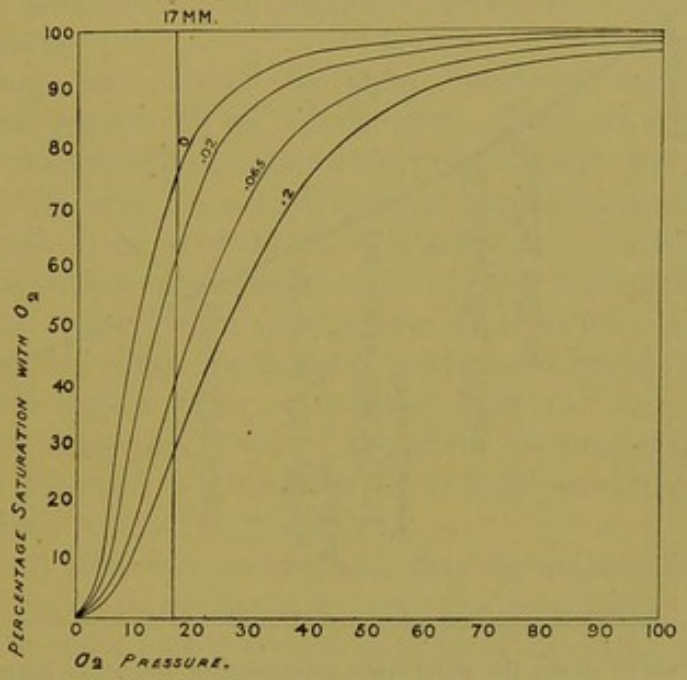


FIG. 125.

acid added to the blood of a particular person. We may express such results graphically: if we do we get a curve such as that shown in Fig. 126. This curve may then be used as the basis of determinations of unknown quantities of acid added to the blood in the body of the person for whom it has been determined. The blood is withdrawn from the finger or the arm, the CO_2 is shaken out, it is exposed to 17 mm. pressure of oxygen at 37° C. and the percentage saturation with oxygen is determined; the amount of acid is then read off from the curve.

It is necessary, of course, to make a curve for the blood of each person; moreover, the method is only approximate, but it has the

advantage as compared with methods of hydrogen ion determination by means of a gas chain battery, that the apparatus is very easily carried and set up. I imagine direct determinations of hydrogen ion concentrations at the Alta Vista hut would be attended with considerable difficulty.

At present I will only deal with one of the party, namely, myself, as I was in most respects a typical case. The changes which took place in my blood may be traced in the various stages of the journey.

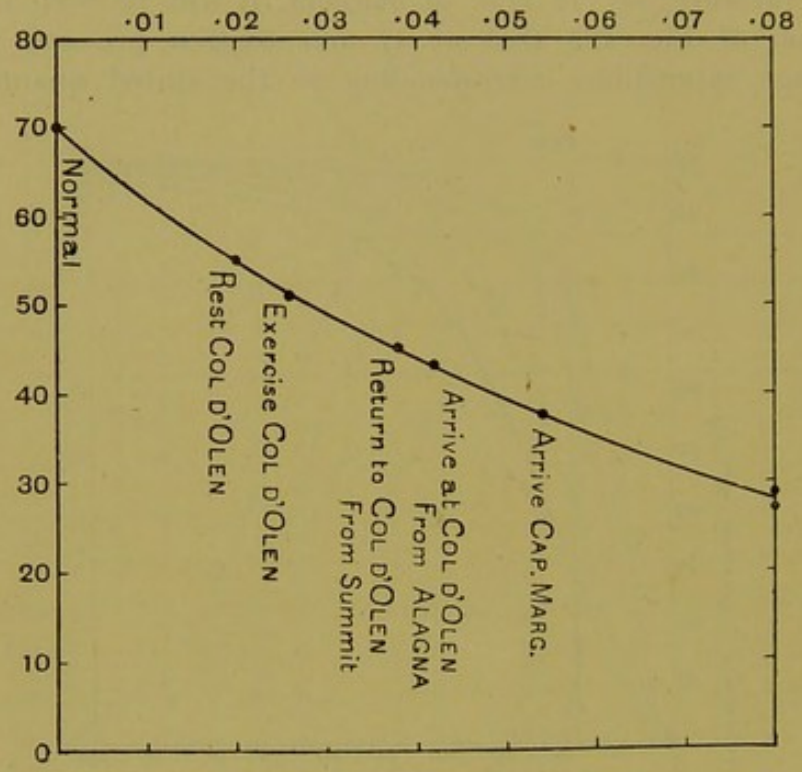


Fig. 126.—The curve shows the percentage saturation of Barcroft's normal blood, with oxygen, when exposed to 17 mm. oxygen pressure with various quantities of lactic acid added as determined at Pisa. Ordinate = °/₀ saturation. Abscissa = °/₀ of lactic acid added. The dots correspond to blood taken under the circumstances indicated: the percentage saturations at 17 mm. were determined by analysis, the points were then referred to the curve and thus the degree of acidosis was found.

We walked from Alagna to Col d'Olen, an ascent of 5500 feet (1700 m.), in about four hours; immediately on arrival some of my blood was drawn and a determination made; the determination showed that, leaving out of account the carbonic acid, the acidosis in the blood amounted to the equivalent of ·042 °/₀ of lactic acid; this however was not a permanent condition, it was a mixed effect due to exercise and altitude together; at rest at Col d'Olen the acidosis amounted to the

equivalent of 0·02 °/₀ lactic acid. After we had been at Col d'Olen some ten days we went up to the Margherita. The ascent is about 5000 feet, we made it in seven hours, of which we rested for perhaps an hour at the Capanna Gnifetti ; on reaching the summit there was again a further large accession of acid to the blood. It reached 0·54 °/₀. This again had ebbed to some extent by the following morning. At present we are only discussing the question of the condition of the blood when the person under observation is at rest or at all events not exerting himself. There is the most distinct and definite evidence that the blood changes at each different altitude, in that apart from the CO_2 present the blood gains in acid, or loses in alkali, at all events that its reaction alters in the direction of decreased alkalinity at the higher altitudes.

(3) Though the method we have described, namely, that of measuring the reaction by the affinity of the blood for oxygen, proved most satisfactory and was operated with great skill and rapidity by Mathison and Roberts, we naturally wished to have some other methods at our disposal. One of these was the method of Boycott and Chisholm[1] for determining the reaction of blood. It depends upon the fact that the haemoglobin precipitates at a definite acidity. The blood is therefore titrated with acetic acid until a precipitate appears, the original reaction being calculated from the amount of acid necessary to bring the blood to the point of precipitation. This method was not used so systematically as that previously employed. It gave qualitatively the same result[2].

Lastly, as it seemed to us probable that the acidosis which we had observed in Teneriffe might have been due to a lactic acidosis, we determined to make actual determinations of the lactic acid present by Ryffel's[3] method (see Chapter XV). Let us state precisely what it is that this method estimates. It is the total quantity of the radicles of the α-OH organic acids. It therefore has to do with something essentially different from the others; the others are a measure of the hydrogen ion concentration, for it has been shown by Mathison that the change in affinity of the haemoglobin for oxygen when acids are added to the blood depends upon the concentration of hydrogen ions, and the same has been shown by Boycott and Chisholm for their method. But Ryffel's method is different; it measures the lactic acid radicle irrespective of the degree of ionisation of the acid or of the bases with which it is united. Moreover it includes any other α-OH acids which may be present in the solution.

Our conjecture that the change in the reaction was due to the increase of lactic acid turned out to be entirely erroneous. Ryffel obtained the following figures for the "lactic acid" in the blood of various members of the party at Pisa, Col d'Olen and the Margherita hut respectively.

Subject	Lactic acid Pisa	Increase of lactic acid Col d'Olen	Increase of acidity* by Mathison's method Col d'Olen	Increase of lactic acid Cap. Margherita	Increase in acidity* by Mathison's method
Roberts	0·018	+0·006	0·023	+0·027	+0·035
Camis	0·017	+0·004	0·026	—	—
Ryffel	0·019	+0·004	0·025	+0·021	+0·048
Mathison	0·013	−0·005	0·016	—	—

* The acidity is expressed in terms of lactic acid.

From these figures it will appear that the lactic acid at Pisa and Col d'Olen was practically the same, whilst at the Capanna Margherita there was a sensible lactic acidosis.

Our Monte Rosa expedition therefore left us in the following position. We found:

(1) In common with previous workers that the higher the altitude the less CO_2 in the alveolar air and presumably less in the blood.

(2) The higher the altitude the more marked the acidosis in the blood when the CO_2 is shaken out[4].

(3) At any altitude the acidosis and the diminution of CO_2 so nearly balanced one another that the reaction of the blood remains practically constant and the dissociation curve is therefore mesectic. This is true at all events on a first approximation. It is only by a statistical treatment of a great number of determinations[5] that a degree of meionexy, corresponding to a fall of about 7 % in K, may be discovered—sufficient to give the respiratory centre the slight stimulation[6] which would account for the increased ventilation observed.

Every man at rest has therefore a dissociation curve which remains approximately constant in spite of changes in those individual factors whose balance preserves the constancy of the curve, its form is a calculable quantity involving almost fixed values of n and K. You might label the man with these letters as a mark of his identity. What acid is responsible for the acidosis in the blood is yet to be ascertained, it is clearly not lactic acid or any of its close relations; on the other hand

it may be that there is no increase of acid at all but rather a diminution in the amount of alkali present. From the point of view of the dissociation curve the matter is of little interest, but from a wider point of view it is of great interest; so far we are left without a knowledge of mechanism of the adaptation which we have discovered. The idea of oxygen want producing lactic acid is familiar enough; were the acid lactic, we should at once say that it had been produced in the tissues as the result of oxygen want—but we should be in another difficulty for lactic acid is secreted by the kidney. If we found it continuously in the blood we should expect to find it in largely increased quantities in the urine. Ryffel tested this point and did not find any great excess.

Normal urine, sleep	0·001	% lactic acid
Col d'Olen 9.15 p.m—7 a.m.	0·0012	,,

There seem to be two alternatives before us; one is that oxygen want under these circumstances produces acids in the tissues which are not readily excreted: the other is that oxygen want so affects the kidney that it excretes alkali more freely. Certainly Verzàr's experiments seemed to show that oxygen want did increase the activity of the kidney. We are now entirely in the region of speculation, however, so let us retrace our steps a little.

A few lines back we spoke of the altered condition of the blood as an "adaptation" to the altered conditions of barometric pressure. In doing so we introduced a fresh idea into our narrative, namely, that the change in the blood was beneficial to the organism. This is true; so far we have treated the matter merely as an interesting observation that the dissociation curve of the man remains constant; but the alteration of the individual factors while the balance is preserved leads to a very important result. Over any considerable interval of time there is always a certain relation between the CO_2 in the alveolar air and the oxygen in the same.

The proportion of the one to the other depends upon the respiratory quotient and ultimately upon the food that is eaten. If the CO_2 changes the oxygen will change, so that if C_V be the pressure of CO_2 in the alveolar air and C_A that in the atmospheric air, while O_V be the oxygen in the alveolar air and O_A that in the atmospheric air, $\frac{C_V - C_A}{O_A - O_V}$ will remain approximately* constant.

* The determinations of CO_2 pressure in the alveolar air were made at the end of expiration. To obtain the respiratory quotient more exactly a slight correction has

For the purpose of the present calculation C_A may be neglected, it is under half a millimetre and therefore we may say as an approximation that $\frac{C_V}{O_A - O_V}$ is constant.

Let us see by a specific example the bearing of this constancy on the question. In Teneriffe at the sea-level Douglas's alveolar CO_2 (C_V) pressure was 41 mm., his respiratory quotient was ·75, therefore $(O_A - O_V)$ was $\frac{41}{\cdot 75} = 51$. The value of O_A was 160 mm., or corrected for the pressure of aqueous vapour in the lung 150 mm., therefore $O_V = 99$ mm. Now at the Alta Vista hut, where the barometer was 514, the value of O_A corrected for aqueous vapour in the lung is 104. Had his CO_2 pressure been as before 41, $O_A - O_V$ would have been 51 and his alveolar oxygen pressure 53; but owing to the acclimatisation which we have described his CO_2 pressure dropped to 32, and therefore $O_A - O_V$ was $\frac{32}{\cdot 75} = 43$, instead of 53, therefore his alveolar oxygen pressure would work out at $104 - 43 = 61$ mm.* He had gained 10 mm. at this altitude, or one-sixth of his whole oxygen pressure by the change of the distribution of acids in his blood.

My own case afforded a control experiment. My carbonic acid was scarcely altered as between the sea-level and 11,000 feet in Teneriffe, with the result that my oxygen pressure at that altitude was only about 49—50 mm., while that of Douglas was 61, a fact which made a great difference to our comparative comforts at the Alta Vista hut. This again was controlled by another observation. For reasons of which I shall speak elsewhere, at Col d'Olen my alveolar CO_2 did drop to 33 mm., my oxygen did reach 64 mm., whilst later at Col d'Olen, after I had been up to the Margherita hut, where my blood had acquired another dose of acid, displacing more CO_2, and had retained it after my return, my alveolar CO_2 pressure was only 30 mm. and my oxygen up to 70 mm. Therefore, even allowing a mm. or two for experimental error, my oxygen pressure was half as much again as it had been at the Alta Vista. On my dissociation curve the difference between 70 and 50 mm. is the difference between

to be made. This may be done from a table found in the appendix copied from Haldane's "Methods of Air Analysis."

* These calculated values agreed very closely with the observed values, which were 58·5—62·5 mm.

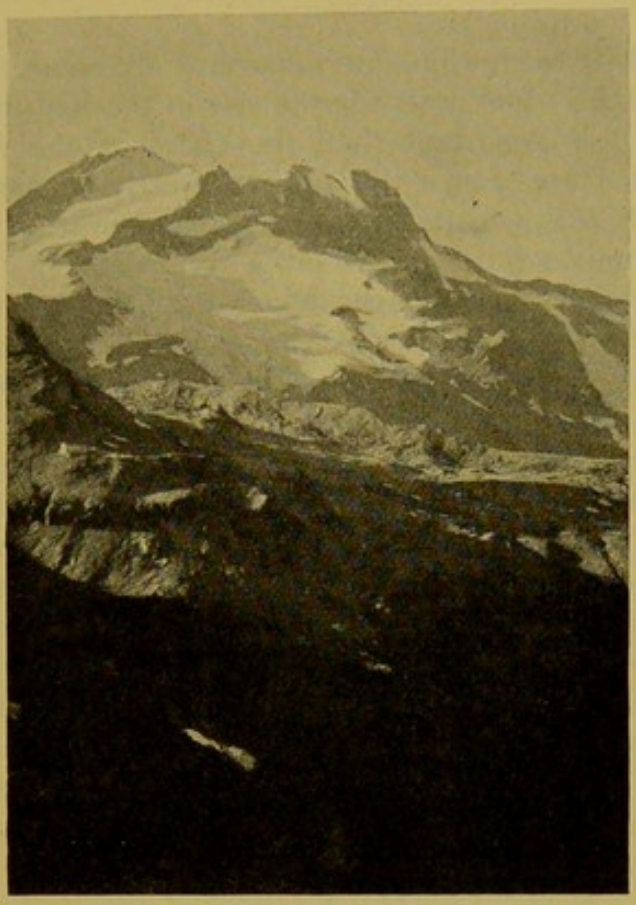


Fig. 127 *a*.—Col d'Olen showing track. (Durig.)

Fig. 127 *b*.—Hut at Col d'Olen. (Aggazzotti.)

85 °/₀ and 93 °/₀ saturation, an important difference in the saturation attainable. But probably the rise in the limiting percentage saturation is less important than the effect on the rate at which oxygen is taken up. Since this rate is directly proportional to the pressure of the oxygen, the oxygen is taken up nearly half as fast again in the lung of the acclimatised person.

There are two points in the above argument which have been passed over rather lightly because the argument itself only demanded the mere mention of them. Yet they are both of considerable interest on their own account. They are the following: firstly, why did I become acclimatised at once on Monte Rosa when I did not change at Teneriffe? and secondly, why did Douglas acclimatise at Teneriffe when I did not do so? The fundamental principle is that the acclimatisation is due to oxygen want. In order that you should acclimatise satisfactorily you must do so gradually, and therefore court oxygen want from the beginning of the ascent, then the acid gradually finds its way into the blood and the CO_2 gradually finds its way out. The most satisfactory way of doing this is to walk up the mountain. The difference between my ascent of Monte Rosa and that of the Peak was that the former was made on foot, the latter on a mule. As regards the acidosis when I reached Col d'Olen there was no doubt. Some of my blood was taken directly I arrived there; it was analysed by Mathison and contained the equivalent of 0·42 °/₀ lactic acid. The following figures, obtained with Roberts' blood and my own, illustrate this point.

Equivalents of lactic acid in blood.

	Barcroft	Roberts	Mathison
Reaching Col d' Olen	0·042 °/₀	0·08 °/₀	—
Living at ,,	0·02	0·02	0·00 °/₀
Reaching Margherita Hut	0·054	0·09	—
Living at ,,	—	0·05	—
Living at Col d'Olen after return from Margherita	0·038	0·02	0·016 °/₀

At each effort of climbing then there was an acidosis which only partially disappeared in the ordinary life of the place. An interesting point was the fact which we discovered on coming down to Col d'Olen from the Margherita hut, that the acidosis which had been induced by going up there remained some time and materially strengthened our position owing to the lowering of the

CO_2 pressure and the corresponding rise in oxygen pressure which we have already discussed. The beneficial effect of exercise at high altitudes is of course commonplace amongst the persons who frequent mountains, and especially the benefit derived from going up a little higher than the point at which one is living, and then coming down again. Our results show that this benefit is no intangible affair which may be vaguely included under the general term "training," but that it is a very definite change in the blood of the individual which may be detected by the chemical analysis of that fluid.

There is no difficulty then in stating the reason why I should have become acclimatised when I walked up the mountain and not when I went up on the mule—in the former case the effort of the climb induced oxygen want and consequently acid production, in the latter case this element was absent.

To answer the other of the two questions set above, why did I not become accommodated at Teneriffe when Douglas did so? is at first sight more difficult, as the consideration of mountain sickness is bound up with that of exercise at high altitudes. I will postpone the answer till the next chapter.

REFERENCES

(1) Boycott and Chisholm, *Biochemical Journal*, v, p. 23, 1911.
(2) This research is published in abstract in the *Physiol. Proc.*; *Journal of Physiol.* XLV, p. xl, et seq. and will be sent for publication *in extenso* to the Philosophical Transactions of the Royal Society.
(3) Ryffel, *Physiol. Proc.*; *Journal of Physiol.* XXXIX, p. v, 1909.
(4) See also Aggazzotti, *Arch. ital. de biol.* XLVII, pp. 54, 66; Galleotti, *Arch. ital. de biol.* XLI, p. 80.
(5) Barcroft, *Physiol. Proc.*; *Journal of Physiol.* XLVI, p. XXX, 1913.
(6) Campbell, Douglas, Haldane and Hobson, *Journal of Physiol.* XLVI, p. 301, 1913.

CHAPTER XVIII

THE EFFECT OF ALTITUDE ON THE DISSOCIATION CURVE OF BLOOD CONSIDERED IN RELATION TO EXERCISE

In each of the two preceding chapters I have tried to draw a picture, in one of the man who is taking exercise at ordinary altitudes, in the other of the man who is at a high altitude but not taking exercise.

I have depicted the man who is taking exercise as meionectic; his blood is unusually acid, it takes up oxygen less readily than usual, it parts with it more readily, while at the same time the change in the reaction of the blood quickens the respiration and the heart beat.

I have pictured the man living at a high altitude as possessing blood of usual or almost usual reaction. Mesectic, or nearly so, his blood contains an unusually small quantity of carbonic acid and an unusually large quantity of other acid radicles. The diminution of carbonic acid in the blood is reflected in the alveolar air with the result that CO_2 pressure in the pulmonary alveoli is unusually low and consequently the oxygen pressure is higher than it would otherwise be.

In the present chapter I wish to consider the effect of altitude upon exercise. In a couple of words it is this: a given degree of meionexy would be produced by a lesser amount of exercise at a high altitude than at a low one, or, to put the matter in another way, a given amount of exercise would produce a greater degree of meionexy at a higher altitude than it would at a low one.

We shall now give some account of the experiments on results of which these statements are based.

Our party had two mountain tracks as similar to one another as we could make them, each was a climb of 1000 feet. The low level course was at Carlingford, co. Louth. It has already been described. It started from practically the level of the sea. Our high level course

commenced at a point 1000 feet below Col d'Olen and ended at the Laboratory. It extended from an altitude of 9000 feet to one of 10,000. Our scheme was to go down 1000 feet from Col

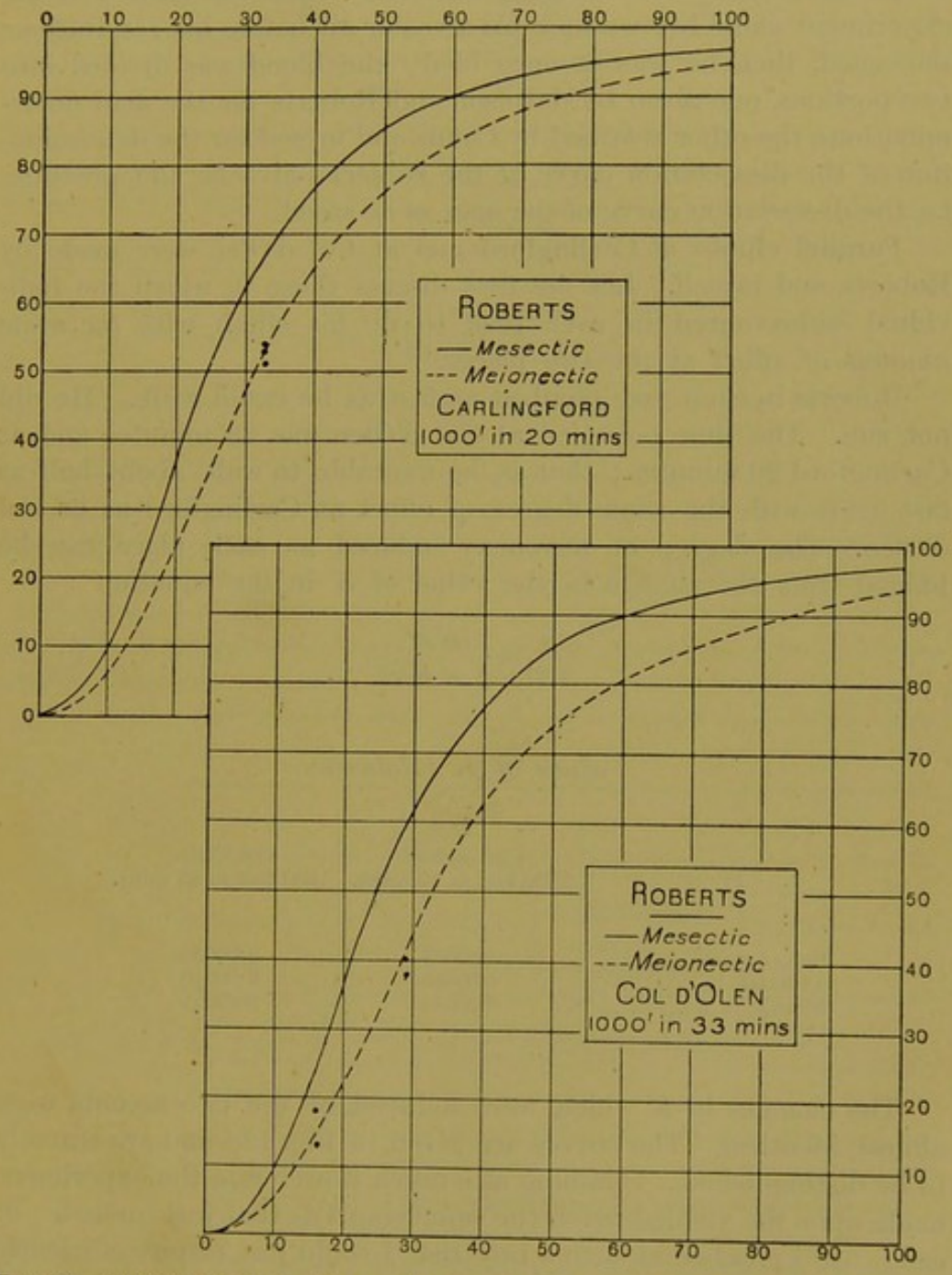


FIG. 128.—Showing approximately equal change in the dissociation curve as the results of 1000 feet climb near sea-level in 20 minutes and in the high Alps in 33 minutes.

d'Olen and ascend at the required speed. The ascent is a steep mountain path all the way from a small pond, right up to the Albergo: thence a few minutes' walk over some rocks to the right brought us to the Laboratory. Everything was ready when the subject of the experiment came in; we took his alveolar air before his respirations slackened, then he was at once bled; the blood was divided into two portions, one given to Mathison and Roberts for the acid determinations, the other retained by Camis and myself for the determination of the dissociation curve at the subject's alveolar CO_2 pressure, i.e. the dissociation curve of the man as he stood.

Parallel climbs at Carlingford and at Col d'Olen were made by Roberts and myself. Let me first discuss those in which the individual endeavoured in each case to do his climb *with the same amount of effort* at the two places.

Roberts in each case climbed as fast as he could walk. He did not run. The time occupied at Col d'Olen was 33 minutes and at Carlingford 20 minutes; that is, he was able to walk about half as fast again with the same degree of effort at Carlingford as at Col d'Olen. The degree of meionexy induced at each place can be judged from the change in the value of K in the equation

$$\frac{y}{100} = \frac{Kx^n}{1 + Kx^n}.$$

Values of K (Roberts).

	Carlingford 1000 feet in 20 mins.	Col d'Olen 1000 feet in 33 mins.
Before start	0·00033	0·00033
At finish	0·00018	0·00016
Change in K.....	0·00015	0·00017

The changes in K which were induced by the two ascents were almost identical. The curves are given in Fig. 128 and are scarcely to be distinguished. Climbing at a much slower rate the experiment made upon me yielded much the same result as that just quoted. In each case I climbed at such a rate that I could just respire efficiently without departing from nasal breathing; had I gone faster I should have had to breathe through my mouth.

The dissociation curves are given in Fig. 129 and were, as in Roberts' case, almost identical at Col d'Olen and at Carlingford.

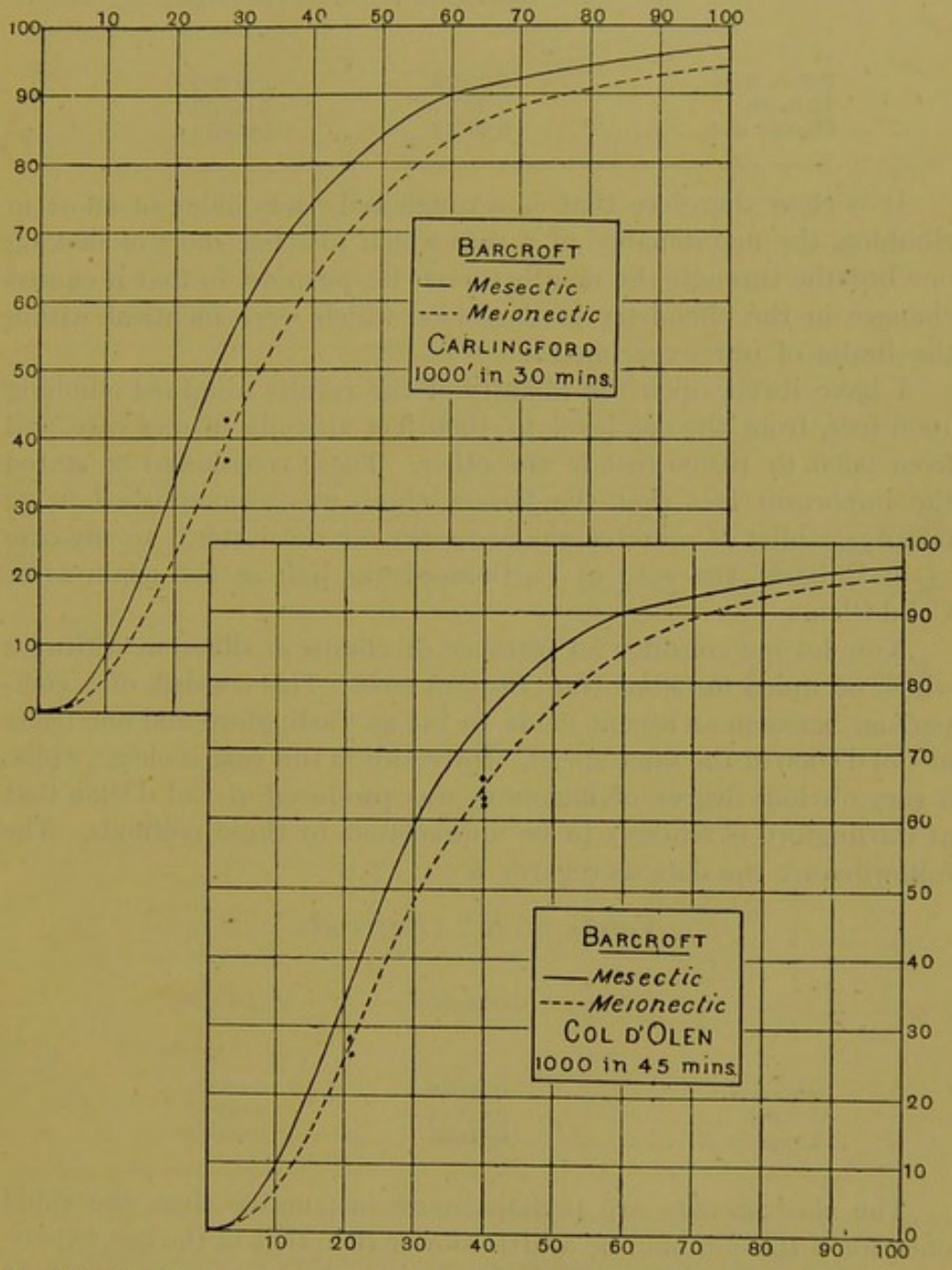


FIG. 129.—Showing approximately equal change in the dissociation curve as the result of 1000 feet climb near sea-level in 30 minutes and in the high Alps in 45 minutes.

Values of K (Barcroft).*

	Carlingford	Col d'Olen
Before start	0·00029	0·00029
At finish	0·00017	0·00019
Change in *K*.....	0·00012	0·00010

It is clear therefore that as a rough and ready index of effort in climbing, the maintenance of a rate which just fell short of making one breathe through the mouth served its purpose, in that it caused changes in the chemistry of the blood which were identical within the limits of our experiments.

I have dwelt upon the identity of the results obtained climbing 1000 feet, from the sea level to 1000 feet altitude in one case, and from 9000 to 10,000 feet in the other. There remains to be stated the important fact that the former climb was accomplished in 30 minutes, whilst 45 minutes was necessary for the latter. In my case as in Roberts' the rate at Carlingford was half as fast again as at Col d'Olen.

Now let me consider an instance of climbs at different altitudes which occupied *the same time in each case.* This consists of a comparison between an ascent made by me at Carlingford and one made at Col d'Olen at the same speed. The result in this case is clear: whilst a very obvious degree of meionexy was produced at Col d'Olen that at Carlingford is scarcely to be appreciated by these methods. The following are the data as regards *K* :

Values of K (Barcroft).*

	Carlingford	Col d'Olen
Before start	0·00029	0·00029
At finish	0·00024	0·00017
Change in *K*.....	0·00005	0·00012

The slow ascents are perhaps more instructive than the rapid ones, for if the climbing be a little slower than that of the last experiment which I have mentioned, the degree of meionexy produced at

* For hydrogen ion values see Appendix III.

low altitudes will become inappreciable. Not so at high altitudes, however. This was most strikingly shown on our ascent from Col

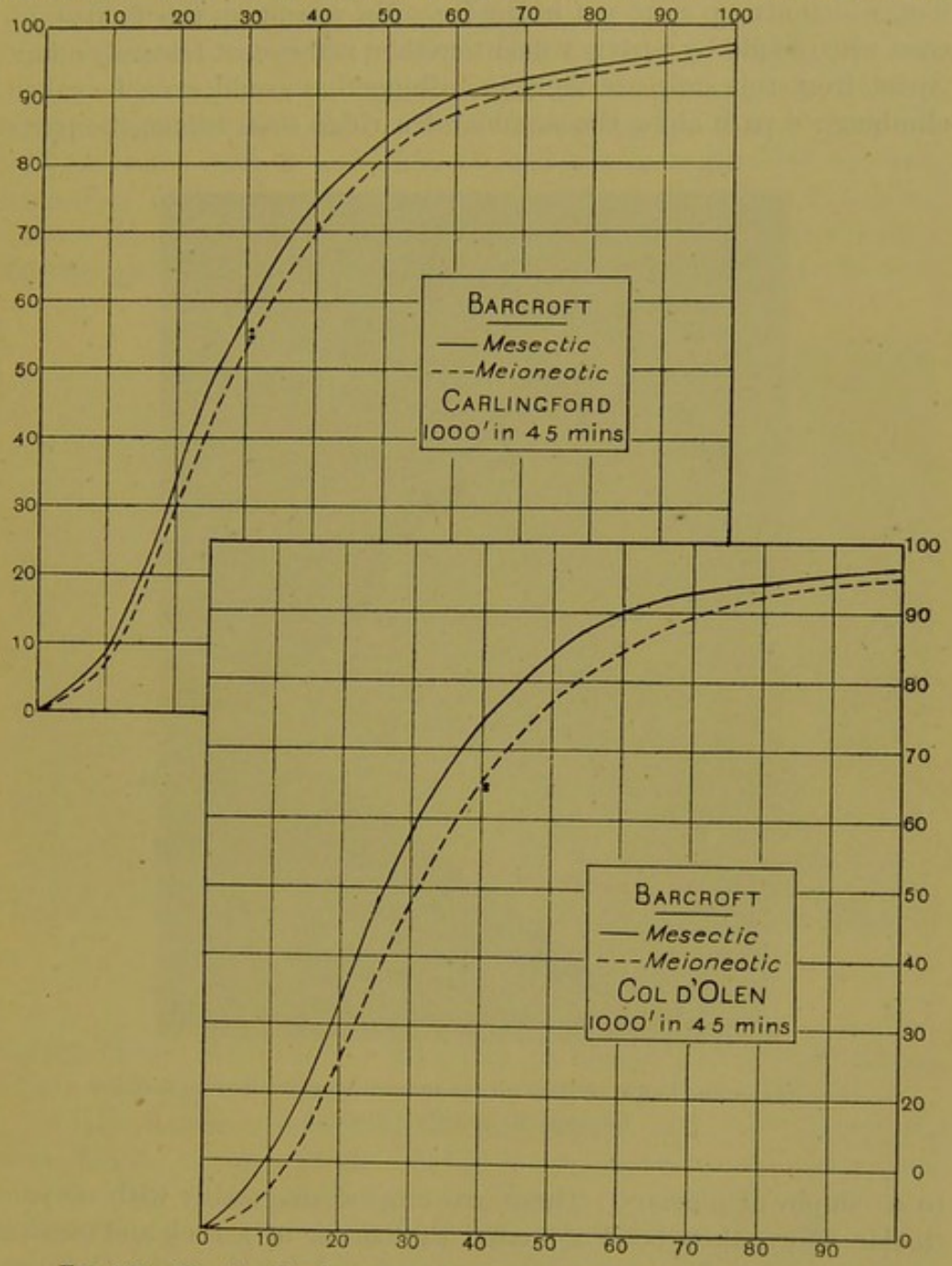


FIG. 130.—Showing the changes in the dissociation curve which result from a climb of 1000 feet near the sea-level and in the high Alps.

d'Olen to the Margherita hut. It is an ascent from an altitude of 10,000 to one of 15,000 feet. For the ascent we took about eight

hours. This time included a rest of an hour at the Capanna Gnifetti. The gradient does not become steep until the last 1500 feet, which are up steps cut in the ice. The ascent up this final staircase, when made by a party roped together, is the most leisurely affair. Apart from this staircase there is nothing that could even be called climbing : a path along the summit of a ridge soon brings the party

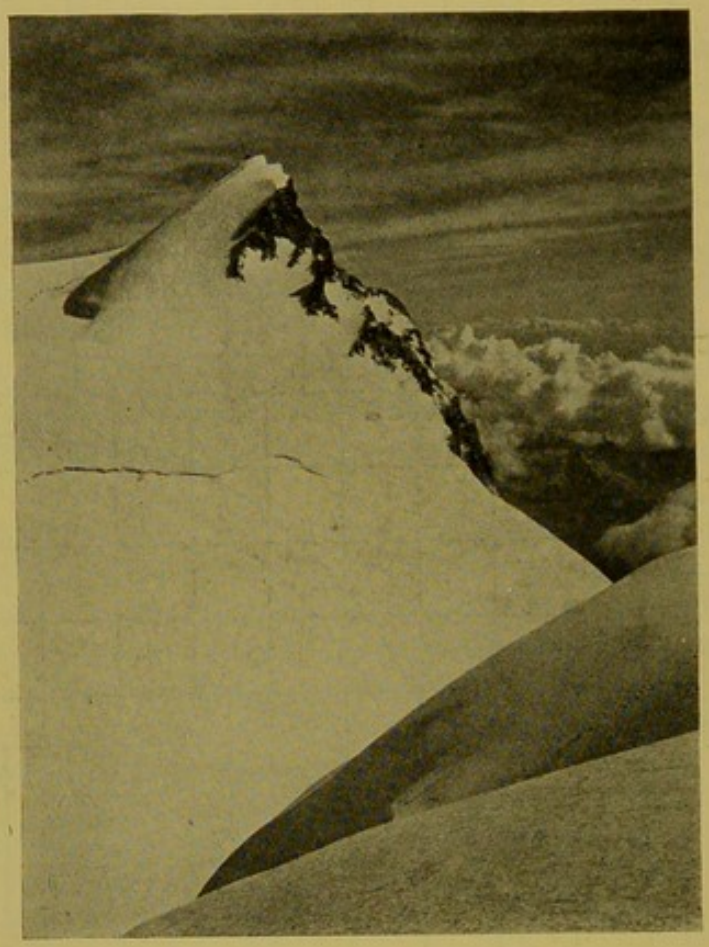


Fig. 131.—The Punta Gnifetti on the summit of which may be seen the Capanna Margherita. (Durig.)

to a couple of glaciers. These are crossed diagonally with no particular effort, then bending round the corner of a rock and making an ascent of a few metres the party found itself at the Capanna Gnifetti, the point at which the route to Zermatt diverges from that which leads to the summit. After this point it is all snow and ice. Immediately in front of the party is the summit of Lyskamm, that

conical peak of pure white which commands the Gressoney Valley. To the right of this was our way, up a snow slope and still up, till we reached the Lysjoch glacier, and then we were on a vast plateau from which rise the peaks which form the crown of the Monte Rosa group. Lyskamm was on the left, the punta Parrot, the punta Gnifetti, the Ludwigshohe, and the Pyramid Vincent on the right; for about half an hour's walk it was level, though the snow entailed a certain amount of effort. Looking down to the left, past Lyskamm, was the Görner Grat and Monte Cervino (Matterhorn) beyond. Our height may be grasped from the fact that we were then about the level of

Fig. 132.—The Capanna Margherita. (Aggazzotti.)

the top of the Matterhorn. Suddenly we turned to the right and as I have said crept slowly up the punta Gnifetti to the summit.

Whilst I cannot hope that my description will really bring the scene before the eye of the reader, it will suffice to show that such a walk would be an intolerably tedious stroll at ordinary levels. Yet it taxed our powers to the full. I cannot for a moment suppose that any appreciable degree of meionexy would be induced by a walk from say Fort William to the top of Ben Nevis in seven or eight or nine hours. The only symptoms from which the pedestrian would suffer would probably be those of cold. Not so when we arrived at

the Margherita hut. At these rates of climbing the effect of altitude to all intents and purposes is quantitative, the effect is clear at 15,000 feet, it is not to be found at low levels. It is in fact an acid intoxication ; its extent may be gathered from the two sets of curves given in Fig. 134. They are those of Roberts and myself. At the present time they are the record cases of meionexy.

To pass from the actual degree of meionexy produced, let me treat of the degree of acidosis, confining this term to the appearance of unusual acids in the blood. We must ask two questions :

(i) To what extent did acid radicles appear in the blood ?

(ii) What were the acid radicles which appeared ?

Fig. 133.—View of Matterhorn from the Capanna Margherita at sunset. (Durig.)

Here again we must recapitulate what has been said about acidosis in the last two chapters. The acidosis due to exercise appears to be a lactic acidosis, that due to altitude does not appear to be a lactic acidosis at altitudes of 10,000 feet, though at altitudes of 15,000 feet there is some degree of lactic acidosis. As regards the degree of acidosis the best determinations are those performed on Mathison and on Camis, but especially the former, and this for the following reasons : firstly Mathison started his climb free from acidosis both at Col d'Olen and at the commencement of his low level station, which was the Sugar Loaf at Abergavenny ; secondly his climb was in each case a very strenuous one, 1000 feet in 20—21 mins. The data are as follows :

Mathison, acidosis expressed in equivalents of lactic acid in blood.

	Before climb	After climb	Due to climb
Abergavenny	0·000	0·021	0·021
Col d'Olen	0·000	0·040	0·040

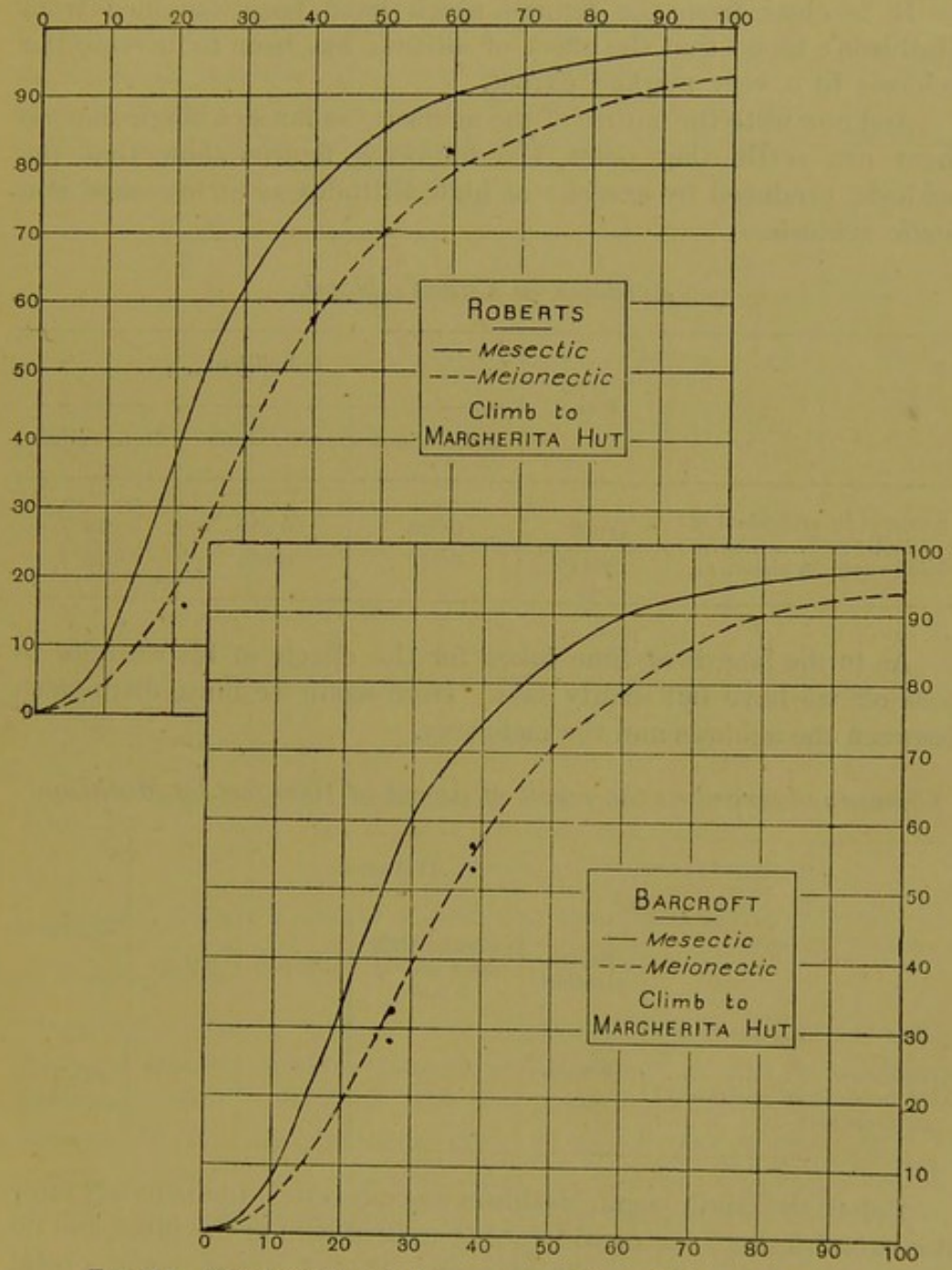


FIG. 134.—Changes in dissociation curve caused by walk from Col d'Olen to the Margherita hut.

Camis, acidosis expressed in equivalents of lactic acid in blood.

	Before climb	After climb	Due to climb
Col d'Olen	0·023	0·051	0·028

It is clear from the figures which have been obtained from Mathison's blood that the effect of altitude has been to increase the acidosis to a very marked extent.

And now as to the nature of the acidosis; so far as a single experiment can settle that point, the following figures show that the acidosis, produced by *exercise* at high altitudes as at low ones, is a *lactic* acidosis.

Acidosis in Camis's blood.

	Pisa	Col d'Olen		
		Before climb	After climb	Due to climb
Acidosis in equivalent of lactic acid.....	0·000	0·023	0·051	0·028 %
Lactic acid in blood.....	0·014	0·017	0·043	0·026 %

As to the length of time taken for the effects of his exercise to pass off we have but scanty data. Here again we must distinguish between the acidosis and the meionexy.

Changes observed as the result of ascent of 1000 *feet by Mathison.*

	11 August				13 August
	Before starting	Immediately after arrival 6 p.m.	6.20 p.m.	8 p.m.	
Alveolar air.....	36 mm.	37 mm.	27 mm.	37 mm.	—
Acid in blood in equivalent of lactic acid	·00	·040	·040	·018	·011

Before the climb began Mathison was mesectic. Immediately after the exercise there was considerable excess of acid in the blood and no diminution of CO_2 in the alveolar air. This of course meant a total reduction in the alkalinity of the blood, and consequently a meionectic

condition, the physical signs of which were increased frequency of the pulse and violent panting. The effect on the dissociation curve is typical. The curve was pushed to the right. Mathison's normal dissociation curve as determined before going to Col d'Olen is shown in Fig. 135. It has the equation $y = 100 \frac{Kx^n}{1 + Kx^n}$, the constants being $K = \cdot 000212$, $n = 2 \cdot 5$. The curve obtained at Col d'Olen immediately after returning from the climb is also shown, together with the points upon it which were actually determined. It has the following constants : $n = 2 \cdot 5$, $K = \cdot 000140$.

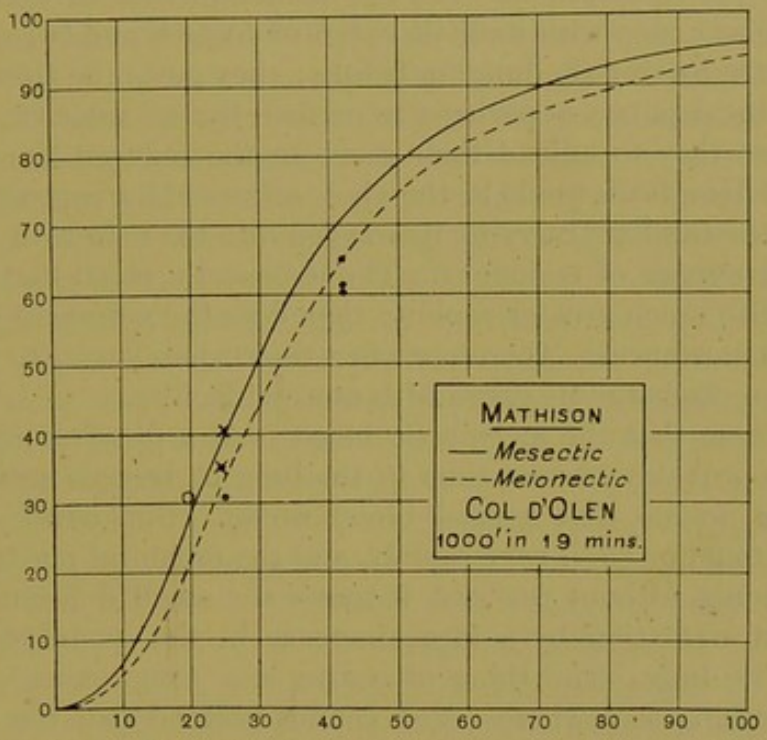


FIG. 135.—Changes in Mathison's dissociation curve caused by climbing 1000 feet in 19 minutes, and their disappearance. • 5 mins. × 20 mins. o 2 hours after exercise.

In twenty minutes Mathison's alveolar carbonic acid pressure had dropped from 37 mm. to 27 mm., and a couple of determinations of his dissociation curve under the new conditions indicated that it was mesectic within the limits of error of the determinations, and by 8 p.m., two hours and a quarter after the experiments, he had settled down to his permanent condition. There was an acidosis equivalent to ·018 % of lactic acid, but from this he scarcely, as far as is known, receded while he was at Col d'Olen.

And now to turn to the physiological significance of the meionexy and of the acidosis.

The essence of any mechanism of adaptation to high altitudes must be a "speeding up" of the whole process of respiration, physical and chemical. The blood in the lung is exposed to a lower oxygen pressure than usual; it is saturated to a less extent with oxygen than formerly. The tissues begin to suffer from oxygen want: a very trifling change in the blood is enough to produce a great change in the circulatory conditions; the pulse quickens, respiration becomes deeper and more rapid, the amount of blood which leaves the lung increases perhaps twofold. Let the reader picture to himself each corpuscle as a ship with its little cargo of oxygen and twice as many of these are leaving the lung as before; they go to the tissue and go through the capillary at perhaps twice their former velocity; but stay —how are they to unload their cargo in the reduced time at their disposal? How futile would be the whole scheme if the corpuscle bolted through the capillary carrying its oxygen into the vein with it. Here is the advantage of meionexy. The meionectic blood parts with its oxygen with much greater rapidity than does the normal blood under given circumstances. Therefore when the corpuscle gets to the capillary it can discharge its cargo with unusual facility.

It is true that the meionectic blood is at a disadvantage in the lung. It probably has not time in the lung to become saturated to quite the degree that normal blood would. But blood saturates itself up to 80 or 85 % very rapidly, and the organism must take the risk of doing without the rest, it meets the small deficiency in the degree of oxidation by a large increase in the quantity of blood leaving the lung. The thing of course is a compromise, you must lose something somewhere. You cannot pretend that the organism is not working at a disadvantage at an altitude of 15,000 feet. It is making the best of a bad business. The best it can make is meionexy. Meionexy involves stimulation of the neuro-muscular mechanism of respiration and quickening of the proportionate rate at which the blood loses oxygen in the tissues.

But at slow rates of climbing the degree of acidosis is much greater than the degree of meionexy. Including the carbonic acid the blood becomes to a small extent more acid than formerly, but the blood becomes much richer in other acids and poorer in CO_2 than before. The carbonic acid is displaced in the blood and after a preliminary rise it ultimately decreases in the alveolar air. Therefore the

oxygen pressure in the alveolar air is higher than it would otherwise be. This relative increase in the oxygen pressure has a twofold effect—it increases the rate at which the blood can acquire oxygen in the lung and it increases the limiting percentage saturation. In effect it brings you thousands of feet down the mountain again. So even from the chemical point of view the blood is not under such bad conditions in the lung after all. The meionexy reduces affinity of the blood for oxygen, but indirectly betters the conditions for its acquisition. What it takes away with one hand it gives back with the other. It is not all loss in the lung and in the tissue it is all gain—to the organism.

The adaptation to altitude, being as I have said a compromise, must break down if the conditions become too severe, that is to say, if the altitude becomes too high for the necessary exercise, or the exercise too great for the altitude ; the result is mountain sickness. I have sometimes been asked the following question : If the effect of altitude is merely to produce a given degree of meionexy with a less degree of exercise, why are the final effects of exercise at low and high altitudes different? No one suffers from mountain sickness in say the University Sports, though the effects of meionexy are evident enough. Mountain sickness is doubtless caused by want of oxygen in the brain itself. It is not merely that the vomiting centre is stimulated by a meionectic condition of the blood in the general circulation. At high altitudes even tissues which are comparatively inert are suffering to some extent from oxygen want ; or at least to prevent their so suffering there is some degree of dilatation. The result of this dilatation, combined with faulty oxygenation of the arterial blood, is to reduce the supply of oxygen of the medulla to a minimum. Then some trifling change takes place ; the wind meets you in the face and you hold your breath ; the digestion of food, or perhaps even the contemplation of it, sends an extra supply of blood to the abdomen ; sleeps comes and the blood tends to leave his brain. Any of these will make you feel sick. The actual symptoms of mountain sickness resemble those of haemorrhage rather than of exercise.

Is it possible to tell those who are likely to suffer from mountain sickness from those who are not? It is difficult to say without studying a much larger number of cases than those which have already been subjects of research. In Teneriffe[1] the individuals whose bloods had the smallest affinity for oxygen at a constant CO_2 pressure suffered least from mountain sickness. Zuntz' and Douglas' blood,

for instance, both had a value for K at 40 mm. CO_2, which was about ·000212, mine was ·000292. Neither of these suffered in the least from mountain sickness; I did, though it never actually amounted to vomiting. In the case of Douglas' and my blood, these values for K represented our actual curves. It appeared that Douglas' more gradual dissociation curve meant a more gradual degree of adaptation. I did not begin to adapt till my alveolar oxygen pressure was 10 mm. below that of Douglas, mine being about 50 mm. of oxygen and his being 60 mm.

At that pressure the percentage saturation in each of our bloods was roughly the same, but I was in a very much worse position than Douglas

(1) Because a trifling further drop in the oxygen pressure in the blood meant a large drop in percentage saturation in my case and a small one in Douglas' case.

(2) Because 60 mm. oxygen as compared to 50 meant that, other things being equal, oxygen could be taken up more rapidly in the ratio of 6/5.

(3) Such a trifling drop in the oxygen pressure to which the actual blood was exposed might be due simply to an increase in the pressure gradient between the outside and the inside of the lung epithelium. An increase of this sort would be the direct result of a little exercise.

Zuntz' case was a little different from that of Douglas. It is true that with the same CO_2 pressure their bloods gave the same dissociation curves, but in point of fact Zuntz had a less alveolar CO_2 pressure and his dissociation curve at this pressure was more like mine than it was like Douglas'. But the lower CO_2 pressure gave him the advantage over me, for it meant of course a higher oxygen pressure.

Between the dates of the Teneriffe and Monte Rosa expeditions two mountaineers of eminence were kind enough to give me blood for analysis, these were Oscar Eckenstein, who claims to have lived at an altitude of 20,000 feet longer than any other man, and Longstaffe, the Himalayan explorer. The fact that the blood of these two persons, when exposed to the standard CO_2 pressure, gave the lower values for K than any other human blood that I have analysed, was an interesting confirmation of my theory.

It became a matter of some little interest to me at Pisa to speculate as to which of the members of the Monte Rosa party would stand the high altitude best. The five persons fell into three groups.

In the first was Mathison, whose blood at 40 mm. gave a value for $K = \cdot 000212$. In the next were Camis, Roberts and myself, K ·00029—·00033, and lastly Ryffel, whose blood had a value $> \cdot 00037$. According to the theory then we predicted that Mathison should suffer least, Ryffel most, and the rest of us should occupy an intermediate position, and whether by coincidence or otherwise our classification proved to be correct.

REFERENCES

The equations of the dissociation curves discussed in the above chapter were published in the *Physiol. Soc. Proc.*, January 1913; the data of acidosis in the Report of the Committee on High Altitudes, British Association *Proc.* 1911.

(1) *Journal of Physiology*, XLII, p. 43, 1911.

CHAPTER XIX

SOME CLINICAL ASPECTS

My concluding chapter will tell of a couple of researches, one at University College Hospital [1], the other at Guy's Hospital [2], in which the dissociation curve "used as an indicator" has revealed new facts with regard to disease and done something to shed a new light on certain pathological conditions.

The first of these researches with which I will deal has been carried out by my two former colleagues, Ryffel and Poulton. Their concern was to explain the dyspnoea which takes place during uraemia. Naturally they pursued the methods with which the reader is now already familiar, the methods which had already given positive results at Carlingford, on Monte Rosa and in the Alta Vista hut. These methods showed clearly enough that the dissociation curve of the patient had shifted in the direction of meionexy, a result which forms the counterpart of the discovery by Schlayer and Straub—the latter an old member of "the firm"—that the alveolar carbonic acid sank during uraemia.

Expressing the degree of meionexy by the depression in the value of K, the reader will see from the following table that the uraemic patients were very meionectic. He must bear in mind that the normal limits of $K \times 10^4$ for active persons are $3{\cdot}6 - 2{\cdot}1$, whilst for middle-aged patients, who may be regarded as control cases, $K \times 10^4$ varies within even narrower limits, $3{\cdot}4 - 2{\cdot}6$.

Case	Age	Alveolar air		Blood				
		CO_2 mm.	O_2 mm.	Dissociation curve O_2 mm.	Saturation per cent.	$K \times 10^4$	Urea per cent.	Lactic acid per cent.
I	51	25	119	40	61	1·57	0·36	0·018
II	21	14	132	29·4	44	1·67	0·34	0·018
III	29	24·5	119	27·4	32·4	1·22	0·30	0·023
IV	61	24	121	26·3	34	1·46	0·21	0·030
Normal	—	35—45	—	30	51—63	3·6—2·1	0·02*	0·015—0·02

* On hospital diet.

Schlayer and Straub's paper, in which they described the low alveolar pressure of CO_2 in uraemic cases, bore the title "Is uraemia an acidosis?" The answer to that at the present time, in so far as it can be given, is as follows. It is not a lactic acidosis, yet the change in the value of K in the equation

$$\frac{y}{100} = \frac{Kx^n}{1 + Kx^n},$$

is such as would be produced by the addition of acid to the blood, and as far as is known this change can be produced in no other way. The addition of urea to the blood does not reduce the value of K. Uraemia therefore is accompanied by a change in the reaction of the blood in the acid direction. This change is not due to excess of CO_2 but to excess of other acids. In this sense it is an acidosis.

Now to turn from uraemia to the class of cases on which I myself have worked. I came upon them in this way. Dr Thomas Lewis, Physician to University College Hospital, London, told me of certain cases of dyspnoea in which the distress was unaccompanied by cyanosis or other physical signs of a sufficiently grave character to account for the degree of dyspnoea. In the absence of sufficient physical signs he inquired if there were chemical signs to which the dyspnoea could be attributed. After a few preliminary experiments we determined to make an investigation on the following lines.

Lewis undertook the selection of cases and their treatment; Ryffel, the estimation of lactic acid in the blood and of abnormal organic acids in the urine; Wolf, the analysis of the blood for urea, ammonia and "rest" nitrogen; Cotton, the analysis of the blood for urea (hypobromite) and the determination of the constant of Ambard*; whilst the tests for meionexy and acidosis and the alveolar air determinations were undertaken by me.

The reader will get the most clear account of these cases of dyspnoea by reading the reports on a typical case. From these he will see that there is:

(1) Considerable acidosis (in the sense in which I have defined the word).

(2) Low alveolar CO_2 and respiratory quotient.

(3) Meionexy.

It is clear then that the dyspnoea is explained: there is no further mystery about it. The meionexy sufficiently accounts for it.

* See Appendix IV.

(4) There is no increase in the amount of lactic acid in the blood. It is about equivalent to the lactic acid found in control cases.

(5) There is no increase in other organic acids above those present in control cases. But after the CO_2 has been shaken out there is a change in the balance of acids to bases in the blood in the acid direction.

(6) The reports on the urea and other forms of nitrogen in the blood and urine have turned out negative.

The following is a report of a typical case.

CLINICAL REPORT

H. L. (Dr Lewis.)

A married woman of 66 years, admitted to hospital on April 22nd, 1913, complaining of great shortness of breath, vomiting and pains in the sternal region.

History. Several members of the family have died of tuberculosis. There is no history of past illness. She had one child (now dead); there have been no miscarriages. The illness began two years before with shortness of breath and this has increased; it has been continuous and is increased by exercise. For several weeks frequent vomiting and pain in the sternal region have been present. She has had a cough for years and there has been a good deal of expectoration; attacks of palpitation and giddiness are common. Her appetite is poor; she has been wasting a good deal. Sleep is very disturbed; she says she wakes repeatedly in the night with a feeling of suffocation. She has had to get up to micturate four or five times each night for two years.

Condition on May 5th, 1913. A frail woman (weight 8 st. 4 lbs.), who sits propped up in bed with pillows. Modified Cheyne Stokes breathing is present; the hyperpnoeic periods are very long (85 seconds, approximate rate 45 to 60), the breaths gasping. During the hyperpnoeic period and especially towards the end of it the lips and tongue are pink or but very slightly cyanosed, during the intermediate periods the breathing is slower (rate 33), shallow and very irregular (from curves taken 1st May). During the apnoeic period, the lips and tongue are but very slightly or only moderately cyanosed. The hands are cold and moderately blue; the finger tips are clubbed. There is a slight yellowish tinge of the conjunctivae and skin; the vessels of the cheek are injected. Dropsy is present in the legs. The right apex of the *lungs* is dull, there are no crepitations. No retinitis.

The *heart's* limits of dullness are increased ($1\frac{3}{4}$ and 7 inches to right and left of the mid line); the apex beat is in the 7th left interspace in the anterior axillary line. A systolic murmur is heard at the apex; the second sound is reduplicated and intensified at the aortic cartilage. The arteries seem a little thickened, the blood pressure is 150 mm. Hg; the pulse rate is 90, extra systoles are present and a trace of alternation follows them.

The liver is enlarged, the edge is hard and feels nodular, and there is tenderness to pressure over it; there is a suspicion of some ascites.

The urine flow is not free; sp. gr. 1017, dark, acid with faint smell of (?) acetone. Albumen and granular casts are present. She has leucorrhoea.

May 5th, 1913. (Dr Cotton's report.)

T (duration of observation, minutes)	65 mm.	D_{25}	$\frac{11{\cdot}97 \times \sqrt{36{\cdot}06}}{5} = 14{\cdot}36$
v (vol. of urine in time T)	15		
V (calculated vol. of urine in 24 hours)	332 c.c.	urea in blood (grs. per litre)	·33
C (concen. of urea in grs. per litre)	36·06		
D (urea in 24 hours)	11·97	K	$\frac{{\cdot}33}{3{\cdot}78} = {\cdot}087$ *.

May 5th, 1913. (Dr Gloyn's report.)

Red blood cells	3,300,000
Haemoglobin	82 °/₀
Colour Index	·9 (nearly)

Red cells showed marked poikelocytosis.

May 5th, 1913. (Dr Ryffel's report.)

Blood: 0·030 grm. lactic acid per 100 c.c.
Excess about 0·015 grm. per 100 c.c.

Urine: day's volume 385 c.c., sp. gr. 1022.
No acetone. Marked excess urobilin. Alb. 0·7 °/₀.
Total N. 1·432 per cent., 5·51 grms. per diem.
Ammonia N. 0·27 grm. per diem.

$\frac{\text{Amm. N}}{\text{Total N}} = 4{\cdot}8$ °/₀.

Total acidity. 279 c.c. $\frac{N}{10}$ per diem.

Lactic acid 0·0062 per cent., 0·024 grm. per diem.
Large deposit of urates and some uric acid crystals.

May 5th, 1913. (Mr Barcroft's report.)

Alveolar CO_2	31 mm.	31 mm. (abnormally low)
Respiratory quotient (corrected)	·63	·53, ·61 (abnormally low)
Blood acidosis	Saturation at 17 mm. 44 °/₀, abnormally low. Evidence of considerable acidosis.	
Meionexy	Gas in tonometer, O_2 pressure 30 mm.	
	,, ,, CO_2 ,,	29 mm.

Saturation of blood exposed to this gas 38·5 °/₀.
Normal limits of saturation at 30 mm. 51—64.
$K = {\cdot}000127$.
Very meionectic—attributable to considerable acidosis.

May 23rd, 1913. (Dr Lewis' report.)

More specimens taken. In much the same condition as regards breathing. Getting weaker. Cyanosis, as before, very slight.

June 11th, 1913. Progressively weaker; evidently dying, but very slowly. C. S. breathing continues as before.

* See Appendix IV.

May 23rd, 1913. (Mr Barcroft's report.)

Alveolar air :	O_2	CO_2	mm.	R. Q. correct
	14·24	4·05	28·6	·56

Blood acidosis : Saturation at 17 mm. 43 °/₀ = ·04 lactic.
Meionexy : Saturation at 29·5 mm. O_2 and 27 mm. CO_2 47·5—48 °/₀.
K = ·00019.
Meionectic.

May 23rd, 1913. (Dr Cotton's report.)

Urea in blood ·78 grm. per litre.
Chlorides in blood 6·10 grms. per litre (normal quantity of chloride per litre is 5·62).

May 23rd, 1913. (Dr Gloyn's report.)

Haemoglobin = 90 °/₀.

With the case of H. L. may be compared that of another patient E. M. with distressed breathing which is of quite a different type. Here also there is meionexy to a slight extent but there is no acidosis, no fall in the CO_2 pressure in the alveoli, but rather a rise, and deep cyanosis. The meionexy is due to the rise in CO_2 which in turn is due to deficient circulation.

CARDIAC CONTROL

E. M. xiv/18. Age 29. (Dr Lewis' report.)

A married woman of 29 years, admitted to hospital for shortness of breath and swelling of the legs and stomach.

There is a history of St Vitus dance at the age of 6 years.

Her illness commenced 11 years ago when she began to get short of breath on exertion, about the same time the legs and abdomen began to swell; she has suffered from these symptoms off and on ever since, having been admitted on several occasions to hospital for exacerbations of the symptoms. On the present occasion the breathlessness has been considerable for 14 days and the swellings have been present for the same period. She has a cough with fairly copious, sometimes haemorrhagic sputum for several months. For two weeks occasional vomiting has been experienced.

Condition June 4th, 1913. A thin subject who sits propped up in bed. Cyanosis is deep, the lips being plum coloured and cheeks dusky and the ears tinged with blue. The conjunctivae and skin generally are tinged with urobilin. The respirations are hurried and irregular. The veins of the neck are very distended and pulsate freely. Slight oedema of the skin is present. Temperature is normal.

The cardiac impulse is very diffuse in the 5th, 6th and 7th spaces. The shock of the heart-beat is seen and felt over a wide area and there is epigastric thrust. An early diastolic or full diastolic thrill is palpable at the apex. R. L. C. D., 7 inches, L. L. C. D., 2 inches, U. L. C. D., 2nd rib. Early diastolic or full diastolic, and systolic murmurs are heard at the apex. The aortic and pulmonary sounds are normal. The

heart's action is grossly irregular. Rates 90—100. Auricular fibrillation is present. The liver and spleen are both enormously enlarged, the former reaching the umbilicus, is pulsatile, the latter occupies the greater part of the left flank.

A few crepitations are heard at the bases and in the interscapular region and axilla; sputum is still present.

The urine is dark of high sp. gr. (1026—35), blood was found in it on one occasion; it contains albumen, but no casts. Quantity reduced.

June 4th, 1913. (Dr Cotton's report.)

Chlorides in the blood, 6·90 grms. per litre (considerable retention, Normal 5·62).
Urea in the blood ·38 grm. per litre.
Red blood cells: 4,420,000.
Haemoglobin content: 92·5.

June 4th, 1913. (Mr Barcroft's report.)

Alveolar air:

O_2	CO_2	mm.	R.Q.
14·05	5·5	39	·77
12·51	6·0	43	·66
12·84	5·7	41	·67

Acidosis: Saturation at 17 mm. 71 °/₀ = O ·006—·008 °/₀ lactic.
Meionexy: Saturation at 31 mm. O_2 and 40 mm. CO_2 49 and 55...52 °/₀ average.
Value of *K*, ·000207.
Slightly meionectic.

June 4th, 1913. (Dr Wolf's report.)

Total non-protein nitrogen	per	100	c.c.	of blood	32·4 mg.
Urea	,,	,,	,,	,,	17·2 mg.
Rest	,,	,,	,,	,,	15·2 mg.

It seems then that these cases of dyspnoea of cardiac or renal origin may be split into two, and that from the obviously cyanotic cases there may be detached a definite type of clinical case in which dyspnoea is a prominent symptom but which differs from the majority of cases of dyspnoea in the absence of what may be regarded as equivalent cyanosis. Clinically and pathologically the cases show cardiac and renal degeneration, though the actual lesions are not destructive. Amongst the common symptoms are those already enumerated, namely, dyspnoea without, or with but a slight grade of, cyanosis, Cheyne-Stokes breathing, restiveness and a relatively high pulse frequency (both of which are more conspicuous in the evening), and also thirst. In its fully developed form it rapidly ends fatally. On the chemical side the symptoms are acidosis (in the sense of an increase of the ratio of acid to basic radicles), absence of abnormal nitrogenous metabolism, and meionexy.

To Lewis, the clinician of our party, I freely leave what is his due —the privilege of providing this particular "asthma" with a suitable

clinical adjective; and I will write it down here in the terms in which I think of it—"Lewis's dyspnoea."

Nevertheless there are certain broad aspects of these cases which seem to justify some comment. The prevalence of acids in the blood coupled with the absence of acids which are abnormal *in kind* suggests that the condition is due rather to renal than to metabolic disturbance.

Had we found excess of lactic we might have supposed that there was general oxygen want in the tissues, had we found β-oxybutyric we might have tried to link the condition with metabolic disorders, but the evidence so far as it goes is that the kidneys, instead of keeping the blood at a certain composition which we call normal keeps it at another and more acid character which we call abnormal. Such a change might be wrought by a functional renal disturbance so slight as to be quite remote from the region of a visible lesion of the kidney.

The analogy between the condition which we have been discussing and the condition of the body at altitudes of about 10,000 ft. is inevitable. Meionexy, fall in alveolar CO_2 pressure, increase in acid character of the blood, are the obvious points of resemblance.

In each case the immediate cause of the change in the blood seems to be renal, beyond this we cannot at present go.

In our study of the cases of Lewis's dyspnoea we compared them with various other cases which served as controls. In addition to cases of dyspnoea referable to evident cardiac trouble such as E. M., we compared cases of "Lewis's dyspnoea" with ones which had no dyspnoea but were in hospital for quite other reasons such as convalescent appendix or gastric cases. In these we found the value of K to be singularly constant, much more so than in the case of persons living an everyday life. This no doubt was due to the fact that these control cases were all of middle age or rather more and were living the same sort of life and eating the same sort of diet. The value of K varied from ·00024 to ·00034, being thus higher than in the cases of dyspnoea from whatever cause they arose.

But the most interesting controls were those of the patients suffering from "Lewis's dyspnoea" whose condition changed considerably whilst they were in our hands.

For instance one case J. P. was seen by me first on February 14, he was then suffering from the complaint, a month later he was sufficiently well to be discharged; just before this I saw him on March 13th. Within a month (April 7) he returned in a very grave condition and died that evening. The day before his death his blood was tested again.

The following changes in the value of K show the degree of meionexy which obtained in each case:

		K
	Normal limits of K	·00024—·00034
1.	J. P. 7 March, a week after admission ...	(1) ·00018 (2) ·00018
2.	,, 13 March, shortly before discharge ...	(1) ·00023
3.	,, 7 April, a few hours before death ...	(1) ·000082 (2) ·00011

Another case which showed variations was that of a female patient, M. P.

She was a mild case on May 23rd when first tested, by June 24th the dyspnoea had disappeared and the case was discharged.

			K
1.	23 May, dyspnoeic	(1)	·00020
2.	24 June, breathing normal	(2)	·000274

Not only had the value of K become normal but the alveolar CO_2 rose from 33 mm. to the normal value of 39 mm. and the blood in the absence of CO_2 on May 23rd was 61 °/₀ saturated with oxygen at 17 mm. oxygen pressure, whilst on June 24th the percentage saturation at 17 mm. had risen to the normal figure of 74 °/₀; the former corresponded to normal blood with ·01 °/₀ of lactic acid added.

One more point: in the account of the case of J. P., given above, when tested just before death the values of K given do not appear to be very concordant. The interpretation of this irregularity, which appears in *very* meionectic curves, e.g. Figs. 30 and 134, where the points observed are below the curve at low pressures and above it at high ones is, in the light of more recent* work, that blood has gained sufficient in acidity to cause a measurable change in the value of n, the average number of molecules of haemoglobin clumped together. The effect is of course in the direction of increased clumping of haemoglobin and, as in other cases of the same phenomenon, its significance lies in the fact that a similar clumping of other protein molecules probably takes place and alters the properties of every cell in the body by a reduction of the velocity of its molecular vibrations.

REFERENCES.

(1) Lewis, Ryffel, Wolf, Cotton and Barcroft, *Heart*, v, p. 45, 1913; *Journal of Physiol.* XLVI, p. liii.

(2) Poulton and Ryffel, *Physiol. Proc.*; *Journal of Physiol.* XLVI, p. xlviii.

* See Appendix II.

APPENDIX I. ON METHODS

ESTIMATION OF THE OXYGEN CAPACITY OF BLOOD BY THE FERRICYANIDE METHOD OF ESTIMATING OXYGEN IN BLOOD

The simplest measurement which it is possible to make of the oxygen in blood is that of the total oxygen capacity of the haemoglobin. For this measurement it is necessary to be careful about three things quite apart from apparatus.

(1) The blood must be fresh so that it does not reduce itself as the result of bacterial action. The length of time which may elapse from the time at which the blood is drawn till the estimation is made depends upon the cleanliness with which the fluid is collected and the temperature at which it is kept. In ordinary weather blood taken by pricking the finger should be estimated within 24 hours. Blood may be kept for some two or three days by placing it in a stoppered tube which is kept in ice in a Dewar's flask. It cannot be kept indefinitely even at 0° C.

(2) The blood must be thoroughly shaken with air in order that it may be fully saturated with oxygen. At room temperature the degree of saturation in air is not measurably less than 100 %.

(3) The blood must be shaken just before it is used in order that no sedimentation may take place.

The theory of the ferricyanide method is obscure, the blood in faintly alkaline solution gives off accurately the quantity of oxygen which could be abstracted from the haemoglobin with a blood gas pump. Nevertheless the substance formed by the reaction is methaemoglobin, a body which is credited with containing the same quantity of oxygen as oxyhaemoglobin, though in a stable form.

The following equation is given by Haldane for the reaction. The alkali in the formula is sodium bicarbonate. The general nature of the formula is the same no doubt if ammonia or other alkali be substituted.

$$\mathrm{Hb}\begin{matrix}\diagup\mathrm{O}\\ \\ \diagdown\mathrm{O}\end{matrix} + 4\mathrm{Na_3(FeCy_6)} + 4\mathrm{NaHCO_3} = \mathrm{O_2} + \mathrm{Hb}\begin{matrix}\diagup\!\!\diagup\mathrm{O}\\ \\ \diagdown\!\!\diagdown\mathrm{O}\end{matrix} + 4\mathrm{Na_4(FeCy_6)} + 4\mathrm{CO_2} + 2\mathrm{H_2O}.$$

Haldane's demonstration of the accuracy of the method was made with an ordinary Dupré's apparatus for the estimation of urea. 50 c.c. of blood and 100 c.c. of dilute alkali were mixed in the bottle of the apparatus; into the small tube in the interior of the bottle were placed 20 c.c. of a saturated solution of ferricyanide of potassium. The blood and the alkali were thoroughly mixed in order to allow of complete laking of the corpuscles. The rest of the operation was conducted just

like an estimation of urea in urine with sodium hypobromite. In theory at all events this was so, in practice very much greater precautions had to be taken (1) to maintain the temperature of the great mass of colloidal fluids constant, and (2) to get off all the oxygen by an immense amount of shaking. These two processes were of course wholly inimical to one another. Nevertheless the method sufficed in the hands of Haldane to establish the theoretical accuracy of the ferricyanide reaction.

The differential method of Blood Gas analysis.

The apparatus consists of a manometer, with a blood gas bottle at the head of each limb. The apparatus is shown in Fig. 136.

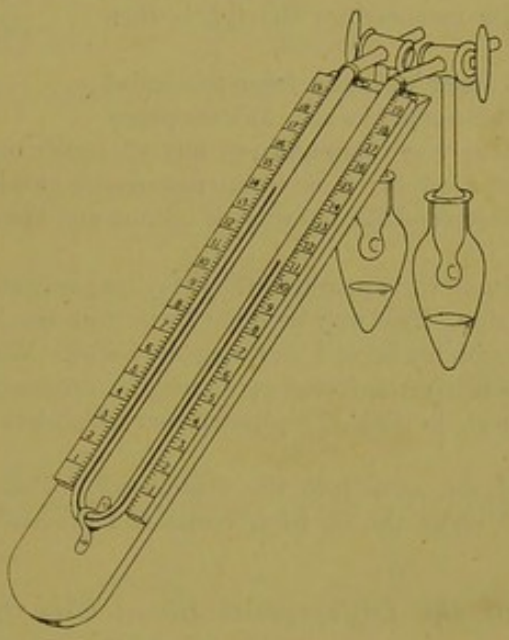


Fig. 136.

The bottles are similar to those of the apparatus previously described.

No control apparatus is necessary, the principle of the differential apparatus being that changes in the temperature of the bath, &c. affect the two bottles alike and therefore counterbalance one another.

The tubing of the manometer should be 1 mm. bore approximately; the bottles each about 25 c.c. in capacity. They should be of the same size within 1 tenth of a c.c.

To fill the apparatus with clove oil.

This fluid is in many ways ideal, it has however one drawback, namely that the apparatus must be chemically dry before the oil is put in. It must also be chemically clean. It is best, I think, to avoid alcohol and ether in the cleaning. I clean the tubes by filling them with potassium bichromate and sulphuric acid and then plunging them in a bath of the same, which I can warm up. The bichromate is then thoroughly washed out with distilled water and the apparatus is dried by aspirating air (which has passed through sulphuric acid) gently through the manometer in a warm air bath.

The clove oil is put in the following way. Starting with clean apparatus, the taps of which are free from grease, a fine pipette is drawn out which will go down the broad portion of the tubing in the vicinity of the tap, the stopper of the tap being removed. The stopper on the other side is turned so that no air can get out of the manometer on that side. The pipette is charged with clove oil, of which some slight excess is put into the broad portion of the tubing, the pipette is then removed, the tap on the other side is opened gently and sufficiently to allow of the oil descending the fine tubing and getting just round the bend of the manometer; the tap is then closed again.

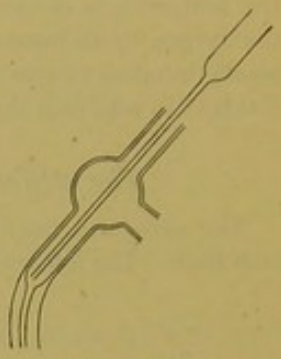


Fig. 137.

All excess of oil is now removed from the broad portion of the tubing with a pipe cleaner. It is necessary that this should be done very thoroughly, as any oil which remains here is apt to creep down and enclose a column of air in the manometer at a later stage. When all excess of oil has been removed the tap may be opened and the oil allowed to find its own level.

Should bubbles form in the apparatus they may be expelled by forcing the fluid up to the top on that side. This may be done by putting the bottle on the opposite side and with the tap suitably turned, warming the bottle. When the fluid has been forced up, or rather as it is being forced up, it may be necessary to break the bubble by making the fluid meet the end of a pipe cleaner which has been pushed down as far as it will go.

In no case should one blow into the manometer. This will inevitably cause a dampness that will make the oil form beads and prevent it rising and falling accurately

To calibrate the Differential Blood Gas apparatus.*

The calibration consists in a determination of the relation between the quantity of gas x evolved in the apparatus and the difference of pressure p, measured in the manometer, $x=kp$. The method depends upon the liberation of a known quantity of gas inside the apparatus; x then is known and p is observed, therefore if k is the constant of the apparatus it is directly determined

$$k=\frac{x}{p}.$$

The reaction which has proved most serviceable so far has been the liberation of oxygen from hydrogen peroxide in the presence of acid by the addition of potassium permanganate. The equation is as follows:

$$K_2Mn_2O_8+3H_2SO_4=K_2SO_4+2MnSO_4+3H_2O+5O,$$
$$5O+5H_2O_2=5H_2O+5O_2.$$

Therefore 316 grams of potassium permanganate yield five molecular weights or 111,000 c.c. of oxygen, at normal temperature and pressure. A solution of hydrogen peroxide must be made up and freshly titrated with approximately decinormal potassium permanganate, of such a strength that 1 c.c. of the H_2O_2 gives off 0·2 c.c.

* See also Hofmann's method, *Journal of Physiol.* XLVII. p. 272.

of oxygen. It must of course when made be accurately titrated and the exact quantity of oxygen which it will give off determined.

The following example may serve to help the experimenter:

Potass. permanganate solution used, strength being 1 c.c. = ·00295 gr.

Pure 20-volume hydrogen peroxide diluted 100 times, then 100 c.c. H_2O_2 required 10·3 c.c. $KMnO_4$ to set the oxygen free,

$$2KMnO_4+3H_2SO_4+5H_2O_2=K_2SO_4+2MnSO_4+8H_2O+5O_2.$$

From this equation it is seen that 316 grs. $KMnO_4$ liberate 160 grs. oxygen.

But ·0896 × 16 grs. oxygen occupy 1000 c.c. at N.T.P.

$$\therefore \quad 160 \quad \text{,,} \quad \text{,,} \quad \text{,,} \quad \frac{160\times1000}{\cdot0896\times16} \text{ c.c. at N.T.P.}$$

Since 100 c.c. hydrogen peroxide contain oxygen liberated by 10·3 c.c. $KMnO_4$,

$$\therefore \quad 1\cdot5 \text{ c.c.*} \quad \text{,,} \quad \text{,,} \quad \text{,,} \quad \text{,,} \quad \text{,,} \quad \text{,,} \quad \frac{1\cdot5\times10\cdot3}{100} \quad \text{,,}$$

which contain $\dfrac{1\cdot5\times10\cdot3\times\cdot00295}{100}$ grs. $KMnO_4$.

Then 316 grs. $KMnO_4$ liberate $\dfrac{160\times1000}{\cdot0896\times16}$ c.c. O_2.

$$\therefore \quad \frac{1\cdot5\times10\cdot3\times\cdot00295}{100} \quad \text{,,} \quad \frac{1\cdot5\times10\cdot3\times\cdot00295\times160\times1000}{100\times\cdot0896\times16\times316} \text{ c.c. } O_2$$

$$= \cdot1537 \text{ c.c. } O_2.$$

Temp. was 13·4° C., pressure 755 mm.

Now 1000 c.c. air, saturated with water vapour at this temp. and press., occupy 932 c.c. at N.T.P.

$$\therefore \text{ vol. of } O_2 \text{ actually liberated was } \frac{153\cdot7}{\cdot932} \text{ cub. mm.}$$

Difference of press. observed was 46·2 mm.

$$\therefore \text{ Constant} = \frac{153\cdot7}{\cdot932\times46\cdot2} = 3\cdot57.$$

But vol. in bottle was 3·5 c.c. which would add ·35 to the constant

$$\therefore \text{ Constant for empty bottle} = \mathbf{3\cdot92.}$$

Perhaps the greatest drawback in this method is due to the difficulty of obtaining pure hydrogen peroxide. The commercial reagent is made up with glycerine or other substances which are added for the purpose of preventing the H_2O_2 from deteriorating in strength. Such peroxide appears to give unreliable results.

The calibration of the apparatus is carried out as follows:

Into each of the bottles put 1 c.c. of H_2O_2 and 2 c.c. of H_2SO_4 strength N/100.

If the left bottle is the one which is to have its constant determined, place ·2 c.c. of permanganate in the reservoir and the same quantity of water in the corresponding reservoir of the other side. After the permanganate is in its place cut a piece of filter paper 2 or 3 cm. in length and 1 mm. in breadth and place it so that it projects a little from the reservoir but does not become wet with the permanganate, till it is

* This was volume of H_2O_2 put in blood gas bottle.

desired to spill the permanganate from the reservoir into the bottle. It will ensure the proper spilling of the permanganate. Put on the bottles, the stoppers being efficiently greased with lard or vaseline. Place the apparatus with its bottles in the water bath.

After five minutes read the meniscus on each side:

Left	Right
120	119·5

Close the taps. See that the meniscus remains constant for two minutes, then remove the apparatus from the bath; upset the fluid into both bottles. Shake for one minute. Replace the apparatus in the bath. After a minute has elapsed shake again in the air for a minute, put back in the bath and after 60 seconds more read

Left	Right
90	159·5

Difference 69·5 mm. $+ \cdot 5 = 70$ mm. $= p$.

Now by calculation 1 c.c. of H_2O_2 gives ·2 c.c. of O_2 at 0° C. and 760.

$$\therefore \text{ it gives } \cdot 2 \times \frac{288}{273} \times \frac{760}{755} \text{ at } 15° \text{ C. and } 755 = \cdot 212 \text{ c. or } 212 \text{ c. mm.}$$

$$\therefore \ k = \frac{212}{70} = 3 \cdot 03.$$

Comparison of this result with another which depends upon the actual measurements of the physical constants (i.e. the volumes of the bottles and the bore of the tubing) of the apparatus, shows a slightly higher constant by this method. As a matter of fact very careful research carried out by Burn has shown that this method gives results consistently higher by about 2 °/₀ than those given by a method which depends upon the measurement of the sizes of the bottle and tubing. This is scarcely surprising. The old method is of course theoretically accurate; but for certain small quantities which are negligible and have been neglected in the calculation, the method was mathematically correct. Nevertheless in practice one was dealing with fluids which leave films on whatever they touch, which exert vapour pressures and so forth, and it is not surprising that a theoretical method when checked by a practical one should depart from it to the extent of 2 °/₀.

To determine the total oxygen capacity of a sample of defibrinated blood by the differential method.

Apparatus: a differential blood gas apparatus, a burette, 1 c.c. pipette (calibrated), 1 finely drawn out pipette, potassium ferricyanide, ammonia solution (4 c.c. of strong ammonia per litre), vaseline.

In each blood gas bottle put 2 c.c. of ammonia from the burette and 1 c.c. of defibrinated blood. Shake up the two together so that the blood becomes thoroughly laked.

Grease the stoppers with vaseline.

See that the taps are open.

With the fine pipette put 0·2 c.c. ferricyanide solution into the reservoir on one side.

Put the apparatus in the water bath.

After five minutes read the meniscus on each side

Left	Right	Zero error in p
120	119·5	·5

Close the taps; after two minutes if the meniscus has not moved upset the ferricyanide on one side with the precautions indicated above; shake for one minute and place in the bath for one minute; read again

Left	Right	p
93	146·5	53·5 + ·5 = 54

Shake again for one minute and allow to stand for one minute; read

Left	Right	p
91	148·5	57·5 + ·5 = 58

Shake again for one minute and allow to stand for one minute

Left	Right	p
91	148·5	57·5 + ·5 = 58

The value of p is now constant, had it not been so the operation of shaking, &c., must have been continued.

The volume of gas given off is $p + k = 57 \times 3{\cdot}03 = 171$ cubic mm. or ·171 c.c.

This must be reduced for temperature and pressure and corrected according to the calibration of the 1 c.c. pipette. Suppose the latter delivers ·96 c.c., that the temperature is 15° C. and the pressure 755 mm. The oxygen capacity is then

$$\cdot 176 \times \frac{273}{288} \times \frac{755}{760} \times \frac{1}{\cdot 96} = \cdot 173 \text{ c.c.}$$

Repeat the operation on the other side of the apparatus.

Correction of Haldane's standard haemoglobinometer.

The importance of the above determination is great, owing to the fact that it is the only practical way of ascertaining the correctness of a standard haemoglobinometer.

Haldane's standard instrument is constructed so that 100 on the scale corresponds to an oxygen capacity of ·185 c.c. of oxygen per c.c. of blood. The reading of any blood on Haldane's scale should therefore be

$$\frac{\text{The oxygen capacity as determined}}{\cdot 185} \times 100.$$

That given above should be

$$\frac{\cdot 173}{\cdot 185} \times 100 = 93\ {}^{\circ}/_{\circ}.$$

If it is not so, as the result of careful determination the standard solution in the haemoglobinometer is incorrect.

Determination of the oxygen in unsaturated blood with the differential apparatus.

Apparatus as above, with the unsaturated blood in addition.

Place 2 c.c. of ammonia in each bottle, put 1 c.c. of the unsaturated blood in the left bottle and 1 c.c. of saturated blood in the right bottle. The blood must in each case be run from the pipette gently to the bottom of the bottle so that it lies in a layer underneath the ammonia and is protected by it from oxidation by the air.

Grease the stoppers, place the bottles on the stoppers, place potassium ferricyanide in the left reservoir. With the tap open place the apparatus in the water bath; after five minutes read the meniscus. Suppose it to be

Left	Right
120	119·5

Shut the taps, see that the meniscus does not move in the next two minutes. Then shake thoroughly so as to lake both samples of blood. The level of the clove oil will have changed, as the unsaturated blood will have taken up some oxygen. This change may be disregarded, however, as this extra oxygen combined with the haemoglobin will be turned out again by the ferricyanide producing a corresponding change in the opposite direction. When the blood is completely laked turn the bottle so as to let down the ferricyanide, shake as in the above example. Let the final reading be

Left	Right	p
110	129·5	19·5 + ·5 = 20 mm.

The volume of gas as measured therefore is $p \times k : 20 \times 3{\cdot}03 = 60{\cdot}6$ cubic mm. or 0·06 c.c. This must be corrected in several ways before a correct result is obtained. The considerations which must be borne in mind are as follows: (1) temperature, (2) pressure, (3) the calibration of the pipette, (4) the fact that the plasma is not saturated with oxygen, (5) the temperature at which the blood was in equilibrium with oxygen and nitrogen. Taking the first three together and using the values given in the determination of the total oxygen capacity the corrected reading would be 0·059 c.c.

As unsaturated blood is usually taken directly from the body or from a tonometer at body temperature we will suppose that it has been exposed to gases at 37° C.

In the lung the partial pressure of nitrogen is about 560 mm., in the air in the blood gas bottle about 590. The question then is how much nitrogen will blood which has been exposed to 560 mm. nitrogen at 37° C. take up when exposed to 590 mm. at say 15° C.

	Exposed to	760 mm.	N_2 pressure	at	15° C.	blood takes up 0·016 c.c.
	,,	760 ,,	,,	37° C.	,,	0·011 c.c.
	,,	590 ,,	,,	15° C.	,,	0·012 c.c.
therefore	,,	560 ,,	,,	37° C.	,,	0·008 c.c.

The blood in the apparatus will therefore take up nitrogen from the air to the extent of 0·012 − 0·008 c.c. = 0·004 c.c. This must decrease the amount of oxygen which appears to have been given out by this amount, therefore 0·004 c.c. must be added to the answer given above, namely 0·059 c.c.; it therefore becomes 0·063 c.c. Blood which is unsaturated at room temperature may be regarded as having no

oxygen in the plasma. Such blood therefore when shaken with air at 15° C. will take up ·006 c.c. of oxygen per c.c. This also must be added to the reading obtained above, which will bring it up to (0·063+0·006)=0·069 c.c. This therefore is the corrected oxygen reading, and represents the oxygen in the blood from this animal, which when cold will be practically entirely in the haemoglobin, when warm it will have been to a trifling extent in the plasma.

Determination of Percentage Saturation of Blood with oxygen by means of the differential method.

The percentage saturation is of course the quantity of oxygen which the blood contains A, divided by the total oxygen capacity C, multiplied by 100, i.e.

$$100 \times \frac{A}{C}.$$

We have already described methods for the estimation of A and C; but as they cannot both be performed upon the same sample of blood it is convenient to make use of the following very simple device.

If the blood in question be simply shaken with oxygen it will take up oxygen until it becomes saturated. If the quantity which it originally held was A, and that which it takes up on shaking was B, then

$$B = C - A \quad \text{or} \quad A = C - B.$$

The percentage saturation then is

$$100 \frac{(C-B)}{C}.$$

We proceed to measure C and B. Of these two measurements B is made first.

Measurement of B.

Place 2 c.c. of ammonia in each bottle, underneath the ammonia run 1 c.c. of the blood to be estimated into the bottle on the left hand side (L) and 1 c.c. of saturated blood (not necessarily from the same animal) in the right hand bottle (R).

Grease the stoppers.

It is most important that the stoppers should be thoroughly clean inside and free from all traces of ferricyanide.

See that the taps are open.

Place the bottles on the stoppers and the apparatus in the bath.

After five minutes read. Suppose the reading to be

	Left	Right
	120	119·5

Close the taps; see that the surfaces do not move for two minutes, then shake for one minute, or until the unsaturated blood ceases to become redder. Replace in the bath, after one minute read, shake again for one minute. Read and shake for alternate minutes until the reading becomes constant. Suppose it then is

Left	Right	p
129·5	110	19·5 − 0·5 = 19 mm.

B then = 19.

Measurement of C.

Take the apparatus out of the bath, open the taps. Take the bottle L. Put potassium ferricyanide into the reservoir, replace the bottle, put the apparatus in the bath, and proceed as in the determination of the oxygen capacity.

Let the measurement of C give a pressure of 60 mm.

The percentage saturation $= 100 \frac{(60-19)}{60}$ i.e. 68 %.

This determination gains considerably in accuracy owing to the fact that neither the quantity of blood used nor the constant of the apparatus enters into the calculation. There is therefore no need to calibrate the pipette used or even to exercise extreme caution in measuring the amount of blood used for the experiment.

It is of course unnecessary to correct for temperature and pressure, the correction affecting B and C alike. On the other hand one must reckon with the fact that the unsaturated blood will take up nitrogen and oxygen from the air of the apparatus in which the blood is shaken in quantities which depend upon the composition and temperature of the gas with which the blood has previously been in equilibrium.

The general principles upon which the calculation for this correction is made are set out under the measurement of oxygen in unsaturated blood. For blood taken from a tonometer at 13 % or from the body it is generally sufficiently accurate to add 4 % to the observed saturation.

Determination of the difference between the quantities of oxygen contained in 1 *c.c. of arterial and* 1 *c.c. of venous blood with the differential manometer.*

If, as is usually the case, the arterial blood is nearly saturated with oxygen, relatively to the venous blood, this determination is one of the easiest in blood gas analysis.

Place 2 c.c. of ammonia in each of the bottles, in L place 1 c.c. of venous blood, in R 1 c.c. of arterial blood.

See that the stoppers are greased and that they are free from all traces of ferricyanide and that the taps are open.

Place the bottles on the apparatus, and the apparatus with its bottles in the water bath.

After five minutes read. Suppose the readings to be

Left	Right	Zero error
120	119·5	0·5

Close the taps; see that the meniscus does not move for two minutes.

Shake for one minute and place in the bath for one minute alternately till a constant reading is obtained. Let this be

Left	Right	Diff.	Zero error	p
127	112·5	14·5	·5	14

If k be the constant of the apparatus, $p \times k$ is the difference between the oxygen in the arterial and venous blood.

A few words may be said about the limitations of this method.

In the first place it measures the difference of the total oxygen in each sample of blood and not merely the oxygen in the haemoglobin.

Secondly it assumes that the two samples of blood are of the total oxygen capacity. The reasoning which underlies the method is as follows. Let the oxygen in the arterial blood be A_A and that in the venous blood A_V. It is required to measure $A_A - A_V$.

Let the amount of oxygen necessary to saturate the arterial blood be B_A, and the venous blood B_V, and the total oxygen capacity C.

$$A_A = C - B_A,$$
$$A_V = C - B_V,$$
$$A_A - A_V = (C - B_A) - (C - B_V),$$
$$A_A - A_V = C - B_A - C + B_V$$
$$= B_V - B_A.$$

The question then arises, Is the method applicable to cases where the oxygen capacity of the arterial and venous bloods is different, such for instance as in the case of blood flowing through an active gland?

There is one special case in which the method may be applied; fortunately it is the most common, but care must be taken to see that it obtains. It is the case in which A_A and C are almost equal, that is to say in which so little oxygen is taken up on the arterial side of the apparatus that a small error in its quantity may be neglected as compared with the difference between A_A and A_V.

Thirdly we must consider the accuracy of measurement of the blood. The guiding principle is the same. So long as B_A is almost nothing it is not material that the arterial blood should be very accurately measured. The practical point that has to be considered is the accuracy of the measurement of the venous blood.

Moreover if any considerable quantity of oxygen is taken up by the arterial blood, one has to allow for any difference which may exist between the constants of the two sides of the apparatus.

For all these reasons it is best when working with arterial blood which is unsaturated to place the arterial and venous bloods each in a different apparatus, and analyse each separately with saturated defibrinated blood in the other bottle of the apparatus, and, having obtained the value of either A or B for each blood, to subtract the one from the other by arithmetic.

A useful check on the measurement of the blood is a determination of C for each sample of blood after B has been determined.

These complications, as Verzàr found, make the estimation of the oxygen used by organs during oxygen want very difficult; for the reasons given above he made separate estimations of arterial and venous bloods.

Differential method with apparatus for 0·1 c.c. of blood.

There is no difference in theory between this apparatus and that which we have just described. The smaller apparatus was designed for work on human blood. With it all the work described in this book on the human dissociation curves has been performed, except where otherwise stated.

The apparatus consists of a manometer of 0·5 mm. bore glass tubing. At the top of each limb of the manometer is a three-way tap with a T boring. Of the three ways of each tap one opens to the open air, the other goes to the blood gas bottle. The blood gas bottles are of the shape indicated in the figure; they are each about 3 c.c. in capacity up to the top, and should of course be identical in size. The apparatus is fixed to a wooden stand, fitted to a clip by which it hangs on the edge of the water bath.

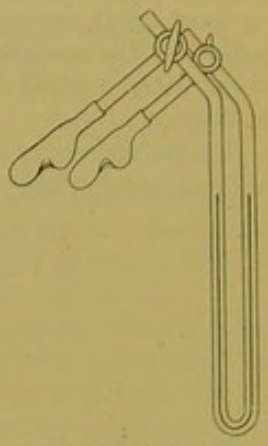


Fig. 138.

There are certain practical details about which the buyer of one of these pieces of apparatus should satisfy himself:

(1) That the tubing of the manometer is not more than 0·5 c.c. bore.

(2) That the point at which the fine tubing of the manometer joins the coarser tubing of the tap should be above, not below the bend of the tubing.

(3) That when the manometer hangs in the water bath with the tubing vertical the bottles should be completely submerged in the water.

(4) That the clip be hung in such a position that the apparatus can stand in the bath with the bottles vertical or nearly so.

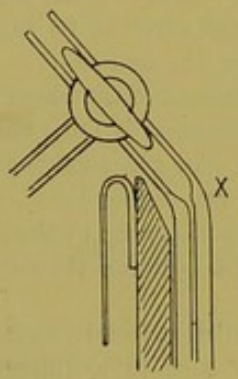


Fig. 139.—Correct position of junction between fine and coarse tubing.

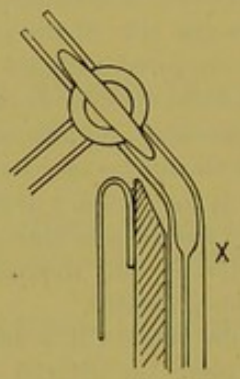


Fig. 140.—Incorrect do.

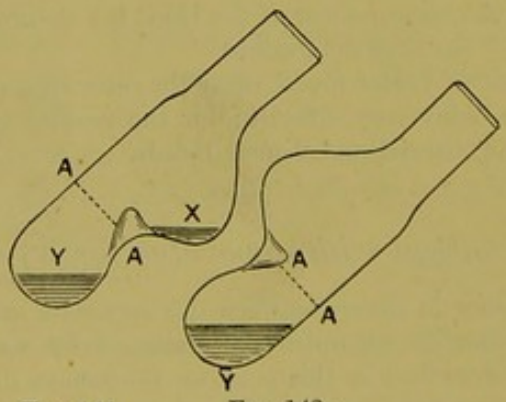


Fig. 141. Fig. 142.

(5) That the shape of the bottles be such that when hanging on the apparatus with the manometer vertical 0·02 c.c. fluid placed in the pouch at *X* has no tendency to find its way down the tubing to *Y*.

(6) That the diameter *A*—*A* of the bottle be such that if 0·2 c.c. of ammonia and 0·1 c.c. of blood be placed at *Y* the fluid will assume the position shown in Fig. 142 when the bottle is placed in that position. If *A*—*A* is too narrow the fluid sticks in the bottom part of the bottle even when the latter is placed on its back.

Calibration of the differential apparatus for analysis of oxygen in 0·1 *c.c. of blood.*

Two methods are available for this calibration.

(i) A direct comparison of the oxygen given out by blood with that given out by the same blood in the larger form of apparatus, the constant of which is known.

(ii) The hydrogen peroxide method. The strengths of solutions necessary and their standardisation are carried out in the small as in the case of the larger apparatus already described. The only point which needs amplification is the use of the solutions in the small apparatus. This is very simple. Into each bottle is placed 0·2 c.c. of *N*/100 sulphuric acid and 0·1 c.c. of standard hydrogen peroxide.

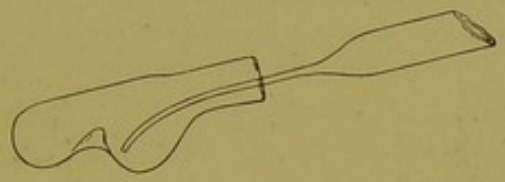


Fig. 143.

These should be run into the bottles from a pipette with a long narrow end, so that the sides of the bottle are not wet. For putting the potassium permanganate into the bottle a special pipette is necessary. It is drawn out and bent at the end. As shown in the figure it may be used to place the permanganate in the pouch.

The danger, of course, is that the H_2O_2 and the permanganate may become prematurely mixed. If the bottle is of the proper shape and reasonable care is taken, this danger should not cause serious preoccupation. The other bottle is filled in the same way except that water, not permanganate—a drop in each case—is placed in the pouch.

The bottles are then placed on the apparatus which has, of course, been adequately greased. Care must be taken to see that the taps are open when the bottles are put on.

The apparatus is placed in the bath and allowed to stand for five minutes the meniscus is read, the taps are closed, if in another minute the meniscus has not changed the bottles may be shaken. Care being taken that both the permanganate and the water become mixed with the acid hydrogen peroxide.

The bottles are then shaken and left in the bath for alternate minutes until constant readings are obtained.

The method of working out the constant is similar to that already given for the larger apparatus. Suppose *k* to be ·38.

To determine the total oxygen capacity of a sample of defibrinated blood by the differential method, using 0·1 *c.c. for each determination.*

Apparatus: a differential blood gas apparatus of the form shown in Fig. 138; a burette holding about 1—5 c.c., which delivers $\frac{1}{10}$ c.c. with fair accuracy; a pipette which delivers $\frac{1}{10}$ c.c. of blood; a curved pipette similar to that shown in Fig. 143; a saturated solution of potassium ferricyanide; ammonia (4 c.c. of strong ammonia per litre); vaseline.

Into each blood gas bottle put 0·2 c.c. of ammonia from the burette and 0·1 c.c. of defibrinated blood. Shake up the two together so that the blood becomes thoroughly laked.

Grease the stoppers with vaseline, see that the taps are open.

Put one drop of ferricyanide into the pouch of the left-hand bottle, place the right-hand bottle with the pouch uppermost. Place the apparatus in the water bath. After five minutes read the meniscus on each side,

Left	Right	Zero error
120	119·5	·5

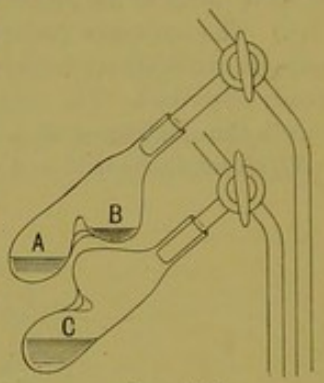


Fig. 144.

After one minute, if the meniscus has not changed, shake up the blood and ferricyanide, being careful that the pouch on the right side does not become wet or soiled. Shake and place in the bath for alternate minutes.

Final reading:

Left	Right	p
91	148·5	57·5 + ·5 = 58

The volume of gas given off is $p \times k = 58 \times 38$ or 22·0 cubic mm.

This must be reduced for temperature and pressure and corrected according to the calibration of the pipette. Suppose the pipette delivers 0·096 c.c., that the temperature is 15° and the barometer is 755 mm., the oxygen capacity is

$$22 \times \frac{273}{288} \times \frac{755}{760} \times \frac{0{\cdot}1}{0{\cdot}096} = 21{\cdot}5 \text{ cubic mm.}$$

Open the taps, take the bottle off the right side, the pouch of which is as yet uncontaminated. Put a drop of ferricyanide in it, replace it on the apparatus, and determine the oxygen capacity of the blood in the right bottle. Two concordant results should thus be obtained.

Correction of Haldane's standard haemoglobinometer.

See page 295.

Determination of the oxygen in 0·1 *c.c. of unsaturated blood with the differential apparatus.*

Apparatus as above, with the unsaturated blood in addition.

Place 0·2 c.c. of ammonia in each bottle, put 0·1 c.c. of the unsaturated blood in the left bottle and 0·1 c.c. of saturated blood in the right bottle, the blood must in each

case be run from the pipette gently to the bottom of the bottle so that it lies in a layer underneath the ammonia and is protected by it from oxidation by the air.

Grease the stoppers, put a drop of ferricyanide in the pouch of the left bottle. See that the taps are open, place the bottles on the apparatus and the apparatus in the water bath. After five minutes read the meniscus on each side. Suppose it to be

Left	Right	Zero error
120	119·5	·5

Shut the taps, see that the meniscus does not move in the next minute. Shake in such a way that the blood becomes thoroughly laked but that the ferricyanide and the blood do not mix. When satisfied that the laking is complete, mix the ferricyanide and the laked blood and shake for one minute. Place in the bath and shake for alternate minutes. Suppose the final reading to be

Left	Right	
110	129·5	19·5 + ·5 = 20 mm.

If k is ·38 the volume of gas which has come off is

$$p \times k = 7{\cdot}6 \text{ cubic mm.}$$

This must be corrected in accordance with the instructions on p. 296.

Determination of the percentage saturation of 0·1 *c.c. of blood with oxygen by means of the differential method.*

The percentage saturation is, of course, the quantity of oxygen which the blood contains (A) divided by the total oxygen capacity (C) multiplied by 100, i.e.

$$100 \times \frac{A}{C}.$$

We have already described methods for the estimation of A and C; but as they cannot both be performed upon the same sample of blood it is convenient to make use of the following very simple device.

If the blood in question be shaken with oxygen it will take up oxygen till it becomes saturated. If the quantity which it originally held was A and that which it takes up on shaking was B, then

$$B = C - A \quad \text{or} \quad A = C - B.$$

The percentage saturation then is

$$100 \frac{(C-B)}{C}.$$

We proceed to measure C and B, of these two measurements B is made first.

Measurement of B.

Place 0·2 c.c. of ammonia in each bottle, underneath the ammonia run 0·1 c.c. of the blood to be estimated into the bottle on the left-hand side, and 0·1 c.c. of saturated blood (not necessarily from the same individual) in the right-hand bottle.

Grease the stoppers.

It is important that the ends of the stoppers should be thoroughly clean inside and free from all traces of ferricyanide.

See that the taps are open.

Place the bottles on the stoppers with the pouches upwards. Place the apparatus in the bath.

After five minutes read, suppose the reading to be

Left	Right	Error
120	119·5	·5

Close the taps and see that the surfaces do not move in one minute, then shake for one minute at least or until the unsaturated blood ceases to change colour; replace in the water bath and shake alternately for a minute until a constant reading is obtained. Suppose this to be

Left	Right	p
110	129·5	19·5 − 0·5 = 19 mm.

$B = 19$.

Measurement of C.

Take the apparatus out of the bath, open the taps. Take off the left bottle, the pouch of which should be clean and dry—place a drop of ferricyanide in the pouch and replace the bottle on the apparatus with the pouch downwards. Proceed to measure the oxygen capacity.

Let the measurement of C give a pressure of 60 mm.

The percentage saturation $= 100 \dfrac{(60 - 19)}{60} = 68\ ^{\circ}/_{\circ}$.

The corrections which must be applied to this will be found on p. 298.

To expose blood to a gas of a given composition.

Apparatus:

A useful form of tonometer for the purpose consists of a cylindrical vessel of 250 c.c. capacity.

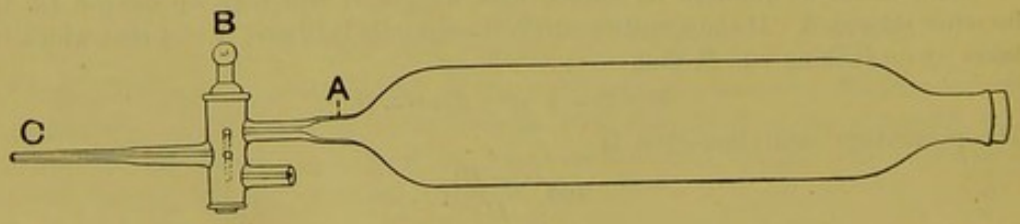


Fig. 145.

At one end the cylinder can be corked with a rubber stopper, at the other there is a three-way tap. The bore of this tap is 1·5 mm. At the point A the tubing is somewhat broader, so that there is a portion of the apparatus which will hold about 1—2 c.c. of blood when the apparatus is placed vertically. That portion of the tubing between B and C requires to be made carefully if, as is sometimes the case, the apparatus is expected to deliver a definite amount of blood into one of the bottles of the small apparatus just described.

The following are the requirements which should be fulfilled for this purpose:

(1) The point of the tube must reach easily to the bottom of the blood gas bottle.

(2) The tubing between *B* and *C* should hold about 0·12 c.c. of blood.

(3) At no place must the bore be so great that bubbles of gas become mixed with the blood as the blood passes down.

(4) At no place must it be so fine that a froth of mercury and blood which sometimes has to be dealt with, will stick in the tube.

(5) The stronger it is the better.

The tubing therefore should be 7 cm. in length, *B* to *C*, 2 cm. from *B* to *D*, and 5 cm. *D* to *C*. The bore should be uniformly 1·5 mm. throughout the whole length of the tube.

The outside diameter of the fine part of the tube should be 4·5 mm.

For many purposes the tonometer is neither required to deliver a given quantity of blood nor to deliver it into a narrow mouthed bottle. A tube 7 cm. long and 1·5 cm. bore will do, without any constriction.

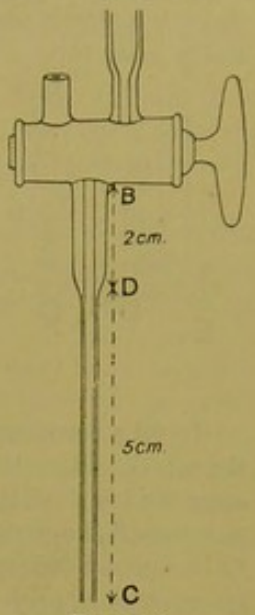


Fig. 146.

Laboratory method of filling tonometers from stock gas mixtures.

The following is a description of the plant which is in the Cambridge Laboratory for this purpose.

For each gas mixture two Mariotte bottles of a capacity of 15 litres each are connected. It is better not to get the bottles with ground glass bungs, these are liable to break and the bore of the taps is much too narrow to be serviceable. Rubber bungs are quite satisfactory. They should be bored and glass taps inserted which have a bore of 1 cm. in the stopper.

Into the top of bottle *B* should be securely fixed with wire a good rubber cork bored to take the glass tube *Y* which goes to the bottom of the bottle. The cork in the bottle *B* has another hole; through this a short glass tube passes which is connected by rubber to a brass tap. This tap connects at right angles to a brass tube *W* (see endwise in Fig. 147) which we shall call the vacuum main. In the installation at Cambridge there are five such pairs of bottles, each forming a gas holder, joined to the vacuum main.

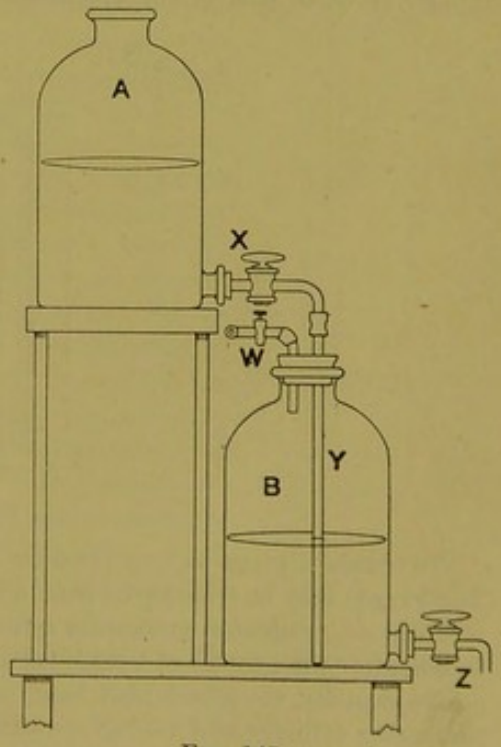


Fig. 147.

At one end the main connects with a glass tap (Fig. 148) *A*, and beyond this there is a Geryk pump. At the other end there is a T-tube *C*, a vacuum gauge *D*, and a glass tap.

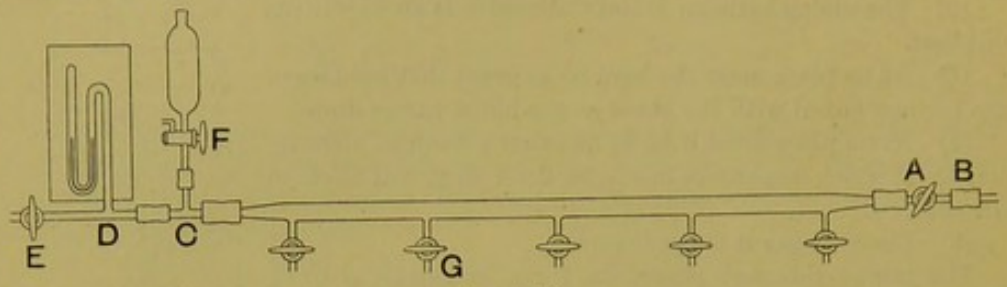


Fig. 148.

To fill a tonometer with gas from one of the gas holders—say the one attached to the tap *G*. Place the tonometer, which is of course securely corked, on the T-tube *C*, open the tap *F* of the tonometer, close all the other taps except *A*. Turn the Geryk pump until the gauge shows a good vacuum. Then close *A* and gently open *G*. If *G* be opened suddenly the gauge may be broken. Close *G*, open *A* exhaust once more, close *A*, open *G* and after a minute or so close *G* again. Close *F* and take off the tonometer.

The holders may be filled with nitrogen through the tap at the bottom of bottle *B*, Fig. 148, from a nitrogen cylinder. This is done with the bottle full of water and just enough water in *A* to cover the tap at the bottom. The water is then displaced upwards into the bottle *A* by the nitrogen—the nitrogen must not be run in too rapidly or some part of the apparatus may blow out.

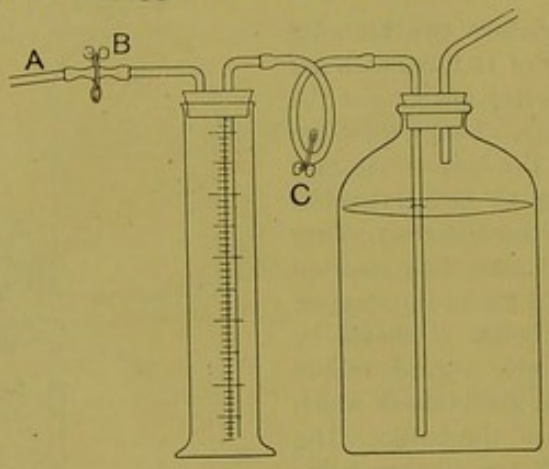


Fig. 149.

To obtain a given oxygen pressure add 20 c.c. of oxygen for each mm. required. The oxygen may be transferred from an oxygen cylinder—in an apparatus consisting of a 250 c.c. graduated measuring cylinder—fitted with a rubber cork and with glass tubing after the manner of a wash bottle. This is attached to a wash bottle of about a litre capacity, the attachment being as shown in the figure. Start with the whole measuring cylinder and tubing connected with it full of water, and the wash bottle partly full. Place the glass tube *A* on a piece of rubber tubing along which oxygen

is gently coming from the cylinder, remove the clamps at *B* and *C*. Let the oxygen displace the water backwards into the wash bottle till the required amount has been obtained. Close the clips and disconnect.

Connect *A* by rubber tubing to the tap *Z*, Fig. 147. See that the tap *X* is closed and that there is no considerable positive pressure in the gas holder by opening *W* for a moment. Then with *W* closed and *Z* open, transfer the oxygen by blowing into the wash bottle.

It is now necessary to analyse the gas, by filling a tonometer in the manner described above and analysing the contents with a Haldane's gas analysis apparatus. By the addition of a little air or nitrogen in calculated quantities it is possible to get the gas in the holder pretty exactly to a known composition. It is best however to have the gas with a trifle less oxygen than what is actually likely to be required. For instance if 20 mm. oxygen is being aimed at one would have the gas in the gasometer 19·5 mm. oxygen. One has to analyse the gas in the tonometer before using it; having made the analysis it is very easy to add a small quantity of air from the gas burette of the Haldane's apparatus. With a 250 c.c. tonometer air is added to the extent of 0·15 c.c. for every tenth of a mm. oxygen pressure required; of this we shall speak later.

We now have a mixture of oxygen and nitrogen in the proportions required; we may want a known pressure of carbonic acid.

It is not possible to keep carbonic acid in the stock mixture on account of its solubility in the water. It is best to add it from a burette. For this purpose I use a burette of the form shown in figure 150. It is of 15 c.c. capacity and is graduated in c.c. and $\frac{1}{10}$ths of a c.c., the gas is manipulated over mercury. The tonometer is fixed at *A* by a junction of rubber pressure tubing. Mercury is run up to the tap of the tonometer, which is shut. The *B* tap is also shut. The mercury reservoir is now placed so that the surface of the mercury is at the level *C*. The tap *B* is opened so as to connect *C* with the interior. Some excess of CO_2 is forced in by placing the nozzle of a tube connected with the CO_2 cylinder and from which CO_2 is gently coming, in the rubber tube at *C*. Close the tap. Take away the tube by which the carbonic acid entered. Level the mercury within and without the burette with the tap shut. Then open the tap towards *C*, gently raise the mercury reservoir and expel CO_2 to the air until the burette contains the required amount. Shut the tap of the burette. Open the tap of the tonometer, raise the mercury reservoir considerably so that when the tap of the burette is opened towards the tonometer the CO_2 may go up into the tonometer and not the mercury and blood from the tonometer come down to the burette. Open the tap of the burette towards *A* and send up the CO_2 into the tonometer.

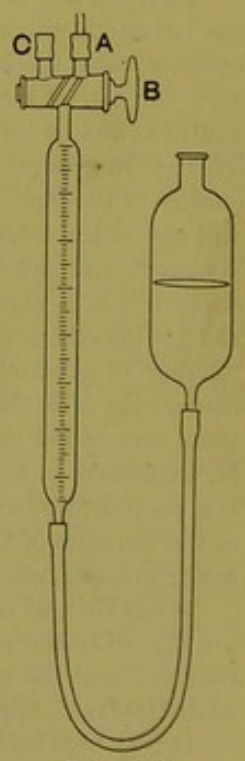


Fig. 150.

It is usual to analyse the gas in the tonometer before using it. If this is to be done remember that some oxygen and carbonic acid are lost in the analysis and therefore to this extent more of these gases must be put into the tonometer than are to be ultimately required.

Alternative method for filling tonometers.

On our expeditions on Monte Rosa and in Teneriffe we had not at hand the plant which has been described in the foregoing paragraph. The tonometers were therefore filled over mercury. This method has the merit of simplicity, otherwise I think it is not particularly desirable. The cork is taken out of the tonometer, which is placed with the mouth downwards in a mercury bath. The pressure is reduced inside the tonometer either by means of attaching a pump to the delivery end or by sucking the air out with the mouth. When the tonometer is filled with mercury the tap is shut. A tube from a nitrogen cylinder (or as was the case in Teneriffe, a phosphorus pipette in which nitrogen was made from atmospheric air) was then attached to the end of the fine tubing and the tap so turned that the nitrogen ran to waste through the tap. When the dead spaces have all been cleared of air and nitrogen is found to be running at a convenient rate the tonometer tap is turned through 180° and the mercury replaced by nitrogen. A few bubbles are allowed to escape through the mouth. The tap is then turned back to its former position, the nitrogen tube removed and the gas shut off. The tonometer is corked up, and the oxygen and carbonic acid are added by the method previously described for the addition of CO_2.

To analyse the gas in a tonometer.

Make ready the gas analysis apparatus by clearing all oxygen out of it and getting the various fluid surfaces to their marks.

Turn the tap *C* of the burette as in the figure. Connect the tonometer with the gas burette in the manner indicated in the diagram. Turn the tap *C* into the vertical position, raise the mercury reservoir till mercury reaches *A*. Then turn the tap *B* through 180° and lower the mercury reservoir till sufficient gas for analysis—8 or 9 c.c.—has gone into the gas burette. There is in general a positive pressure in the tonometer so that the surface of the mercury in the gas burette and that in the reservoir will not be the same. This pressure is an essential factor in the experiment and must therefore be measured. The simple way of doing this is to place the mercury reservoir as close to the stem of the gas burette as possible and read off the pressure in terms of the number of graduations on the stem. These graduations are, of course, $\frac{1}{100}$ of a cubic centimetre, but if it be known how many of them correspond to 1 mm. in height the measurement may readily be expressed in terms of mm. of mercury. In finally calculating the partial pressure of the gases from the percentage composition of the contents of the tonometer, this positive pressure in the tonometer must of course be added to the height of the barometer.

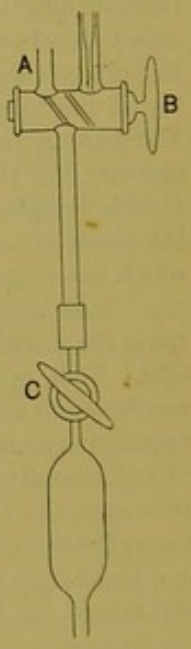


Fig. 151.

When the gas has been transferred to the gas burette, the taps are turned to the positions shown in the figure. The tonometer is removed and the gas in the burette is analysed in the usual way with Haldane's apparatus.

It may here be helpful to give an actual calculation of the partial pressure of oxygen as obtained from the data at which we may be supposed to have arrived.

Let the percentage of oxygen in the air of the tonometer be *O*, the height of the

barometer P, the positive pressure in the tonometer p, the absolute temperature of the bath in which the blood will be exposed to the gas T, that of the room t, and the required partial pressure of oxygen x,

$$x = O \times (P+p) \times \frac{T}{t}.$$

There is a slight correction which is sometimes worth making at low pressures. It has reference to the fact that the air in the tonometer acquires oxygen from the blood. Suppose 1·5 c.c. of blood are placed in the tonometer and the partial pressure is such that half of the oxygen is given off. This will amount to 0·15 c.c., which in 250 c.c. will exert a pressure of upwards of half a millimetre.

In analysing the gas after an experiment, as is frequently necessary, there are some additional points to be considered.

(1) The tonometer contains blood and this must not be allowed to foul the gas burette. It is therefore advisable to connect the two by a long rubber tube of 1 mm. bore. The tonometer is placed with the cork downwards; the air is cleared from the dead space of the rubber tubing, etc., by running mercury out to the open air through the tonometer tap; this is now turned and a few drops of mercury run into the tonometer; the mercury reservoir is then lowered and the gas is drawn into the burette.

(2) The positive pressure must be measured as before, but it must be borne in mind that in taking the gas into the burette the pressure in the tonometer has become reduced. If 8·3 c.c. are taken out of a 250 c.c. tonometer the value $(P+p)$ will be 3 % below the pressure which existed in the tonometer.

Measurement of composition of alveolar air.

There are two methods, each of which has warm adherents, for measuring the composition of alveolar air. (1) The method of Zuntz and Loewy; (2) the method of Haldane and Priestley. Before any detailed description of either of these is given a few words may be said about the principles on which each rests.

The method of Zuntz and Loewy consists in estimating the composition of the inspired and expired air, and computing that of alveolar air from a number of factors of which the most important is the "dead space of the lung." The dead space really is the volume of the nose, throat, trachea, bronchi and bronchioles. There is no profession that the alveolar air is ever actually obtained, and the correctness of the method really hinges upon the accurate measurement of the dead space.

In the method of Haldane and Priestley it is claimed that if the most sudden and violent expiration possible be made, the volume of air expired is so great as to clear the dead space entirely and to give a considerable volume of actual alveolar air at the end of the expiration. In a sense this involves a knowledge of the size of the dead space too, but in practice this may be evaded. The further one gets down the passages which lead to the alveoli, the more does the composition of the air resemble that of the alveolar air and the more does it depart from that of the atmospheric air. If, then, as the respiration comes out, one gets to a point after which successive portions of the respiration are of constant composition it may be assumed that one has arrived at the alveolar air—that at least is the contention; and further it is the contention of Haldane and Priestley that such a point is reached, and that about the last third of the respiration consists of alveolar air, which may be collected.

Within the last few years a good deal of energy has been expended by the protagonists of each theory; the general *motif* of which has been to shake confidence in the results obtained by the opposing theory.

In so far as I have had an opportunity of seeing the two methods at work on the same individual (and I believe that this test has rarely been performed) the two methods gave an identical result, that being so it seemed to me rather a work of supererogation for the supporters of one method to be so suspicious as to the validity of the other.

This however was at or near the sea-level, and it is at great altitudes that the two methods are described as giving most divergent results, the method of Zuntz giving lower readings for the oxygen alveolar air than the method of Haldane and Priestley. We may therefore make some criticisms upon the two methods.

(1) There is underlying the method of Zuntz and Loewy, or at all events underlying their application of it, the assumption that the dead space is a constant quantity. This, as Haldane has pointed out, is probably very far from being the case. The respiratory passages, at all events the smaller ones, all have a rich musculature in their walls. They are, in fact, like arteries which admit air instead of blood. The physiology of the bronchial muscles leaves much to be desired. It has mostly been studied from the point of view of asthma, and therefore it is the contraction of the muscles as compared with the normal tone which has claimed attention. It is, however, not improbable that our notions about the bronchial muscles are as distorted as those of the arteries would be if we merely considered the changes from the normal arterial tone of the resting organ to that of pathological arterial spasm. In the case of the artery the functional significance of the musculature of the arterial wall is that it can relax to let the organ have the amount of blood which it requires when it is in full activity. There is no reason to doubt that the functional significance of the bronchial muscles is to relax during conditions of exalted respiration and allow perhaps ten times the ordinary quantity of air to pass through the tubes without let or hindrance. When we recollect that the volume of a cylinder increases with the square of its diameter it will be easily seen that the dead space of the lung may increase without difficulty two or three-fold under conditions such as those obtaining at high altitudes, in which the respiration is quicker than usual. Of the mechanism of such dilatation we are in ignorance, it may be medullary; it may be the effect of changes in the blood*.

The neglect of this factor may be one reason why Zuntz's method diverges from that of Haldane's at high altitudes. This neglect would tend to make Zuntz and Loewy's results for the oxygen pressure in alveolar air too low.

If however one concedes that the dead space of the lung may be two or three times as great during conditions of deep or rapid respiration, as under ordinary circumstances one must ask oneself whether by a violent deep expiration such as is made for the purpose of Haldane's alveolar air determinations, it is possible to clear the air space as efficiently as one does under normal circumstances.

It would be most desirable that someone should make himself thoroughly conversant with both methods and make a more systematic comparison of the two than has yet been done.

* Since this was written the work of Krogh has appeared (*Journal of Physiol.* Oct. 1913).

I have always used Haldane's method, and it is by that method that all the results in the foregoing chapters have been obtained. Indeed the simplicity of the apparatus required and the ease and rapidity with which the determinations can be carried out by it, makes it much the more suitable of the two methods for work outside the laboratory.

I will therefore proceed to describe the method. A rubber tube six feet in length and ¾—1 inch in bore, is fitted with a glass mouthpiece round which the lips fit tightly. This mouthpiece must be at once large enough to offer no appreciable resistance to the respiration and small enough to be easily occluded by the tongue. About one inch from the end of the rubber tubing is a hole about 4 or 5 mm. in diameter, just large enough to take an ordinary piece of glass tubing.

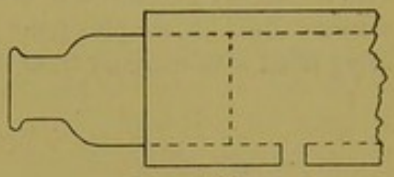


Fig. 152.

In the laboratory this piece of tubing is put directly on to the burette of a Haldane's gas analysis apparatus. The tap is turned diagonally as shown in Fig. 153, after the whole burette has been filled with mercury up to the point *A*. The mercury reservoir of the burette is then lowered almost to the bottom of the burette. (The operator must be careful that he has a tap good enough to stand this procedure.) The apparatus is now ready for the determination. The subject of the determination applies his mouth to the mouthpiece, taking care to breathe normally through his nose all the while—this is the most difficult part of the experiment—then suddenly either at the beginning or at the end of one of the normal respirations he shuts his nose and makes an expiration so forcible through the tube that he expels all the air he possibly can along the tube and at the end closes the tube with his tongue. Either he or an assistant then turns the tap to the vertical position for a moment, the burette fills itself with alveolar air and the stopper is then turned back to the position shown in the figure. The analysis is carried out in the manner described.

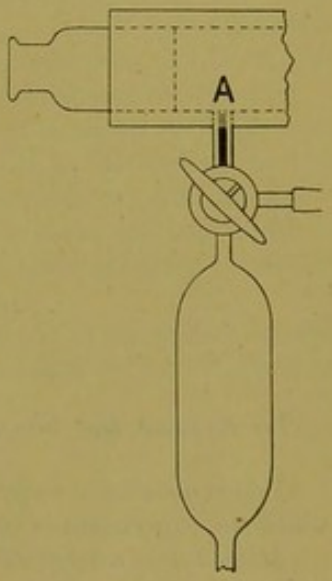


Fig. 153.

It is best to take one sample at the end of a normal inspiration and one at the end of a normal expiration and average the two.

In many cases it is not convenient to carry a gas analysis apparatus to the place where one wishes to make a determination of the alveolar air. One then takes the sample into a Haldane's gas sampling tube instead of directly into the gas burette.

This tube is shown in Fig. 154 attached to the gas burette.

The taps of the tube are adequately greased, and if it is necessary to carry the tube about it is best to be able to prevent all possibility of the stoppers either coming out or accidentally turning by fixing them in position by means of stout rubber rings (for this and similar purposes I use the rings sold for use on umbrellas). The tube is rendered vacuous in the laboratory and the taps placed so that the vacuum is shut off from the air. When it is necessary to take the sample of alveolar air this sampling

tube is put on to the large rubber tube. The operation is that already described except that the upper tap of the sampling tube, not that of the burette, is that which is rapidly opened and closed.

The gas is transferred to the Haldane's apparatus for analysis in the following way. A small piece of bent capillary glass tubing is joined by rubber junctions to the sampling tube and the gas burette of the Haldane's apparatus. The dead space is filled with mercury. The lower tap of the sampling tube is connected with a mercury burette such as shown in Fig. 150; the dead space here is filled with mercury also. A sample of the gas is then taken over into the gas burette, its place being filled with mercury from the mercury burette.

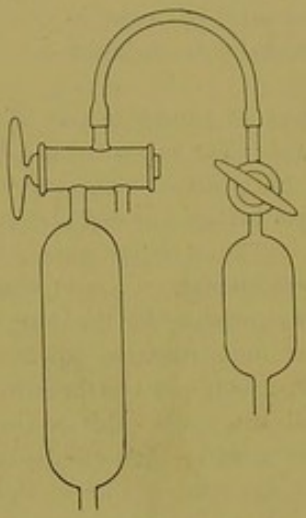


Fig. 154.

To expose the blood in a tonometer at a known temperature.

This is done in a water bath. According to the circumstances and the scale on which the experiment is being performed, one of two methods may be adopted.

Method I is a laboratory method which is best adapted for use where power can be obtained and is suitable for the investigation of a number of different samples at a time. The plant which I have is for five tonometers; these might for instance contain the samples of the same blood exposed to gas containing five different oxygen pressures and thus five points on a dissociation curve might be obtained; or on the other hand the tonometers may all be filled with different samples of blood and exposed to similar atmospheres as in experiments for the determination of the alkalinity of the various samples of blood.

The bath consists of a brass cylinder or barrel 44 cm. in height and 16 cm. in diameter. This cylinder is mounted horizontally on bearings from which it can be taken by simply lifting it, it being kept in place by a weak spring. One end of the cylinder screws off, the spindle attached to this and which rests on the bearing is hollow; through this a thermometer may be inserted. On the spindle at the other end a V-pulley is attached by which the cylindrical bath is geared by a belt to some

source of power, the gearing being driven in the case of the bath at Cambridge by $\frac{1}{25}$ horse-power motor.

Into the cylinder fits a rack, this rack can stand vertically on a metal base, from the centre of which rises a hollow tube which is concentric with the bath itself. Attached to this tube are five spring clips, into each of which a tonometer can be fixed.

The bath is placed on its end and filled almost full of water at about 1—2° C. higher than is desired for the experiment.

The rack with the tonometers is then put in the water bath. The lid containing the thermometer is screwed on, the bath is then placed horizontally on its bearings and the belt put on the driving wheel. It is then rotated at about 15 revolutions per minute by the motor. The thermometer is just put in so far that the mercury surface can be read from without the apparatus. A spirit flame playing on the bath is sufficient to keep the apparatus up to the required temperature, should the tendency for the temperature to fall be too great.

After ten minutes the motor is stopped, the bath is taken off the bearings and allowed to stand vertically on the driving wheel for two minutes, so that the blood may drain down into the small receptacle near the tap of each tonometer. The lid is then unscrewed and the tonometers taken out one by one. It is of course essential that the tonometers be carried vertically and not shaken, otherwise the blood will acquire oxygen from the atmosphere of the tonometer at the reduced temperature.

If the composition of the blood is required the tonometer is placed vertically with its lower end touching a duster, the tap is gently turned and any air and mercury in the tubing together with a drop of blood are allowed to find their way out; the tap is then closed and the end placed under the ammonia of a blood gas bottle, and if a known volume of blood is not required, the tap is simply opened again cautiously and a suitable amount of blood is allowed to flow gently out.

If the delivery tube of the apparatus is to act as a pipette of known volume, the tap is turned in the opposite direction, in which case the amount of blood which is in the pipette will run out by its own weight. Should it fail to do so it may be assisted by placing a rubber tube of large bore in the position shown in the figure and blowing down it; the air pressure resulting will probably be adequate. It is a risky proceeding to attach a rubber tube to the glass one and then blow.

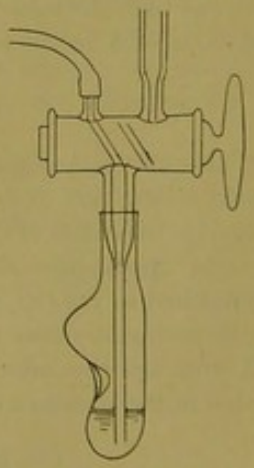


Fig. 155.

Method II, which is very useful for work outside the laboratory as well as in it, consists of using a water bath from the side of which project two arms, each of which has a crook at the end which acts as a bearing. The arms are placed at such a distance apart that the bearings catch the tonometer, one close to the cork, the other close to the tap. When the tonometer is immersed in the bath it of course tends to rise, but finding itself caught by the bearings which are above it, is unable to do so. A piece of string is put round it, by pulling the ends of the string alternately the tonometer is rotated. The bath is heated and kept warm from underneath. The tonometer is rotated for ten minutes and then held for a short time in the bath with the cork at the surface

and the end of the delivery pipette on the bottom, the blood can then drain into the cup for it before the tonometer is allowed to cool.

In emergencies the domestic bath fitted with hot and cold water serves excellently, it is large enough to accommodate the tonometer vertically as well as horizontally.

The method of determination of the percentage saturation of the blood has already been described. It is usually desirable to analyse the air in the tonometer after the blood has been taken out. For this purpose the tonometer must be placed with the cork downwards, the nozzle being connected to the gas analysis apparatus by a bent tube (either of glass or rubber) of fine bore. The dead space is cleared of blood by forcing some mercury into the tonometer, gas is then withdrawn. In calculating the pressure allowance must be made for the expansion of the gas into the analysis apparatus.

APPENDIX II. ON THE AGGREGATION OF HAEMOGLOBIN

Whilst this book has been in the press some further information has been acquired relating to the concentration of carbonic acid to which a dialysed solution of haemoglobin is exposed to the degree of aggregation of the haemoglobin molecules. It has been shown in Chapter V that alterations in the concentration of CO_2 have very little effect on the value of n in the case of blood, n being the average in number of molecules (each containing one atom of iron) in a clump, and having the value 2·5. In the case of high concentrations of CO_2 there seemed to be some evidence of a slight increase in the value of n. The most natural interpretation of this was that the salts present in the blood determined the value of n and the CO_2 the value of K in the equation

$$\frac{y}{100} = \frac{Kx^n}{1+Kx^n},$$

the two effects being therefore quite different in kind. From this supposition it would follow that the dissociations of dialysed haemoglobin in the presence of increasing quantities of CO_2 would be a series of rectangular hyperbolae presenting the general appearance shown in Fig. 13 in which n in each case was unity and K diminished as the CO_2 pressure increased.

Experiment shows that this conception is quite at variance with the facts. The following are the values of K and n for the dissociation curves of dialysed haemoglobin in the presence of the stated pressures of CO_2 at 40° C.

CO_2 pressure	n	K
0 mm.	1	·111
8 ,,	1·78	·0062
33 ,,	2·5	·00049
67 ,,	2·7	·000192

A better conception of the effect of CO_2 will be derived from the following figure:

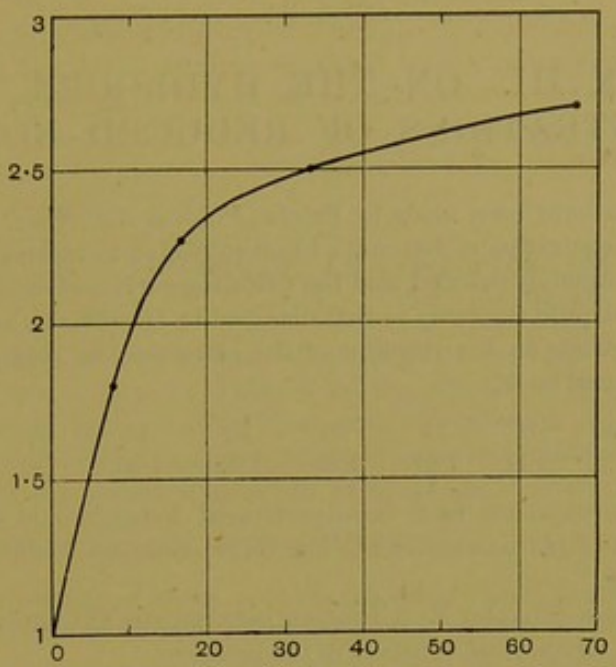


Fig. 156.—Relation of n (ordinate) to CO_2 pressure (abscissa) in dissociation curve of dialysed haemoglobin.

It is clear that the reason why the value of n is almost unaffected by CO_2 in blood is because 2·5 is nearly the maximal value. This has been effected by the salts present.

It is clear also that extreme concentrations of CO_2 may raise the value of n to a slight extent as it appears experimentally to do in the body. The effect of the salts then is to render the system stable and prevent large changes in the clumping of the molecules which would otherwise occur as the result of changes in reaction. The relative stability of the two systems may be judged from the following data:

	Haemoglobin		Blood	
	no CO_2	40 mm. CO_2	no CO_2	40 mm. CO_2
n	1	2·5	2·5	2·5
K	·111	·00043	·00258	·00029

APPENDIX III. ON THE HYDROGEN ION CONCENTRATIONS OF REDUCED BLOOD

Measurements have been made by Peters (*Physiol. Soc. Proc.*, Jan. 1914) of the hydrogen ion concentration of Barcroft's blood subjected to various pressures of CO_2. The blood was completely reduced, and the following curve was obtained. The result was nearly but not quite the same as that obtained by Hasselbalch and his colleagues, whose admirable work on the reaction of the blood may be read parallel with the later chapters in this book.

CO_2 pressure mm.	0	10	20	30	40	60	80	100
P_H	8·11	7·67	7·52	7·39	7·30	717	707	699

Till further experiments have been performed Peters would not insist on the *absolute* accuracy of his measurements but there seems no doubt about their *comparative* accuracy.

We already have (Chapter V) a relation between the CO_2 pressure in Barcroft's blood and K, the equilibrium constant in the equation

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

We may therefore directly compare K and the hydrogen ion concentration:

Log K	$\bar{3}$·40	$\bar{3}$·20	$\bar{3}$·00	$\bar{4}$·80	$\bar{4}$·60	$\bar{4}$·40	$\bar{4}$·20	$\bar{4}$·10
K^x, $x = -2{\cdot}60$	2·80	3·00	3·20	3·40	3·60	3·80	3·90	
P_H	−8·10	7·933	7·72	7·59	7·42	7·25	7·075	6·99

The comparison is instructive enough for it appears that the product of $C_H + K$ is constant as shown by the fact that the figures in each column of the last 2 lines in the above table add up to $-10{\cdot}8 \pm {\cdot}1$. Should this relation prove on further examination to be substantiated, Hill suggests that it is evidence of actual chemical combination between the hydrogen ion and the haemoglobin. The hydrogen ion would then have two actions: (1) a tendency to cause the globin molecules to aggregate and (2) an actual union with the haematin portion.

We are not so much concerned with the theoretical question as with the fact that we can state a figure for P_H at the various places at which we have measured K in Barcroft's blood.

	K	P_H
Normal	·00029	−7·29
Carlingford after climb of 1000 feet in 45 min.	·00024	7·22
,, ,, ,, 30 ,,	·00017	7·09
Col d'Olen ,, ,, 45 ,,		7·14
Margherita Hut arrive	·00013	7·02

APPENDIX IV. ON THE CONSTANT OF AMBARD

The determination of the Constant of Ambard (Ambard and Weill, *Journal de physiol. et path. gén.* XIV, p. 73, 1914) is for the purpose of discovering whether there is retention of urea or not. When the concentration of urea in urine is 25 grams per litre of urine, the concentration of urea in the blood (ur) divided by the square root of the output of urea per 24 hours (D) is nearly constant, varying between 6·3 and 8·0, i.e. $\frac{ur}{\sqrt{D}} = K$ (constant of Ambard).

When the concentration of the urea in the urine is not 25 grams per litre but C grams per litre, then D must be corrected for the change in concentration. It is multiplied by the root of C and divided by the root of 25; this gives a factor termed D_{25} which replaces D in the above equation, so that

$$\frac{ur}{\sqrt{D_{25}}} = K \text{ (constant of Ambard)}$$

and

$$D_{25} = D \times \frac{\sqrt{C}}{\sqrt{25}} \text{ or } \frac{\sqrt{C}}{5}.$$

An actual observation does not last for 24 hours, but for a time T which is about $\frac{3}{4}$ of an hour, the volume of urine secreted in this time is v c.c. and the volume of urine as calculated for the 24 hours at this rate of secretion is V,

$$V = \frac{v \times 24 \times 60}{1000 \times T} \text{ litres,}$$

the other measurements necessary are of course the concentration of urea in the urine C, and in the blood ur. If the final answer does not come between 8 and 6·03 the secretory action of the kidney is upset.

INDEX

CAMBRIDGE: PRINTED BY JOHN CLAY, M.A. AT THE UNIVERSITY PRESS