XCII. THE OXYGEN CAPACITY OF THE BLOOD IN ONE HUNDRED CASES OF MENTAL DISORDER.

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It is probable that the most important factor responsible for the abnormal reactions in cases of mental disease can be traced to some variety of defective oxygen metabolism [Pickworth, 1929].

Irregularities of the normal oxygen supply or its utilisation may occur at any point in the respiratory cycle. The present paper deals with deficient oxygen supply due to defective blood-pigment. From pathological considerations it is highly probable that toxic substances absorbed from the intestinal canal (and evidence of blood infection with intestinal organisms is found almost invariably in association with acute mental disorder) might effect a chemical change in the haemoglobin of the blood, rendering it useless for respiratory purposes.

The present investigation was therefore undertaken to ascertain whether inactive forms of haemoglobin occurred in the blood in cases of mental disorder.

The active haemoglobin of a large number of specimens was determined by direct measurement of the oxygen liberated from fully oxygenated blood, and these values were compared with the total haemoglobin content found by other methods of analysis.

Since it was required to measure very small quantities of inactive pigment, it was necessary to devise an accurate and reliable method of analysis specially suited to the work and quantity of material available. A modification of Haldane's blood-gas apparatus [1900, 1920] was made which was adaptable for a small quantity of blood. Control experiments of each factor influencing the analysis were made in order to secure consistency and reliability of the results.

Methods.

Estimation of the oxygen capacity of blood.

The blood was collected in sterile tubes containing a trace of potassium oxalate and sodium fluoride as an anti-coagulant. Carbon dioxide was removed by exposing in films to a current of moist, carbon dioxide-free air in presence of a phosphate buffer solution of p_H 7·5. A measured quantity

(1 to 2 cc.) of this CO_2-free blood-buffer mixture was introduced into the reaction flask of the apparatus; this was immersed in a water-bath at 37° and gently shaken until the blood had attained equilibrium with the atmosphere in the flask. 1 cc. of ferricyanide with saponin was then admitted from the separate compartment of the apparatus into the reaction flask. The oxygen was completely expelled by shaking the apparatus for 3 minutes, and the volume of gas evolved measured and calculated to 100 cc. of blood.

Description of blood-gas apparatus.

The apparatus consisted of a narrow bore U tube containing clove oil tinted with safranine as a pressure indicator; there was a third limb of wider bore acting as a reservoir for the oil which was also convenient for measuring the gas under definite barometric pressure.

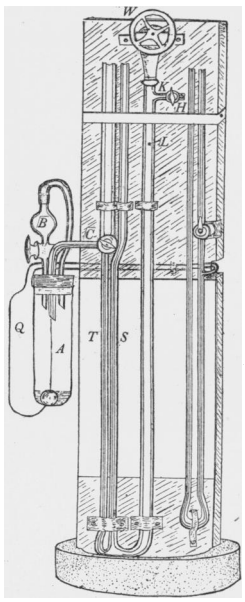

Fig. 1.

The burette T (Fig. 1) was fitted with a two-way tap by means of which it could be connected either with the side limb C and the reaction flask A, or opened to air for adjustment to barometric pressure.

The ferricyanide was contained in a separate compartment B, which was connected above with the reaction flask so that it could be admitted without altering the pressure. The barometric pressure and temperature control bulb Q was of similar size (about 30 cc.).

The burette tubes T and S were of identical bore, the former being accurately graduated to a volume of exactly 0·250 cc. and divided into 100 equal parts.

The apparatus was rigidly mounted on a very heavy base and was designed for use completely immersed in a water-bath at 37°. The temperature was controlled by a thermostat and the water was kept thoroughly stirred by a current of air.

The rigid nature of the connections in the apparatus was an important feature and allowed thorough shaking in order to mix the reacting fluids. Complete and rapid expulsion of the liberated oxygen was secured by the introduction of a single large glass marble 1·5 cm. in diameter into the reaction flask.

Accurate and reliable analyses could be made with as little as 0·5 cc. of blood.

A. *Collection and preservation of specimens.*

The blood was collected in sterile tubes containing 0·02 g. of a finely powdered mixture of potassium oxalate and sodium fluoride, 5 : 1.

This anti-coagulant did not cause haemolysis. Estimations were carried out immediately after collection of the specimens when possible. Otherwise

they could be stored satisfactorily for a short period in an ice-chamber. It was found that sterile blood, so kept, did not change in oxygen capacity in 4 days, although after 6 days an appreciable diminution was noticeable.

B. *Preliminary treatment of blood samples.*

In order to eliminate errors caused by varying hydrogen ion concentrations of the blood specimens, and also to prevent liberation of carbon dioxide during the test, the p_H of the blood was controlled by means of the addition of strong buffer solution, and the carbon dioxide was removed from the mixture before introducing it into the flask. An equal volume of phosphate solution of p_H 7·5 (containing 8·184 g. $Na_2HPO_4, 12H_2O$ and 0·608 g. NaH_2PO_4 in 50 cc. of distilled water) was added to the blood. A steady stream of air purified from traces of CO_2 by bubbling through dilute sodium hydroxide was passed over the mixture which was gently rotated in a tonometer. This treatment removed about 20 cc. of CO_2 per 100 cc. of blood in 20 minutes. Analyses conducted with the blood so treated were proved to be quite consistent.

C. *Equilibration of samples at 37°.*

For each estimation, 1·6 cc. of the blood-buffer mixture was introduced into the flask *A* by means of a standardised delivery pipette; approximately 1 cc. of recently made ferricyanide (12·5 % dissolved in the same buffer solution, diluted five times in order to bring the salt concentration to a suitable amount), with 0·005 g. of white saponin, was placed in the bulb *B*.

In order for the blood to attain complete equilibrium with the air of the flask, it was necessary to shake the apparatus in the bath for 15 minutes, equilibrium being evidenced by the clove-oil levels remaining quite stationary for 2 minutes.

D. *Process of analysis.*

The levels of the clove oil in the burette tubes were brought to a convenient position near to the zero point by adjusting the pressure in the tube *L*, the temperature gauge was closed, and the ferricyanide solution admitted. The apparatus was gently shaken for 3 minutes. The large glass marble greatly facilitated rapid expulsion of the liberated oxygen in a manner much superior to a number of smaller beads, or broken glass. The levels were adjusted to the gas increase and the temperature of the bath was noted. After obtaining equilibrium the volume of gas was observed and calculated to N.T.P. per 100 cc. of blood. A further shaking of the apparatus was then carried out which should cause no further evolution of gas.

Accuracy of the analysis. We were able to eliminate the following errors by a careful investigation into the conditions under which the test was carried out, and in every case considerable latitude could be allowed in the quantities and times of the tests without introducing these or other errors.

(*a*) Incomplete liberation of oxygen due to faulty haemolysis of the corpuscles (see experimental section E′).

(*b*) Blood laked with dilute ammonia may retain a portion of the liberated oxygen.

(*c*) CO_2 may be liberated owing to the insufficient alkalinity of the solution.

(*d*) Litarczek [1928], and Parsons and Parsons [1927] have described a reducing action which occurs in specimens of pathological blood which results in an absorption of some of the oxygen evolved in the test.

Our experiments under similar conditions demonstrated that some specimens of human blood absorbed a quite appreciable amount of the liberated oxygen. It was found that plasma or serum from normal animals reacted similarly, and even washed rabbit or sheep corpuscles possessed this reducing property to a slight degree. These errors were eliminated by adequately buffering the test mixture, the advantage of which is illustrated by experiments in section B′ and Figs. 2 and 3.

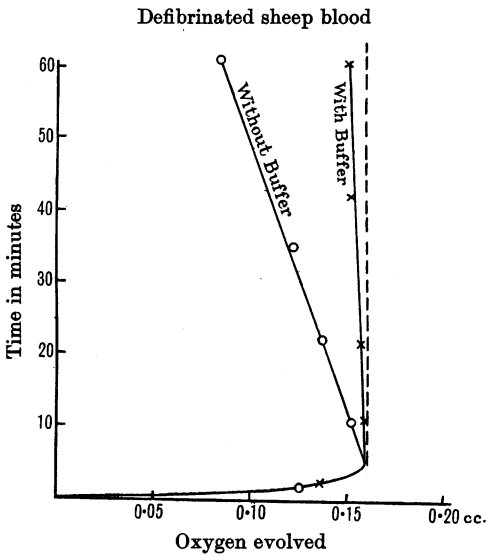

Fig. 2. To show effect of buffer in reducing oxygen absorption in test.

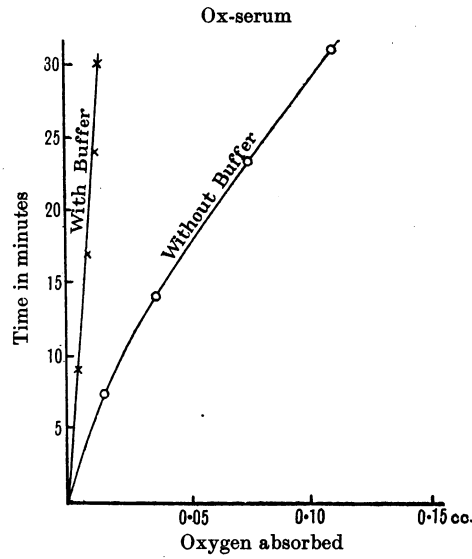

Fig. 3. To show effect of buffer in reducing oxygen absorption in test.

The accuracy of the method was carefully checked by other tests which included Stadie's cyanohaemoglobin colorimetric method, and an estimation of the iron content of the haemoglobin by a micro-titration method using titanium chloride. The quantity of haemoglobin was also estimated by the Fleischl-Miescher haemoglobinometer using a calibrated carbon monoxide wedge.

Experimental.

A′. *Preservation of specimens.*

The following typical experiment demonstrates the change in the oxygen capacity of blood containing oxalate-fluoride anti-coagulant, after storing in the ice-box for various periods.

Specimen No. 59. Sterile rabbit blood + 0·5 % oxalate-fluoride mixture.

Time of storing	Oxygen capacity per 100 cc.
Fresh	15·1 cc.
24 hrs.	15·0 „
48 hrs.	15·0 „
4 days	15·0 „
6 days	12·3 „

No change occurred in 4 days, after which a distinct fall in oxygen capacity took place.

B′. *Effect of buffering the sample.*

1. A large number of sera from various sources, when mixed with an equal quantity of phosphate buffer, were found to have a p_H of 7·5 by the capillator method. The clear fluid obtained by centrifuging a mixture of equal parts of blood and buffer also had a similar p_H value.

2. No alteration in the volume of gas, due to changes in vapour pressure, was caused by the addition of buffered ferricyanide to blood-plasma plus an equal volume of buffer.

Exp. 80. 0·81 cc. human plasma + 0·81 cc. buffer.

Time	Burette reading
Equilibrium	0·185 cc.
3 mins.	0·185 „
Ferricyanide added	
7 „	0·185 „
15 „	0·185 „

3. The addition of acid or alkali to blood did not affect the oxygen capacity, provided the buffer solution as described above was added before saturation with air previous to the test.

Exp. 82. 1 cc. blood + 0·5 cc. 2 % Na_2CO_3; oxygen evolved after buffering etc. = 0·120 cc.

1 cc. blood + 0·5 cc. 0·3 % lactic acid; oxygen evolved after buffering etc. = 0·120 cc.

1 cc. blood + 0·5 cc. isotonic saline; oxygen evolved = 0·121 cc.

4. The addition of a small amount of lactic acid to blood has a very marked effect at low oxygen pressures; when this lactic acid-blood is treated with buffer prior to saturation with the low pressure of oxygen, the calculated theoretical result is obtained.

Exp. 83. 1 cc. corpuscles + 1 cc. isotonic saline + 0·5 cc. 0·1 % lactic acid exposed to 2·6 % oxygen in nitrogen.

Oxygen evolved = 0·194 cc. = 67·5 % saturated.

(Calculated saturation = 84 %.)

1 cc. corpuscles + 0·5 cc. lactic acid + 1 cc. buffer solution, then exposed to 2·6 % oxygen in nitrogen.

Oxygen evolved = 0·225 cc. = 84 % saturated.

(Calculated saturation = 84 %.)

5. The effect of the buffer in preventing the re-absorption of oxygen by the blood-ferricyanide mixture is shown by the following experiment.

Exp. 85. 1 cc. sheep plasma + 1 cc. 2 % Na_2CO_3, after equilibrium established with its atmosphere in the reaction flask, 1 cc. aqueous ferricyanide added. Readings taken at intervals.

Time	Burette reading	Time	Burette reading
0 mins.	0·229 cc.	12 mins.	0·193 cc.
2 ,,	0·220 ,,	18 ,,	0·183 ,,
5 ,,	0·212 ,,	25 ,,	0·170 ,,
8 ,,	0·200 ,,	30 ,,	0·162 ,,

Absorption in 30 mins. = 0·067 cc.

Exp. 86. 1 cc. sheep plasma + 1 cc. buffer p_H 7·5; after equilibrium established, 1 cc. buffered ferricyanide added; readings at intervals.

Time	Burette reading	Time	Burette reading
0 mins.	0·190 cc.	15 mins.	0·188 cc.
5 ,,	0·189 ,,	30 ,,	0·188 ,,
10 ,,	0·189 ,,	40 ,,	0·187 ,,

Oxygen absorbed = 0·003 cc.

Similar tests comparing alkaline with buffered solutions are shown in Figs. 2 and 3.

It will be noted that the serum is responsible for the greatest re-absorption of oxygen. A further similar test with washed sheep corpuscles gave a much smaller absorption deviation.

C'. *CO_2 in the sample.*

When considerable amounts of carbon dioxide are present in the blood sample, a portion of it passes into the air above the mixture in the reaction flask. This quantity may vary slightly on adding the reagent, and samples must therefore be previously treated to remove excess CO_2.

Exp. 87. (*a*) 1 cc. sheep blood-plasma previously exposed to CO_2-free air current for 20 minutes (CO_2 content = 20 cc./100 cc.); 1 cc. buffer added; ferricyanide admitted when in equilibrium.

	Time	Burette reading
	0 mins.	0·147 cc.
	3 ,,	0·147 ,,
	Equilibrium established	
Ferricyanide added	5 mins.	0·147 cc.
	10 ,,	0·146 ,,
	25 ,,	0·146 ,,

No change on adding reagent.

(*b*) 1 cc. sheep plasma (untreated), CO_2 content = 40 cc./100 cc., 1 cc. buffer added; ferricyanide admitted when in equilibrium.

Time	Burette reading
0 mins.	0·135 cc.
3 „	0·135 „
Ferricyanide added	
5 „	0·135 „
8 „	0·120 „
10 „	0·116 „
12 „	0·116 „

When blood-plasma containing a considerable quantity of carbon dioxide is used, there is a distinct change in the volume of the gas on addition of the reagent, due to absorption of CO_2.

Exp. 88. The quantity of CO_2 in the atmosphere of the reaction flask is shown in the table below. Blood + equal volume of buffer p_H 7·5 exposed for varying periods to a current of moist, CO_2-free air; 2 cc. of the resulting mixture introduced into the apparatus. After the volume had become constant, the CO_2 in the flask was absorbed by admitting 0·2 cc. of 5 % sodium hydroxide, the contraction in volume being the amount of CO_2 present.

Time of exposure to CO_2-free air	CO_2 in gas of reaction flask	Equivalent mm. of CO_2
0 mins.	0·57 %	3·9 mm.
5 „	0·31 %	2·2 „
10 „	0·21 %	1·5 „
20 „	0·14 %	1·0 „
30 „	0·13 %	0·91 „
60 „	0·12 %	0·85 „

Laboratory air = 0·4 mm. CO_2.

Exp. 89. Elimination of excess CO_2. The blood at p_H 7·5 was exposed in a tonometer through which a current of CO_2-free air was passed for varying periods.

Time of exposure	CO_2 per 100 cc. blood
0 mins.	42·5 cc.
1 „	37·5 „
5 „	28·0 „
15 „	22·5 „
20 „	21·2 „
30 „	20·0 „
3 hrs.	17·5 „

An exposure of 20 minutes was sufficient to remove the excess CO_2.

D′. *Equilibration of gas pressures.*

It was found that whole blood required about a quarter of an hour to attain equilibrium with the air of the reaction flask, gentle shaking of the apparatus being continued during the whole time. This period was much increased for plasma, whilst only a few minutes were required for a suspension of washed blood-corpuscles in saline.

E′. *The ferricyanide-saponin reagent.*

This consisted of 12·5 g. ferricyanide + 0·2 g. white saponin in 100 cc. phosphate buffer diluted five times with distilled water.

1. *Amount of ferricyanide.* A large excess of the ferricyanide mixture is necessary in order to decompose the oxyhaemoglobin completely. (Conant and Fieser [1924] have calculated that at least 13·5 equivalents per g.-molecule of haemoglobin are required when in neutral solution.)

The following test shows that the amount used (0·125 g. for each estimation) was amply sufficient, and that this amount could be varied considerably either way without error.

Exp. 90. (1) 2 cc. buffered ox blood + 1·5 cc. ferricyanide reagent: oxygen evolved = 0·216 cc.
(2) 2 cc. buffered ox blood + 1·0 cc. ferricyanide reagent: oxygen evolved = 0·216 cc.
(3) 2 cc. buffered ox blood + 0·5 cc. ferricyanide reagent: oxygen evolved = 0·216 cc.

2. *Amount of saponin.* The addition of an amount of saponin equivalent to that in the reagent (*i.e.* 0·002 g. per cc.) to a sample of blood caused complete haemolysis in 2 minutes. Microscopic examination of a drop of the fluid failed to show the presence of corpuscles. The addition of strong salt solution to this clear fluid did not cause the re-appearance of corpuscles (microscopically or by turbidity) as occurs with blood laked with a small quantity of distilled water.

The following test shows that the accuracy of the analysis was not affected if the amount of saponin added to the blood varied considerably from the quantity actually employed.

Exp. 92. (1) 2 cc. buffered ox blood + 1·0 cc. ferricyanide containing 0·4 % saponin: oxygen evolved = 0·203 cc.
(2) 2 cc. buffered ox blood + 1·0 cc. ferricyanide containing 0·2 % saponin: oxygen evolved = 0·203 cc.
(3) 2 cc. buffered ox blood + 1·0 cc. ferricyanide containing 0·15 % saponin: oxygen evolved = 0·203 cc.

The rate of absorption of oxygen by haemoglobin was markedly slower in the presence of saponin.

Exp. 93. (1) Human blood exposed to 2·1 % oxygen. Then exposed to air for 15 mins.
Oxygen content = 17·8 cc. per 100 cc. = 100 % saturated.
(2) Human blood exposed to 2·1 % oxygen; saponin added. Exposed to air for 15 mins. exactly as in test (1).
Oxygen content = 16·4 cc. per 100 cc. = 92 % saturated.

Other methods of estimation of haemoglobin content.

1. *Cyanohaemoglobin colorimetric determination.* A slight modification of the process described by Stadie [1920] and Van Slyke and Stadie [1921] has been utilised. The pigments are converted to methaemoglobin by adding a trace of ferricyanide to the diluted blood and then, by adding a little dilute

potassium cyanide solution, cyanohaemoglobin is produced which has a brownish-red colour suitable for colorimetric estimation. A control is used, similarly prepared from pure blood. Blood containing small amounts of methaemoglobin shows a higher value for the colorimetric, as compared with the gasometric, method.

0·2 cc. blood is diluted to 5 cc. with distilled water; 1 drop of 10 % ferricyanide containing 2 % lactic acid is added. After standing for 15 mins., 1 cc. 0·1 % KCN is added and the solution diluted to 20 cc. The resulting colour is matched with the standard similarly prepared.

The lactic acid greatly facilitates the rate of conversion to methaemoglobin, and the colour is stable and unaffected by the amount of acid present.

2. *Fleischl-Miescher haemoglobinometer method.* A thin film of blood was exposed to coal gas in a tonometer until saturated with carbon monoxide. It was diluted 1 : 200 in a haemocytometer pipette with water saturated with carbon monoxide and the solution placed in one-half of the comparing chamber, the other half being filled with distilled water. The haemoglobin values were read directly from a carbon monoxide wedge.

3. *Micro-titration of the iron content.* This method of analysis was used to check the other determinations and is similar to that used by Peters [1912].

The blood was weighed into a small nickel crucible (about 0·3 g.) and ashed by direct heat. The residue was dissolved in 2 drops of distilled water and 1 drop of strong iron-free hydrochloric acid. A drop of dilute hydrogen peroxide was added, the solution diluted to about 0·5 cc. and evaporated almost to dryness in order to ensure decomposition of all traces of peroxide. The solution was transferred to a titration tube having a constricted neck and bulb of capacity 3 cc. with a little acidulated water, and the volume of solution and washings made up to 2 cc. The titrations were made from a micro-burette, capacity 1 cc., with approx. $N/200$ titanium chloride. The reagent was made by adding 15 cc. of 15 % commercial titanium chloride to 15 cc. of hydrochloric acid, boiling for 3 mins. and diluting to 2500 cc. with well-boiled water. This volume completely filled the containing vessel.

It was stored in contact with an atmosphere of CO_2, and standardised immediately before making analyses.

In order to prevent re-oxidation by the oxygen in the titration tube, the air was completely displaced before the analysis by means of a rapid stream of CO_2 and the surface of the liquid was covered with a thin layer of liquid paraffin.

One drop of 10 % KCNS was used as indicator and the titanium chloride was added from the micro-burette until the red colour just disappeared. When the ashing process was correctly performed and the precautions indicated above were observed, no return of the red tint occurred for some time and accurate analyses were obtained.

Results.

Blood specimens from a number of normal healthy persons and 130 specimens from 100 patients have been analysed. Also 40 specimens from healthy animals, including the sheep, ox, pig and rabbit, have been determined, and a number of analyses have been made with the blood of cats and rabbits whilst under treatment with drugs.

All the analyses were performed in duplicate, the results agreeing to within 2 % for the colorimetric tests and 1 % with the other determinations. The following is a summary of the results obtained. Values have been expressed on the basis of 18·5 cc. oxygen = 100 % haemoglobin (Haldane's standard for normal male).

A. *Normal human blood.*

The following tables show the results obtained in analyses of the blood of a number of healthy persons. A repeat of No. 1 is included to show the consistency of results obtainable after a few hours' interval.

Table I. *Oxygen capacity (normal human specimens).*

Specimen	Oxygen evolved per 100 cc.	Corresponding % Hb. content
1	19·0 cc.	102·6
2	18·6 „	100·7
3	17·6 „	95
4	18·4 „	99·5
1 (repeat)	19·05 cc.	102·9

Table I *a*. *Haemoglobin content (comparison of methods).*

Specimen	% Hb. by gasometric method	% Hb. by Stadie method	% Hb. by CO-Hb. method
1	102·6	103	102
2	100·7	101	100
3	95·1	Standard	94
4	99·5	99·2	98

Table II gives the results of analyses of a number of specimens from healthy animals.

Table II. *Haemoglobin content of animals' blood.*

Animal	% Hb. by gasometric method	% Hb. by $TiCl_3$ titration	% Hb. by CO-Hb. method	% Hb. by Stadie method
Sheep	108	106·5	106	108
Ox	86·5	86·8	87	Standard
Pig	71·4	72·2	70	71·2
Ox (corpuscles)	99·4	—	99	99·3
Rabbit	59·5	—	59	59·2
Cat	57·8	—	58	57·2
Sheep	110·8	109	110	110·7
Pig	84·3	86	85	84

The values calculated from the gasometric method agreed very closely in all cases with those by the cyanohaemoglobin colorimetric method. The values by the Fleischl-Miescher method varied slightly owing to a greater difficulty

in matching the colour. (The tints of the blood solution, when fully saturated with carbon monoxide, compared satisfactorily with the standard wedge with the exception of rabbits' blood which gave a yellower tint.) Determinations of the exact colours were also made by the Rosenheim-Schuster colorimeter, and some of the results are given which may possibly be useful for reference.

Colour values of haemoglobin solutions.

Normal human blood diluted 1 : 100

Specimen	Tintometer units
Oxyhaemoglobin	Red 15·0; yellow 1·5
Carboxyhaemoglobin	Red 20·2; yellow 0·2
Cyanohaemoglobin	Red 12·0; yellow 4·2

B. *Patients' blood.*

The absolute haemoglobin content as found by the gasometric method is given in Table III.

Table III. *Haemoglobin content of patients' specimens.*

	Males		Females	
Hb. content	No. of specimens	%	No. of specimens	%
Over 100 %	6	16	9	10
90–100 %	20	53	30	32
80– 90 %	9	23	39	42
70– 80 %	3	8	11	12
Below 70 %	0	0	3	3

In only comparatively few cases was there any serious deficiency in the quantity of available haemoglobin. The average haemoglobin content for the whole series was 93 % for males and 89 % for females.

Table IV gives the results in 20 unselected cases, 10 male and 10 female.

Table IV.

Sex	Oxygen from 100 cc. blood cc.	% Hb. by oxygen evolved	% Hb. by cyano-Hb. method	% Hb. by CO-Hb. method	Notes
M.	16·2	87·6	88	86	—
,,	16·1	87·0	88	88	—
,,	17·6	95·3	95·6	95	—
,,	16·5	89·2	96·5	96	7·3 % inactive Hb.
,,	18·0	97	97	98	—
,,	19·8	107	107	108	—
,,	17·5	95	94	93	—
,,	17·0	92	98	96	6 % inactive Hb.
,,	17·1	92·6	93	93	—
,,	16·6	90·0	91	90	—
F.	17·0	92	92	92	—
,,	16·0	86	86	86	—
,,	15·9	86	87	87	—
,,	20·1	108·5	110	110	1·5 % inactive Hb.
,,	15·5	84	84·8	85	—
,,	19·6	106	106	106	—
,,	16·4	88·5	92	91	3·5 % inactive Hb.
,,	17·5	95	93·5	95	—
,,	15·9	86	86	86	—
,,	16·5	89	89	88	—

Three of the above specimens gave low results by the oxygen method as compared with the colorimetric and titrimetric methods; this indicates the presence of inactive haemoglobin.

Among the total 100 cases, 28 showed deficient oxygen capacity compared with the theoretical. In all these the difference exceeded 2·5 %, the greatest deficiency being 12·5 %, which was observed in two specimens; neither of these patients had received treatment with sulphonal or allied drugs.

In accordance with previous supposition of the effect of intestinal disease, the 100 cases contained a large number whose blood showed a positive agglutination by the Widal test. (See Table VI.)

A number of cases showing deficient active haemoglobin were examined at intervals of several weeks. In most cases the deficiency persisted for a long period. The following table is included to illustrate this important finding.

Table V. *Haemoglobin in blood of special cases.*

Name	Date	Sex	% Hb. by gasometric method	% Hb. by colorimetric method	% difference
H.F.	14. iii. 29	F.	95	100	5
„	3. v. 29	„	89	97	8
„	30. v. 29	„	99·3	109	9·7
„	14. vi. 29	„	101	113	12
A.S.	24. v. 29	„	72·6	83	10·4
„	14. vi. 29	„	74	80	6
A.J.	14. vi. 29	„	80	86·5	6·5
„	16. v. 29	„	71·5	82	10·5
B.W.	19. vii. 29	„	90	99	9
„	25. vii. 29	„	81	87·3	6·3
„	30. viii. 29	„	85·1	92	6·9
„	5. ix. 29	„	91	102·8	11·8
D.G.	30. viii. 29	„	82·4	92	9·6
„	5. ix. 29	„	84	89	5
„	18. ix. 29	„	101·3	107·1	5·8
L.S.	1. viii. 29	„	90	100·8	10·8
„	5. ix. 29	„	79	88	9
D.R.	5. vii. 29	M.	92	98	6
„	2. viii. 29	„	92·5	98	5·5
R.P.	27. vi. 29	„	89·2	96·5	7·3
„	2. viii. 29	„	85	91	6·0
J.G.	5. ix. 29	„	95	107	12·0
H.H.	26. ix. 29	„	103	110	7·0

Table VI.

Widal agglutination reaction	No. of cases	Cases with over 2 % of inactive pigment	Average % of inactive pigment
+	52	27	4·2
−	38	1	0·38

A simple test for methaemoglobin.

The following test demonstrates the presence of methaemoglobin when 5 % or more is present in blood; with the simple type of spectroscope about 20 % of methaemoglobin must be present for reliable detection.

0·20 cc. of blood is saturated with carbon monoxide (coal-gas) in a tonometer and then diluted with 40 cc. water saturated with carbon monoxide. 10 cc. are transferred to each of two similar tubes and 1 drop of 2 % ammonia

is added to each. The air is displaced by coal-gas from the tubes, which are corked and shielded from bright light. To one tube 0·5 cc. of 1 % KCN is added, and to the other 0·5 cc. of freshly made solution of 1 % sodium hydrosulphite, avoiding undue exposure to air. The stoppers are replaced and the tubes gently inverted. The methaemoglobin of the one tube is converted to brownish-red cyanohaemoglobin, and the methaemoglobin in the other is first reduced to haemoglobin followed by formation of carbon monoxide-haemoglobin. The colour difference is recognised by naked-eye comparison. The colours produced were quite stable when air was excluded.

Discussion.

The presence of inactive haemoglobin has been found to occur in one quarter of the cases examined, the amount in two cases being as much as 12 %. It is submitted that the amount of inactive haemoglobin under certain circumstances is very much higher than this, and further work is being continued with acute cases.

The presence of inactive haemoglobin has been correlated with diseased conditions of the intestinal tract. Such diseased conditions are very frequent in cases of mental disorder, and evidence of blood infection apart from clinical disease can be obtained from the agglutination reaction of the blood in about one quarter of the cases admitted to the mental hospitals investigated (Birmingham) [Pickworth, 1927, 1928].

Table VI shows the relation of inactive haemoglobin to a positive agglutination reaction. The close correlation is obviously more than coincidence and tends to confirm the view that an infected intestinal tract allows the absorption over a large area of bacteria and toxic substances which deprive the blood of its primary function, that of oxygenation. Other visceral conditions of focal sepsis of the upper respiratory tract, the fatty liver and kidneys and the atheroma of the blood vessels, so often found in cases of mental disorder, support this view.

Buscaino [1923, 1926] has also described lesions of the small intestine and holds that a toxic substance is absorbed, especially in acute confusional insanity and in dementia praecox. He suggests that in these cases the liver does not function normally and that amino-acids become decarboxylated, giving rise to poisonous amino-compounds. Stewart [1929] suggested that this toxic compound is tyramine and demonstrated that tyrosine was destroyed with production of the amine by the intestinal bacteria from cases of acute insanity.

Boyd [1923] found histamine in the intestines of children dying from intestinal toxaemia. Looney [1924] has described toxic amines in the blood of mental patients in the depressed phase.

A number of animal experiments have been conducted with a view to reproducing some of these conditions.

1. *Effect of chemical reagents.* (*a*) A rabbit, 2000 g., was injected intravenously with the following quantities of sodium nitrite at intervals of 30 minutes: 0·25, 0·5, 1·0, 1·5, 2·0 cc. of 2 % solution, 2·0 cc. of 5 % solution. Blood samples were taken after 12 hours. The haemoglobin value was 70 %, but no inactive haemoglobin was demonstrated. (Sodium nitrite readily produces methaemoglobin *in vitro*.) This experiment shows how quickly methaemoglobin disappears from the blood. Haldane found that methaemoglobin disappeared in about 4 hours.

(*b*) A rabbit, 1500 g., was injected intravenously with 5 cc. of 5 % sodium nitrite solution. A blood sample taken 40 minutes later contained 64·2 % of blood-pigment by the Stadie method. Only 21·6 % was oxyhaemoglobin, the capacity being 4·0 cc. O_2 per 100 cc. Thus 42·6 % of methaemoglobin was present. The blood was dark and when saturated with carbon monoxide yielded a brown-coloured solution. The animal died shortly after.

(*c*) A cat, 900 g., was given 5 grains phenacetin by mouth on five successive days. The total haemoglobin content of samples taken after the last dose was 45 %, and the active pigment amounted to only 34 % (8·4 cc. oxygen per 100 cc. blood). Thus 11 % of inactive material was present. The blood was dark and the animal anaemic. The presence of the inactive pigment was also demonstrated by the special test described. (Young [1926] asserts that aniline and certain derivatives do not produce methaemoglobin when administered to animals and that the dark colour of the blood in such cases is due to *p*-aminophenol.)

2. *Effect of bacterial injections.* (*a*) Experiments were made in which emulsions of *Streptococcus mitis* and *S. faecalis* were injected intravenously into rabbits. In neither case was there any indication of inactive pigment in the blood.

(*b*) An emulsion of 0·2 cc. of *B. nitrosobacter* was injected intravenously into a rabbit. A specimen of blood taken 2 days later had an oxygen capacity of 11·7 cc. O_2 per 100 cc., *i.e.* 63 % of the normal Hb. content. The total haemoglobin by the Stadie method was 70 %, *i.e.* 7 % of inactive haemoglobin was present.

(*c*) The following injections of T.A.B. vaccine (2000×10^6 organisms per cc.) were made intravenously into a rabbit of 2000 g. weight. 3 cc. on 3. xii. 29, 6 cc. on 9. xii. 29, 12 cc. on 17. ix. 29, 20 cc. on 23. ix. 29. After each injection samples of blood were examined, but in no specimen was inactive pigment demonstrated. In spite of the enormous doses the animal remained apparently well.

(*d*) A rabbit, 2000 g., was injected with 5 cc. of emulsion of *B. enteritidis* Gaertner (20×10^9 organisms per cc.). A blood sample taken 24 hours after the injection contained 15 % of inactive haemoglobin, the active oxyhaemoglobin being 54 %. The animal died 40 hours after the injection.

The nature of the inactive pigment.

The chemical properties of the inert pigment are similar to those of methaemoglobin, and differences, if they exist, can only be ascertained by complicated quantitative spectroscopic analysis. It does not yield oxygen to ferricyanide or a vacuum, is unaffected by carbon monoxide, and has a brownish colour; it is reduced by sodium hydrosulphite.

The occurrence of similar pigment in pathological blood has been described by a number of workers. Harrop [1919], in describing two cases of pneumonia, concluded that methaemoglobin was responsible for the deficiency in oxygen capacity. Haldane [1929] attributed the fatal character of the influenza epidemic of 1918 to the production of methaemoglobin by the infecting organisms.

Parahaematin or cathaemoglobin has been described by Arnold [1900], v. Klaverin [1901] and by Keilin [1926]. This substance does not yield oxygen to a vacuum or to ferricyanide, but possesses a spectrum very similar to oxyhaemoglobin.

Loeb, Bock and Fitz [1921] describe two cases of nitrobenzene poisoning in which the oxygen capacity by the colour should have been 20 cc. O_2 per 100 cc. blood. Actually the values 6·2 and 8·9 cc. were obtained, but no methaemoglobin could be detected spectroscopically. Peabody [1913] also found a fall in the oxygen capacity of guinea-pigs after injection with massive doses of pneumococcus. No spectroscopic evidence of methaemoglobin could be found.

Ray [1928], Ray and Stimson [1927], Stimson [1927], and Stimson and Houbitz [1928] have observed an inactive substance in the blood of rabbits and dogs after removal of the spleen or 80 % of the liver and also following administration of nitrobenzene into the stomach. The maximum amount found was 15 %. It disappeared from the blood after 7 hours in the nitrobenzene experiments and not until 5 days following splenectomy.

Carbon monoxide-haemoglobin, sulph-haemoglobin, and nitric oxide-haemoglobin, which are useless for respiratory purposes, have been sought for but were not found in any of the specimens.

Summary.

The oxygen capacity of blood has been compared with the total haemoglobin content in order to find out whether derivatives of haemoglobin, useless for respiratory purposes, occur in the blood of mental patients.

A special form of apparatus of the Haldane type has been devised, yielding results of greater accuracy and reliability than heretofore.

The haemoglobin was determined by the Stadie cyanohaemoglobin method, the Fleischl-Miescher haemoglobinometer and a titration of the actual iron content by the titanium chloride method.

130 specimens from 100 cases have been examined, and in 28 cases deficient oxygen capacity has been recognised. The difference between the active and total haemoglobin varied from 2·5 to 12·5 %.

The presence of inactive pigment can, in many instances, be correlated with positive Widal agglutination reactions to organisms of the enteric group, and these infections may prove of importance in the aetiology of this pigment.

The above work has been carried out in the laboratories of the Joint Board of Research for Mental Diseases, City and University of Birmingham.

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