EXPERIMENTS ON THE REGULATION OF THE BLOOD'S ALKALINITY. I.

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THE following experiments were carried out in Dr J. S. Haldane's laboratory, Cherwell, Oxford, and our thanks are due to him for suggesting many of the experiments, and for constant advice and criticism. The majority of the urine analyses were carried out by E. L. K. at the Bland Sutton Institute of Pathology, Middlesex Hospital. We are indebted to Mr Ralph Segnit for the drawings of Figures 1, 2 and 3.

Methods. All gas mixtures were analysed with the small type of Haldane's apparatus. Alveolar CO_2 was estimated by Haldane and Priestley's method, each determination being the mean of an inspiratory and expiratory sample. The volumes of CO_2 taken up by 1 c.c. of defibrinated blood were estimated with Brodie's modification of the Barcroft-Haldane apparatus by the method described by Christiansen, Douglas and Haldane(1). This apparatus was also used for estimating the bicarbonates of the urine by the volume of CO_2 given off on adding acid. The excretion of acid and alkali was also arrived at by titrating the urine to P_H 7·4 as follows: a box, with the back replaced by white paper, is prepared to hold three test tubes of equal calibre. Two of these placed at the sides contain 10 c.c. of a phosphate mixture of P_H 7·4 (19 vols. M/15 KH_2PO_4 and 81 vols. M/15 $Na_2HPO_4 2H_2O$) to which is added 12 drops of neutral red solution (0·05 p.c. in alcohol). The acid urines were titrated with 0·1 *N* NaOH. If the alkaline urines were titrated directly the results were enormously too low (sometimes 1/15th of the true value) owing to retention of CO_2, which comes off very slowly. They were therefore titrated as follows: in a wide test tube is placed—

(*a*) 5 c.c. of urine.

(*b*) 0·1 *N* H_2SO_4 in amount sufficient to produce a strongly acid reaction to neutral red, and water if necessary to make up to 15 c.c. If the urine so diluted shows any appreciable colour in a test tube water is added and the amount of indicator increased proportionally.

(*c*) 24 drops of the neutral red solution.

A strong current of air is then drawn through for 10 minutes (5 minutes were actually sufficient), more acid being added if the reaction does not remain distinctly acid. Liquid paraffin is used to prevent frothing, as caprylic alcohol extracts neutral red near the neutral point. The excess of acid is then titrated with 0·1*N* NaOH; the more alkaline urines neutralised 12 or 13 c.c. of the 15 c.c. acid added. Towards the close of the titration 10 c.c. of the fluid is poured from the titration flask into the middle test tube in the box between each addition of alkali, and is returned to the flask after comparison with the phosphate solution, and so on until the reaction required is reached. The end-point is extremely sharp.

The weakly alkaline urines were titrated as above except that ·02*N* solutions were used. The gas method and the titration method agreed within 5 p.c. or less for high concentrations, but somewhat less accurately for low concentrations, probably owing to the presence of Na_2HPO_4. Hence in the more alkaline urines nearly all the alkali was excreted as bicarbonate.

Total nitrogen was estimated by Kjeldahl's method, and the ammonia by the air-current method. The presence of aceto-acetic acid was determined by Rothera's test, but was never estimated quantitatively. The concentration probably never reached ·1 p.c.

CO_2-carrying capacity of the blood. As a preliminary the CO_2 dissociation curve of the normal blood of H. W. D. was determined. The results are given in Fig. 1. In this figure the combined CO_2 only is given; the dissolved CO_2 is calculated, using Bohr's (2) value of ·511 for the solubility of CO_2 in blood, and the value thus obtained is deducted from the total volume given off. The exact figures obtained are given in Table I.

To obtain the points for high pressures of CO_2 we used mixtures of CO_2 and O_2, as owing to the tendency of CO_2 to dissociate oxyhæmoglobin it appeared desirable to guard against any possibility of incomplete oxidation of the hæmoglobin. Some experiments which are not yet complete indicate that at 400 mm. pressure of CO_2 reduced blood takes up only about two to three volumes p. c. more CO_2 than oxygenated blood. It was also considered possible that the high pressures of CO_2 might cause some irreversible changes in proteins resulting in an increased CO_2 capacity. To settle this question, a sample of blood was saturated at a pressure of 497 mm. of CO_2. A portion of the sample was then placed in the blood gas apparatus and the volume per cent. of CO_2 absorbed was found to be 121·1. The remainder of the sample was then resaturated at 48·2 mm. pressure of CO_2, when the volume p.c. of CO_2

absorbed (after correction for the additional time spent in the bath, *vide* Christiansen, Douglas, and Haldane's paper) was found to be 53·6, a normal result. Hence any important changes of the proteins must be reversible.

The results obtained agree with those of Christiansen, Douglas,

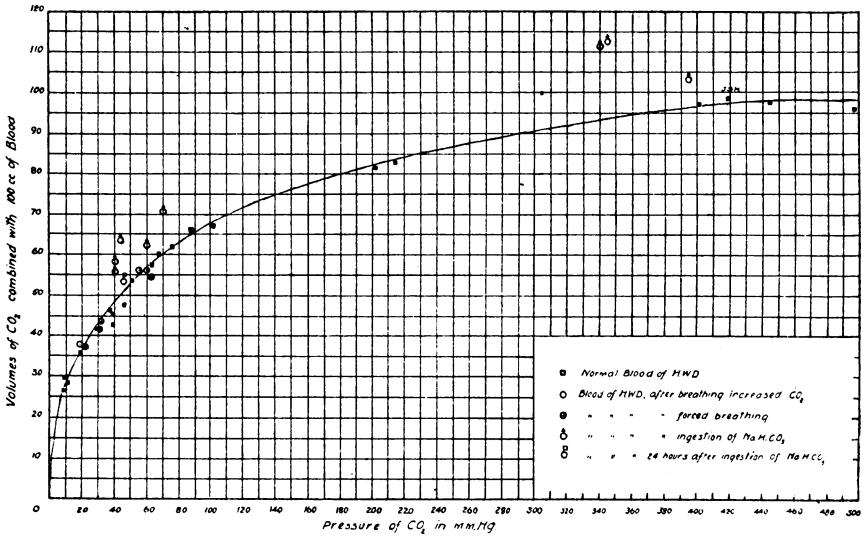

Fig. 1

TABLE I. CO_2-carrying capacity of normal blood of H. W. D.

mm. pressure of CO_2	Vols. of CO_2 taken up by 100 vols. of blood	Vols. of combined CO_2	mm. pressure of CO_2	Vols. of CO_2 taken up by 100 vols. of blood	Vols. of combined CO_2
8·3	26·9	26·3	64·9	61·8	57·4
9·02	30·2	29·6	68·5	64·6	60·0
11·2	29·4	28·7	76·7	67·2	62·0
19·3	36·8	35·5	89·9	71·7	65·7
29·8	43·4	41·4	102·9	73·4	66·5
38·9	49·0	46·4	202	95·4	81·8
39·0	48·1	45·5	215	97·0	82·6
39·1	45·7	43·1	411	125·0	97·3
46·6	50·4	47·3	455	128·2	97·6
51·3	56·6	53·2	497	129·1	95·7

and Haldane(1) and with Parsons'(3) experimental results, being on the whole very slightly lower than the former, but the volume of CO_2 taken up was invariably a good deal higher than the value found by Joffe and Poulton(4). The high values did not agree with those calculated from Parsons'(3) equation. Thus the blood of J.S.H. took up 98·6 volumes of combined CO_2 at a pressure of 428 mm. According to

Parsons' equation it should only take up 90·5 volumes of CO_2 at this pressure, a pressure of 796 mm. being required before 98·6 volumes are taken up. This fact shows that the proteins (of which no doubt hæmoglobin is the most important) cannot quite correctly be taken as equivalent to a single weak acid. If we supposed, as is *a priori* probable, that the different acid side chains of the hæmoglobin molecule have somewhat different dissociation constants, this would account for the divergence of our results from those expected on Parsons' comparatively simple theory.

Since the CO_2 taken up in combination by the blood does not vary appreciably (only between 95·4 and 97·5 vols. p.c.) between CO_2 pressures of 411 and 496 mm. it is clear that at these pressures almost all the available base is combined with CO_2. If then we saturate blood with CO_2 at a high pressure we shall obtain a true measure of the available base or "alkaline reserve." At lower pressures a certain amount of this base is combined with protein, and as Parsons' equation is not quite exact, it is impossible to determine exactly how much. If on the other hand we find that 100 c.c. of abnormal blood take up 16 vols. more of CO_2 than 100 c.c. of normal blood we know that they contain 16 N/2240 or N/140 base above the normal content. In the living subject some of this would be combined with protein.

Effects of breathing increased percentages of CO_2. These experiments were carried out in a large air-tight respiration chamber of about 260 cubic feet capacity. CO_2 was passed in through a meter in amounts sufficient to give approximately the required percentage (6 p.c.). The subject then entered the chamber having previously passed urine. Samples of air were taken for analysis immediately after entering, and just before leaving, the chamber. Three separate experiments were performed on H. W. D. the duration of each being about 2 hours. After entering the chamber the respirations gradually increased, in a few seconds reaching a maximum depth, with frequency varying between 22 and 30, and continued so throughout. No marked rise of pulse rate was observed. A second sample of urine was collected about half an hour after entering the chamber, and a third just before the conclusion of the experiment. The blood sample was also taken just prior to leaving the chamber. In the first experiment the percentage of CO_2 breathed at the commencement was 5·22, rising to 5·59 just before leaving the chamber. In this experiment no samples of urine were taken. About eight minutes after entering the chamber a slight headache came on which lasted throughout the experiment. In the two subsequent experiments the CO_2 percentage

varied between 6·01 and 6·45. No headache occurred in the chamber, but headache of moderate severity, with sudden onset and lasting several hours, was observed immediately after emerging. In none of these three experiments was there any appreciable alteration of the CO_2 dissociation curve of the blood (*vide* Fig. 1). There was possibly some slight increase in the CO_2-capacity of the blood but the differences from the normal hardly exceed the errors of experiment. This result contrasts with the previous results of Yandell Henderson and Haggard(5) in their experiments on animals. Probably the percentage of CO_2 breathed in our experiments was insufficient (they used over 10 p.c.), or the duration of the experiments was too short, to allow of the redistribution of alkali in the body as discovered by these observers.

Table II shows the results of urine analyses in the last two experiments, the main features of which are:

(*a*) Diuresis—the volume per hour increasing almost fourfold.

(*b*) Increased excretion of acid—the faintly alkaline urine of 26/9/19 becoming acid, and the strongly alkaline urine of 3/10/19 becoming almost neutral. (In these titrations no attempt was made to remove CO_2 as in the method described above.)

TABLE II. Analyses of urines. Breathing CO_2.

Date	Time	Time mins.	Volume c.c. Total	Volume c.c. Per hour	To bring urine to P_H 7·4 requires c.c. 0·1 N: Per 100 c.c. urine Acid	Per 100 c.c. urine Alkali	Per hour Acid	Per hour Alkali
26/9/19	9 a.m.— 1.50 p.m.	290	305	63	1·60	—	1·01	—
	1.50 p.m.— 2.25 ,,	35	155	266	—	1·10	—	2·92
	2.25 ,, — 3.50 ,,	85	320	226	—	5·60	—	12·70
3/10/19	11 a.m.—12.30 p.m.	90	185	124	14·90	—	18·40	—
	12.30 p.m.— 1 ,,	30	130	260	6·00	—	15·60	—
	1 ,, — 2.45 ,,	105	778	445	1·00	—	4·40	—

Date	NH_3 c.c. 0·1 N: Per cent.	NH_3 c.c. 0·1 N: Per hour	Total N Mg: Per cent.	Total N Mg: Per hour	NH_3 N p.c. of Total N	Remarks
26/9/19	9·66	6·08	613	387	2·20	H.W.D. entered chamber at 1.54 p.m. CO_2 5·35 p.c.
	5·20	13·80	274	728	2·65	2.30 added 4 cu. ft. CO_2 2.40 CO_2 6·17 p.c.
	5·30	12·00	195	440	3·80	3.50 CO_2 6·17 p.c.
3/10/19	3·14	3·88	554	685	0·79	H.W.D. entered chamber at 12.27
	2·80	7·28	270	702	1·45	12.30 CO_2 6·01 p.c.
	1·88	8·34	138	613	1·90	2·45 CO_2 6·47 p.c.

Rothera aceto-acetic acid—nil throughout.

(*c*) The ammonia excretion per hour was doubled in both experiments.

The increase of ammonia is analogous to that found by Walter (6) in dogs to which HCl had been given; and to that of diabetic acidosis. It is presumably due to the action of increased acid of the plasma in shielding ammonia from conversion into urea by the liver. The increased output of ammonia per hour was of the same order of magnitude as the increase of acid or decrease of alkali excreted per hour in the urine, and in one experiment was equivalent to about half the increased acidity and in the other to about one-third. The liver was therefore from half to one-third as efficient as the kidneys in compensating the acidosis.

Forced breathing experiments. It has previously been shown by Henderson and Haggard (5) that excessive pulmonary ventilation in animals, whether induced by ether, by mechanical means, or by shock, causes a disturbance of the CO_2-carrying capacity of the blood. In addition Leathes (7) has shown that in the human subject a moderate degree of forced breathing can be maintained for considerable periods and that under such circumstances there is increased excretion of alkali by the kidneys.

We have endeavoured to extend the observations of Henderson and Haggard to man, but find that with the amount of forced breathing voluntarily possible in the human subject, there is no alteration of the CO_2-carrying capacity of the blood as evidenced by alteration of the CO_2 dissociation curve (*vide* Fig. 1). Three experiments were performed. In the first two the duration of the forced breathing was one hour. The first sample of urine was taken 15 minutes after commencing forced breathing, and the second, together with the sample of blood, at the conclusion. Considerable discomfort was felt by the subject, the main symptoms observed being numbness and tingling of the extremities, fibrillary twitching of the orbicularis palpebrarum, and slight Rombergism. These symptoms were mostly relieved by the inhalation of a few breaths of pure oxygen. The results of the urine analyses of the first two experiments are shown in Table III.

Details of the later experiment are given in the protocol. Outstanding features are:

(*a*) Diuresis.

(*b*) Marked increase in the alkalinity of the urine (*vide* protocol).

(*c*) Lessened excretion, and, on 4/10/19, complete absence of ammonia in the urine.

(*d*) The excretion in the first two experiments of doubtful traces

of acetone bodies, and in the later experiment of a moderate amount of these substances.

TABLE III. Forced breathing experiments.

Date	Time	Time mins.	Volume c.c. Total	Volume c.c. Per hour	NH_3 c.c. 0·1 N Per cent.	NH_3 c.c. 0·1 N Per hour	Total N Mg Per cent.	Total N Mg Per hour	NH_3 N p.c. of total N
4/10/19	9 a.m. to 12.43 p.m.*	223	147	40	17·00	6·72	920	364	2·60
	12.43 p.m. to 1.40 p.m.†	57	245	258	0·00	0·00	213	550	0·00
5/10/19	9 a.m. to 12.47 p.m.‡	227	268	71	22·00	15·60	852	603	3·61
	12.47 p.m. to 1.47 p.m.†	60	345	345	1·20	4·14	261	900	0·64

The alkalinity of these urines is not given in this table as it was estimated only by titration without removing CO_2. The increased excretion of bicarbonates is shown in the protocol of the subsequent experiment.

* F.B. commenced 12.30 p.m. Rothera, aceto-acetic acid, nil.
† Rothera, aceto-acetic acid, ?
‡ F.B. commenced 12.48 p.m.

Protocol of subsequent forced breathing experiment.

Date 17/3/20. Subject H. W. D.

12.37 Alveolar CO_2 Insp. 5·10 p.c. Exp. 5·09 p.c. Mean, 5·09 p.c.

1.7 Urine A acid to litmus 189 c.c. (10.10 a.m.—1.7 p.m. *i.e.* 64 c.c. per hour). Rothera negative.

1.10 Commenced forced breathing.

1.30 Urine B alkaline 49 c.c. (1.7—1.30 *i.e.* 128 c.c. per hour) containing equivalent of ·219 grams of $NaHCO_3$*. Alkalinity equal to ·053 normal. Rothera negative.

1.33 Feeling thirsty—skin moist. Drank 200 c.c. water.

1.40 Alveolar CO_2 Insp. 1·59. Exp. 1·70. Mean, 1·65.

2.2 Thirsty, sweating. Urine C 43·5 c.c. alkaline (1.30—2.2 *i.e.* 82 c.c. per hour) containing equivalent of ·184 gram $NaHCO_3$*. Alkalinity equal to ·051 normal. Rothera shows moderate amount of acetone bodies.

2.30 Urine D 103 c.c. alkaline (2.2—2.30 *i.e.* ·221 c.c. per hour) containing equivalent of ·154 gram $NaHCO_3$*. Alkalinity equal to ·018 normal. Traces of aceto-acetic acid. Alveolar CO_2 Insp. 1·55. Exp. 1·81. Mean, 1·68.

2.37 Stopped forced breathing.

2.57 Alveolar CO_2 Insp. 4·87. Exp. 4·96. Mean, 4·92.

3.0 Urine E 149 c.c. acid to litmus (2.30—3 *i.e.* 298 c.c. per hour). Traces of acetone bodies.

3.33 Urine F acid 19 c.c. (3.0—3.33 *i.e.* 35 c.c. per hour). Rothera negative.

4.30 Urine G acid 19 c.c. (3.33—4.30 *i.e.* 20 c.c. per hour). Rothera negative.

4.35 Tea.

6.48 Urine H acid 88 c.c. (4.30—6.48 *i.e.* 38 c.c. per hour). Rothera negative

* *Determined volumetrically from CO_2 given off.*

The excretion of $NaHCO_3$ in these forced breathing experiments appears to us to be inconsistent with the theory of renal secretion briefly described by Cushny (8) in the following terms: "The function of the kidney may thus be shortly defined as the filtration of the non-colloid constituents through the capsule and the absorbtion of Locke's fluid through the tubule cells."

During forced breathing the alveolar CO_2 was reduced to between 1 and 2 p.c., but the total CO_2 capacity, and therefore the total "alkaline reserve," as shown by the CO_2 dissociation curve, after drawing a sample of blood and saturating *in vitro*, was not appreciably diminished. As the arterial CO_2 tension was reduced there must have been less $NaHCO_3$ in the plasma, *i.e.* more Na ions combined with proteins and other "buffer" substances[1]. But there was an increased amount of $NaHCO_3$ in the urine. This negatives the view of Palmer and van Slyke (9) that excretion of $NaHCO_3$ depends only on excess of it in the blood. It also seems to negative the idea of filtration through the glomeruli and the return to the blood through the tubule cells of a fluid of constant composition. The "buffer" proteins, like Congo red, would keep back some of the Na ions so that the hypothetical filtrate would contain less $NaHCO_3$ than normal. If, then, a fluid of constant composition were reabsorbed through the tubules, the urine, contrary to our results and to those of Leathes, would become less alkaline, *i.e.* more acid. That the alkalinity is mainly due to bicarbonate is shown by the fact that these urines, collected during forced breathing, when treated with acid gave off from 80–100 vols. p.c. of CO_2.

The diminution or disappearance of ammonia seems to be a normal response on the part of the liver to "alkalosis" and was shown by Hasselbalch (19) and by Haldane, Kellas, and Kennaway (10) to occur in the alkalosis caused by the hyperpnœa due to oxygen want.

The appearance of acetone bodies is possibly due to the fact that they are shielded by some of the alkali and are excreted before the normal oxidative processes occur. It has previously been shown by Stäubli (11) that in diabetic acidosis the administration of 60 grams per day of $NaHCO_3$ caused the excretion of β-hydroxybutyric acid to rise from 17 to 45·2 grams. In our experiments the amount of aceto-acetic acid was so minute that it could not have had any appreciable effect in neutralising the alkali.

[1] Joffe and Poulton (4) showed that the plasma at a CO_2 pressure of 10 mm. (1·3 p.c. of an atmosphere) contains only 30 vols. p.c. of CO_2 against 53 at 40 mm. pressure (5·3 p.c. of atmosphere). Thus the bicarbonate content was reduced from ·023 N to ·013 N.

According to the results of Parsons(12) the P_H of H.W.D.'s arterial blood must have been increased on the occasion when his mean alveolar CO_2 reached its lowest recorded value of 1·42 p.c. from a normal 7·41 to 7·79, or by ·38. There would however have been comparatively little alkalosis in the tissues, as Yandell Henderson has shown in numerous papers on acapnia and shock that forced breathing decreases the peripheral circulation. The bad effects noticed by us were relieved by inhalation of oxygen and were presumably due partly to increased stability of oxyhæmoglobin, partly to vaso-constriction.

Effects of ingestion of $NaHCO_3$. On eight occasions H.W.D. (weight 75 kilos) or J.B.S.H. (weight 97 kilos) ate quantities of $NaHCO_3$ varying from 30 to 57½ grams. It was found best to take the bicarbonate shortly after a massive breakfast as otherwise it acted as a strong purgative. Enough water was drunk to wash down the $NaHCO_3$ and to satisfy thirst. The following were the principal results observed:

(1) Increase of the CO_2 capacity of the blood, demonstrating the presence in it of additional $NaHCO_3$.

(2) Increase in the alveolar CO_2.

(3) Moderate diuresis.

(4) Rapid excretion of $NaHCO_3$ in the urine, the concentration rising to a definite maximum in each experiment which rarely exceeded 1/3rd normal or 2·8 p.c. The rate of elimination rose to 7·3 grams per hour.

(5) Great decrease or complete disappearance of the NH_3 in the urine.

(6) Sometimes but not always the appearance of acetone bodies in the urine.

Most of these results are illustrated by Figs. 2 and 3 and by Table V. The weight of bicarbonate excreted is given as $NaHCO_3$ though doubtless some of it was present as $KHCO_3$. The bicarbonate in Fig. 2 was estimated by titration, in Fig. 3 by measuring the CO_2 given off.

The results of experiments on the CO_2-capacity of the blood are given in Table IV and in Fig. 1. The rise in this varies from 5 to 19 volumes p.c. and in one case there was a small but probably significant excess after 24 hours. The increase of 19 volumes in the total CO_2 capacity (*i.e.* the capacity at a high pressure) corresponds to an increase of ·071 gram of bicarbonate per 100 c.c. or 3·2 grams in the whole blood of H.W.D. whose blood volume is about 4½ litres. At the time this blood was taken 41·5 grams remained in his body, and taking the weight of his skeleton as 12 kilos we should expect to find 3·0 grams in his blood

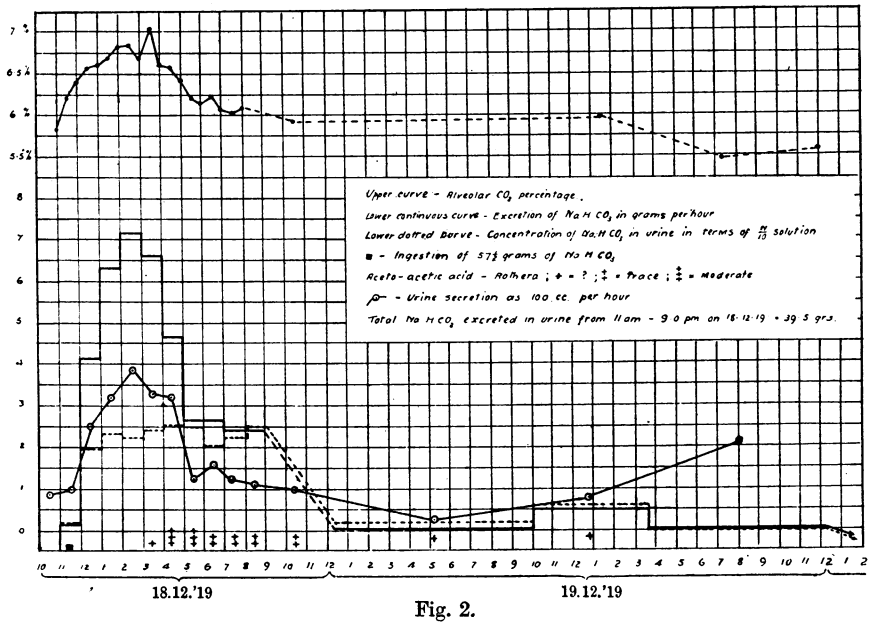

Fig. 2.

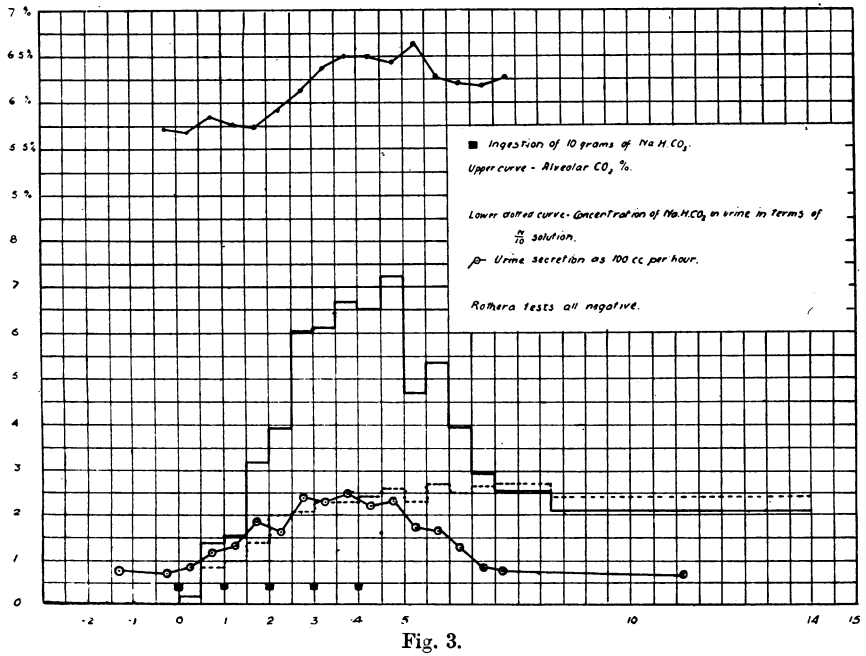

Fig. 3.

Lower continuous curve. CO_2 given off on adding acid per total half hour's urine in 100 c.c. Total $NaHCO_3$ excreted in urine from 0—14 hours = 31·46 grms.

TABLE IV. CO_2-capacity of blood of H. W. D. after ingestion of $NaHCO_3$.

Date	Dose of $NaHCO_3$	Time between 1st dose and drawing of blood	Pressure of CO_2 in mm. Hg	Vol. of CO_2 from 100 vols. of blood	Vols. of CO_2 combined	Excess vol. over normal
6/10/19	30 grms.	2½ hours	71·3	75·7	70·9	10
,,	,,	5 ,,	60·2	66·3	62·2	5
7/10/19	,,	24 ,,	46·3	56·3	53·1	2
10/10/19	15 at 0 hr.	1 ,,	41·3	60·7	58·0	9
,,	15 ,, 1 ,,	4 ,,	405	130·8	103·6	6·5
,,	15 ,, 2 ,,	6 ,,	41·1	58·5	55·7	7
30/10/19	45 grms.	3¼ ,,	339	134·0	111·2	18
10/11/19	,,	2 ,,	345	135·5	112·3	19
,,	,,		43·5	66·4	63·5	13·5

if the ingested bicarbonate were evenly distributed throughout his body. Of course in the living blood the alkali was not all present as bicarbonate, some being combined with protein.

In order to make sure that the changes in alkaline reserve were not due to an increase of the ratio of plasma to corpuscles, hæmoglobin percentages were taken throughout one experiment by the Gowers-Haldane method, and only varied between 94·5 p.c. and 96·5 p.c., although the CO_2-capacity increased by 18 volumes p.c.

The alveolar CO_2 increased by amounts seldom much exceeding 1 p.c., the maximum being generally reached three to four hours after ingestion of the bicarbonate. Typical results are shown in Figs. 2 and 3. The highest point on the former is probably erroneous, as the inspiration sample apparently contained more CO_2 than the expiration. Regular breathing was sometimes rendered difficult by the slight digestive disturbances produced by liberation of CO_2 in the alimentary tract.

An increase of the alveolar CO_2 from 5·5 p.c. to 6·9 p.c. corresponds to a decrease of the breathing by about 15 p.c. According to the results of Campbell, Douglas, Haldane and Hobson(13) this corresponds to an increase of about ·0018 in the P_H of the blood. There was thus a slight, but extremely slight, alkalosis, about 1/200th of that caused in the arterial blood by the forced breathing. In accordance with this there was no appreciable discomfort apart from that due to irritation of the digestive system.

The diuresis never exceeded 400 c.c. per hour, and disappeared long before all the bicarbonate was eliminated. The excretion of water was invariably much larger than the quantity drunk. The concentration of bicarbonate in the urine rose to a maximum which varied on different dates, those of H.W.D. being ·308 normal, ·308 N and ·374 N, those of J.B.S.H. ·358 N and ·269 N. This is in accordance with the results

of Ambard and Papin(14), who in a similar experiment obtained a maximum concentration of ·31 N for total sodium salts.

TABLE V.

$NaHCO_3$ ingested	Urine collected	NH_3 c.c. 0·1 N Per cent	NH_3 c.c. 0·1 N Per hour	Total N Mg Per cent.	Total N Mg Per hour	NH_3 N p.c. of total N	Rothera
11.25							
15 grms.	8.15—11.30	21·5	11·2	873	456	3·440	nil
12.25							
15 grms.	11.30—12.30*	1·50	2·47	419	691	0·501	nil
1.25							
15 grms.	12.30— 1.30*	0·40	0·74	357	660	0·160	nil
	1.30— 2.30*	0·00	0·00	364	575	0·000	?
	2.30— 3.30*	0·80	1·06	419	557	0·270	faint
	3.30— 5.30	0·80	0·66	621	509	0·180	strong
	5.30— 6.30	2·20	1·54	861	603	0·360	trace
	a.m. next day 8.30—12.30	5·10	2·40	966	456	0·739	nil

* Diuresis.

The maximum concentration was gradually attained during the latter part of the diuresis or just after its close, but the concentration does not fall appreciably until many hours after the diuresis has passed. The particular value of the maximum reached did not appear to depend on the magnitude of the dose of bicarbonate or on any other obvious cause. The rate of elimination was greatest during the diuresis, and was roughly proportional to the increase in alveolar CO_2. The greatest quantities eliminated in an hour were 5·80 grams by H.W.D. and 7·32 grams by J.B.S.H. The former represents more than twice the total excess of alkali in his entire blood. In the experiment recorded in Fig. 2, 39·5 out of 57·5 grams were eliminated in the first 10 hours, in that illustrated in Fig. 3 31·46 out of 50 were eliminated in the first 14 hours, yet the concentration had fallen very little. Certainly the excess of alkali in the plasma must have been much less during the early part of the diuresis and yet the concentration was higher. This is in accordance with Ambard and Papin's conclusion—that the kidney requires a certain time to adapt itself to the production of urine of a high concentration, whereas it can produce large quantities of comparatively dilute urine at a moment's notice.

The concentration of ammonia, its rate of elimination per hour, and the ratio of ammonia N to total N all fell rapidly, reaching a minimum and sometimes disappearing completely during the fourth hour after ingestion. Table V gives the results of a typical experiment on H.W.D. It will be seen that the total N excreted per hour remained fairly steady.

Denis and Minot(17) have obtained similar results in cases of nephritis but attempts to duplicate them in normal subjects were unsuccessful. This failure they attribute to excretion of alkali before its neutralising effect on acid radicals has been exerted. They consider speculations regarding "residual ammonia fractions" superfluous, the only function of ammonia being the neutralisation of acid radicals. A similar conclusion has been reached by Janney(18) although he has never succeeded in obtaining ammonia-free urine. The lowest recorded amount of NH_3 nitrogen obtained in one day was ·0086 gram after the ingestion of 60 grams of $NaHCO_3$. Our results confirm the conclusions of these observers and seem to indicate that if sufficient alkali can be introduced into the body all the ammonia will be converted into urea.

Aceto-acetates were generally, but not always, found in the urine. They never appeared in any quantity before the fourth hour after ingestion and their maximum excretion was always after the diuresis and lessened breathing had begun to pass off.

Macleod and Knapp(15) found that lactates appeared in excess in the urine after alkali ingestion. Rough estimations of the lactates in H.W.D.'s urine during one experiment by Ryffel's (16) method seemed to show a slight increase, but exact estimations were not made, as the subject had taken a certain amount of exercise, *e.g.* shaking the blood gas apparatus, during the course of the experiment. In any case the extra lactic acid found would not have neutralised any appreciable proportion of the alkali ingested.

Summary.

1. The complete CO_2 dissociation curve of human blood is given. It does not altogether agree with Parsons' theory at high CO_2 tensions.

2. This curve cannot be altered in man by short periods of forced breathing or breathing moderate excess of CO_2. It is greatly altered by ingestion of alkali.

3. In the acidosis of breathing excess of CO_2 the urine becomes more acid and its NH_3 increases. In the alkalosis of forced breathing or bicarbonate ingestion it becomes alkaline; the NH_3 decreases or disappears; and the rate of bicarbonate excretion may be very rapid.

4. Acetone bodies generally appear in the urine of alkalosis.

5. The concentration of sodium bicarbonate in the urine has a limiting value which is independent of its rate of excretion.

6. The excretion of alkali during forced breathing does not appear

to be compatible with the theory that the kidneys reabsorb a fluid of constant composition from a glomerular filtrate.

7. The alveolar CO_2-percentage is greatly increased by ingestion of alkali.

8. As bicarbonate plays an important part as a buffer in alkaline urine, a special method of titration was employed, and is described, as well as a method for directly determining bicarbonates in urine.

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