A STUDY OF THE CHLORINE INTERCHANGE BETWEEN CORPUSCLES AND PLASMA.

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In 1861 Zuntz(1) showed that after blood is saturated with carbon dioxide the plasma becomes capable of taking up more carbon dioxide in combination than is the case if it be separated from the corpuscles before saturation. He concluded that in the presence of an increased partial pressure of carbon dioxide alkali passes out from the corpuscles into the plasma. Later Gürber(2) showed that what really happens is that hydrochloric acid passes into the corpuscles, thus leaving the plasma more alkaline, and therefore more capable of taking up carbon dioxide. Hamburger(3) obtained a similar result, but interpreted it as due to the passage of chlorine ions into the corpuscles, and further showed that when carbon dioxide is removed from blood the reverse reactions take place. Parallel with this phenomenon the corpuscles increase in diameter as may be shown by a corresponding increase of corpuscular volume by means of the hæmatocrit. More recently de Boer(4) and Hamburger(5) have shown that a similar transference of SO_4 ions occurs on the addition to blood of carbon dioxide and of hydrochloric acid. Van Slyke and Cullen(6) and Fridericia(7) have recently described series of careful experiments and have confirmed the essential points of the phenomenon, viz. the passage of chlorine ions through the corpuscular membrane under the influence of carbon dioxide.

The experiments described in the following pages were undertaken for the purpose of confirming some of the previous conclusions with regard to carbon dioxide and in addition of ascertaining the influence of "acidosis" produced in various ways upon the passage of chlorine into the corpuscles. It is this last question which has principally engaged our attention. Under normal conditions of the circulation it seems probable that the reduction of hæmoglobin is the most important factor in minimising variations of *p*H when arterial blood becomes venous. The transference of chlorine ions caused by changes in carbon dioxide tension within physiological limits must be very small. It seemed to us to be important to determine whether any such transference may occur in conditions of "acidosis."

The experiments were carried out in the Department of Therapeutics, Edinburgh University. Our thanks are due to Prof. Meakins and Mr C. R. Harington for assistance and advice, and to Dr J. S. Haldane for helpful criticism of our results.

Methods. In all the experiments normal human blood was used, clotting being prevented by the addition of a constant small amount of neutral potassium oxalate (0·1 p.c.). In all the experiments with carbon dioxide and with other acids, the blood was fully oxygenated. A few c.c. of whole blood were reserved for estimation of total chlorides, the remainder within a few minutes of being drawn was placed in a saturator similar to that used by Christiansen, Douglas and Haldane (8). Carbon dioxide or other acid was added and the saturator was then rotated in a water bath at 37° C., the excess of pressure being released after the first five minutes. After 15 minutes the saturator was removed from the bath and quickly wrapped in warm cloths. Samples of blood were then taken for estimation of carbon dioxide by the method of Haldane (9), duplicate determinations agreeing to within 0·5 volume p.c. A sample of air from the saturator was also analysed with the small type of Haldane gas analysis apparatus. In addition two samples of blood each of 10 c.c. were placed under paraffin in graduated centrifuge tubes. The blood was then centrifuged at 33° C. for 45 to 60 minutes, 45 minutes having been found sufficient for complete separation, after which the relative volume of plasma and cells was read off and reduced to percentage. Chlorides in plasma and cells were then immediately estimated separately and in duplicate by the method of Wetmore (10), the proteins being precipitated by copper hydroxide, phosphates and oxalates being subsequently removed by shaking the filtrate with a small quantity of calcium hydroxide and refiltering. The few experiments where the difference was of more than 10 mg. (*i.e.* 2 p.c.) in duplicate determinations were eliminated. *p*H was not measured directly but calculated from the formula of Hasselbalch: $pH = pK + \log \frac{NaHCO_3}{H_2CO_3}$ taking 6·1 as the value of *p*K.

Experiments with carbon dioxide. Fridericia (7) has shown that for samples of blood taken from the same animal at the same time and exposed to varying pressures of carbon dioxide, the chlorides of plasma vary in inverse ratio to the pressure of carbon dioxide. The curve so constructed could be superposed upon the dissociation curve of carbon dioxide in blood. In our endeavours to extend these observations to human blood at 37° C. we found it impossible to obtain a consistent curve for plasma chlorides at varying carbon dioxide pressures when

experiments were performed on different days. The results when plotted fell only very approximately on the theoretical curve. The reason for this can readily be seen from the tables, which show that the amount of total chlorides in blood varies in different people and in the same individual from day to day, even in spite of the fact that in our experiments the blood was always taken at the same time of the day (between 11 a.m. and 12 noon). It appears however that when the partial pressure of carbon dioxide increased above 150 mm. no further transference occurred.

The carbon dioxide dissociation curve of H. W. D. has already been published(16). The points obtained for the blood of H. W. D. in the present experiments fall exactly upon this curve. This fact is of some

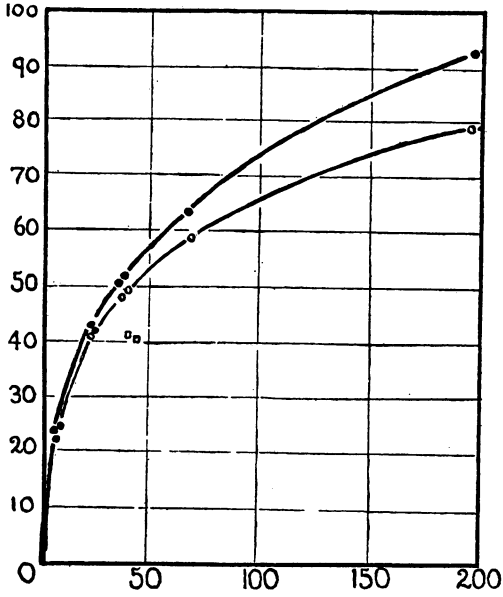

Fig. 1. Carbon dioxide dissociation curve for fully oxygenated whole blood of L. D. Upper curve—total carbon dioxide. Lower curve—combined carbon dioxide. Abscissa—pressure of carbon dioxide (mm. of Hg). Ordinate—c.c. vols. p.c. of carbon dioxide. Points marked □ are for blood after exercise of H. W. D.

interest, as the curve of H. W. D. was determined some eighteen months previously, since when he has travelled round the world, experiencing great variations of climate. A complete carbon dioxide dissociation curve for the blood of L. D. is given (Fig. 1). This agrees almost exactly with the curve of H. W. D. and with the original curve for Haldane(8). We have always found that the corpuscular volume as shown by hæma-

tocrit readings increased with increasing tensions of carbon dioxide. However in our experiments where other acids were added to blood the hæmatocrit readings were somewhat irregular. Table I shows results obtained on exposing blood from three different subjects to varying partial pressures of carbon dioxide. These results will be further discussed below.

TABLE I. Blood exposed to varying partial pressures of CO_2.

Exp.	Subject	CO_2 pressure mm. Hg.	$pH=pK_1+\log\frac{NaHCO_3}{H_2CO_3}$ $pK_1=6{\cdot}1$	Vol. % of CO_2 Found	Vol. % of CO_2 Normal (Haldane)	Hæmatocrit. Cells %	Chlorides mg. % Cells	Chlorides mg. % Plasma	Total chlorides mg. %	% of total chlorides in plasma	O_2 saturation of blood
1 *a*	L.D.	—	—	55·62	—	39·8	—	579	480	72·6	Venous
1 *b*	„	35·2	7·40	48·3	48·5	33·5	—	603	480	83·5	100 p.c.
2 *a*	W.A.	52·04	7·00	31·2*	56	35·7	324	—	514	—	„
2 *b*	„	34·5	7·14	27·9*	48	35·4	302	—	509	—	„
3 *a*	H.W.D.	42·5	7·33	51·6	52	36·7	318	662	519	80·7	„
3 *b*	„	143·3	6·96	78·8	80	37·3	360	587		70·9	„
4 *a*	L.D.	14·3	7·65	35·5	33·5	37	275	626	483	84·9	„
4 *b*	„	674·0	6·41	140·0	—	43·3	345	557	489	75·8	„
5 *a*	L.D.	6·8	7·80	23	24	36·6	258	663	510	82·4	„
5 *b*	„	195·0	6·87	92·3	—	39·3	326	600		73·3	„

*Normal acidosis. Growing boy (see Schloss, *Amer. J. Dis. of Child*, 13, p. 210).

TABLE II. Blood at different degrees of oxygen saturation.

6 *a*	L.D.	24·3	7·50	43·2	42	40·6	294	593	468	73·1	19 %
6 *b*	„	24·3	7·50	43	42	40·8	271	594	462	73·9	100 %
7 *a*	L.D.	32·1	7·47	52·7	47	34·5	289	608	512	77·7	Reduced blood. 2 %
7 *b*	„	38·6	7·37	51·6	50·6	33·1	286	607	500	81·2	100 %
8 *a*	H.W.D.	37·9	7·45	51·97	49·8	—	284	578	460	—	0 %
8 *b*	„	43·0	7·30	50·98	52·4	—	282	575		—	100 %
9 *a*	H.W.D.	22·6	7·54	44·57	40·5	33·1	235	481	402	78·6	4 %
9 *b*	„	28·4	7·42	41·67	44·4	35·6	232	490		79·3	100 %

TABLE III. Addition of acid to blood.

10 *a*	L.D.	36·8	7·25	38·1	49·5	27·4	—	579	512	82·1	·03 c.c. lactic acid
10 *b*	„	36·1	7·39	50	49	28·1	—	581		81·5	Normal
11 *a*	H.W.D.	7·6	7·57	15·5	25·5	35·2	329	631	509	80·1	·03 c.c. lactic acid
11 *b*	„	7·6	7·79	25·8	25·5	33	335	634	507	83·7	Normal
12 *a*	„	2·7	—	0	16	36·3	423	620	520	75·9	·13 c.c. lactic acid
12 *b*	„	6·2	7·85	24	23	36·3	309	689	527	83·2	Normal
13 *a*	L.D.	79·3	6·97	45	66	38·6	364	605	515	72·1	·05 c.c. oxybutyric acid
13 *b*	„	67·5	7·21	63	62·5	38·5	341	625		74·6	Normal
14 *a*	H.W.D.	0·91	—	0	9	36·6	399	612	536	72·3	·15 c.c. oxybutyric acid
14 *b*	„	2·74	8·06	16·9	16	35·1	267	675		81·7	Normal

TABLE IV. Severe exercise.

15 *a*	H.W.D.	46·4	7·29	52	53·5	32·4	305	633	525	81·5	Normal
15 *b*	„	41·5	7·24	41·9	52	31·9	344	639	545	79·8	After severe exercise
16 *a*	„	48·3	7·27	54	54	35·1	320	612	499	79·6	Normal. Hb. 96 %
16 *b*	„	43·0	7·21	40	52·5	38·1	343	615	500	76·1	After severe exercise. Hb. 103 %

Each pair of observations, 1 *a*, 1 *b*, etc., were made on the same day, using different portions of the same sample of blood except in Table IV.

Observations with reduced hæmoglobin. Christiansen, Douglas and Haldane(8) have shown that oxyhæmoglobin behaves as if it were more

acid than reduced hæmoglobin. We have endeavoured to ascertain whether this property has any influence on the transference of chlorine from plasma to corpuscles or *vice versa*. The blood in the saturator was exposed to a current of hydrogen freed from acid by being first passed through a wash bottle containing caustic soda. In order to accelerate reduction, the saturator was placed in the bath at 37°. Five to ten minutes were sufficient for almost complete reduction. As can be seen from Table II, blood with hæmoglobin reduced to 2 p.c. of oxygen saturation and then exposed to carbon dioxide has a *p*H (calculated) of 7·47 while with fully oxygenated hæmoglobin the *p*H is 7·37. In spite of a difference of carbon dioxide pressure in the experiments 3 and 4 of 6·5 mm. of Hg in favour of the oxygenated blood, it still took up less carbon dioxide than the reduced blood. The calculated *p*H changes from 7·37 to 7·47, yet there is no difference in the chlorine percentage of the plasma. These results were confirmed later in experiments 5 to 8. In spite of increased carbon dioxide combining power in reduced blood, the chlorine transference does not take place. This seems to indicate that in the living body, where the oxygen desaturation of mixed venous blood is about 30–40 p.c., and the increased carbon dioxide pressure about 8 mm. of Hg, the interchange of chlorine when arterial blood becomes venous would be very small, scarcely beyond the limits of experimental error. Our results for carbon dioxide content and calculated *p*H given in Table II fall exactly upon the curves given by Parsons(12). Exps. 6 *a* and 6 *b* are interesting in that there appears no increase of carbon dioxide capacity when the hæmoglobin is reduced. This result accords with those of Henderson and Haggard(17), which have been discussed by Van Slyke(18) and also by Douglas and Haldane(19). We attribute this result to delay (34 minutes) while the blood was being reduced and consequent lactic acid formation facilitated probably by the low partial pressure of carbon dioxide as shown by Lovatt Evans(20). Our later results where precautions were taken against delay show results which accord with those of Christiansen, Douglas and Haldane(8).

Acids other than carbon dioxide. (1) Experiments with lactic acid were performed in two manners—firstly by the addition of lactic acid to blood *in vitro* and secondly by the production of lactic acid *in vivo* by severe muscular exercise. Ryffel(13) has shown that blood after severe exercise may contain ·07 p.c. of lactic acid, so for an experiment *in vitro*, to 25 c.c. of blood we added ·03 c.c. of 9·7 N lactic acid in order to obtain an increase of hydrogen ion concentration without exceeding the physiological limits. In Exp. 10 we added this amount of acid, firstly with

blood exposed to partial pressures of carbon dioxide within physiological limits and afterwards (Exp. 11) with a very low carbon dioxide pressure, but sufficient however to leave some of the "bicarbonate reserve" intact. In none of these experiments were we able to detect any chlorine transference between plasma and corpuscles. In Exp. 10 the carbon dioxide capacity is diminished by 23 p.c. which agrees sufficiently with the value of 24 p.c. given by Mellanby and Thomas(15) for the same quantity of acid. In Exp. 12 it is diminished by 39 p.c. It is noteworthy that in Exp. 12 where the quantity of acid was the same as in Exp. 10 the fall of carbon dioxide combining power is less (10 vols. p.c. instead of 11·5 below the normal curve).

Van Slyke and Cullen(6) have shown that when the acid added to plasma corresponded with about half of the bicarbonate present, the fall of "alkaline reserve" corresponded approximately in molecular equivalents to the quantity of acid added. If the acid increases the fall of bicarbonate is not corresponding. In Exp. 13 *a* we added a quantity of acid sufficient to neutralize all the bicarbonate; in this case there was a considerable transference of chlorine.

Hamburger(5) has shown that an interchange of chlorine ions was produced on the addition of acid to the blood. One must remark, however, that he used far greater quantities of acid than were used in our experiments; and, just as in our Exp. 13 *a* the whole of the available alkali must have been neutralised.

When introducing strong acids to blood in order to prevent any precipitation on the sides of the saturator or other gross damage to corpuscles, our technique was as follows: The blood was introduced by means of a 25 c.c. pipette into the bottom of the saturator which was held vertically, the acid having been previously spread as diffusely as possible on the upper part of the sides. The saturator was then corked and the whole contents suddenly and violently agitated. By this method it was possible to avoid obvious damage to cells by the strong acid. When the blood was subsequently centrifuged, no hæmolysis and no layer of precipitated proteins at the bottom of the tube were observed. The general technique of our experiments with lactic acid (0·13 c.c. of 9·7 normal acid with 25 c.c. of blood) as well as with β-oxybutyric acid (0·15 c.c. 7·6 normal acid for 25 c.c. of blood) differed slightly in some cases from our usual technique described at the beginning of this paper in that the air of the saturator, after the addition of the acid, was as far as possible freed from carbon dioxide by means of a current of air sucked through by a filter pump. The saturator was placed in the bath for five mins. after which it was taken out and again connected with the filter pump in order to remove any further carbon dioxide liberated from the blood by the acid. After this the procedure was the same as described above. By this means the blood could be equilibrated with an atmosphere almost completely free of carbon dioxide.

As regards muscular exercise two experiments (Table IV) were undertaken, both on the same subject (H. W. D.) and differing only as regards

the duration of the exercise. The first consisted in pedalling on the Martin bicycle ergometer for an hour at the rate of 592 kilogram-metres per minute. At the end of each quarter of an hour the work was increased to 1576 kg.-metres per minute for 5 mins. After the exercise the subject experienced great exhaustion accompanied by nausea and dizziness. The second experiment was more complete, samples of urine being collected before and after the exercise, and the alveolar air being estimated. The duration was less and the exercise more violent, the object being to obtain a maximum acidosis by minimising the compensatory processes which probably occur with less severe exercise over a longer period. So as can be seen in the following protocol the acidosis was greater.

Subject H. W. D. Barometer 753.

11 a.m.	Resting. 11.15. Bladder emptied.
11.30	Blood drawn—at 48·3 mm. CO_2 pressure, blood took up 54 vols. p.c. of CO_2. (Normal 54 ∴ alkaline reserve ± 0.)
12.30 p.m.	Resting on bicycle ergometer.
12.45	Alveolar air CO_2 5·87 p.c. = 41·4 mm. CO_2 pressure.
12.47	Urine collected. 11.15 a.m. to 12.47 p.m. 370 c.c. containing 14 % N/10 alkali. Ammonia nitrogen 0·0 mg. %. Urea nitrogen 224 mg. %. Ammonia: urea nitrogen ratio 0. NaCl 635 mg. %. Albumen 0. Acetone bodies 0.
12.48	Pulse 70. Respirations 13.
12.50	Commenced pedalling on ergometer. Rate of work 982 kg.-metres per min. Pulse 111. Resp. 25.
12.52	982 kg. metres per min. Pulse 112. Resp. 26
12.54	982 „ „ „ 110. „ 26.
12.56	982 „ „ „ 110. „ 26.
12.58	Resting. Pulse 120. Abundant perspiration.
12.59	„ „ 100. Resp. 19.
1.0–1.2	1629 kg.-metres per min. Maximum possible effort.
1.2	Work stopped. Pulse 120. Exhausted. 1.3. Pulse 100. 1.4. Pulse 88. 1.5. Pulse 100.
1.7	Alveolar air CO_2 5·70 % = 40·2 mm. CO_2 pressure.
1.8	„ „ 4·94 % = 34·9 „ „ „
1.10	Blood drawn. At 43 mm. CO_2 pressure blood took up 40 vols. p.c. of CO_2. (Normal 52·5 ∴ alkaline reserve − 22 %.)*
1.15	Urine collected. 12.47–1.15 p.m. 54 c.c. containing 6·6 % N/10 acid. Ammonia nitrogen 19·6 mg. %. Urea nitrogen 246·4 mg. %. Ammonia: urea nitrogen ratio ·079. NaCl ·635 mg. %. Albumen 0. Acetone bodies 0.
2.15	Urine collected. 1.15–2.15 p.m. 58 c.c. containing 24 % N/10 acid. Ammonia nitrogen 50·6 mg. %. Urea nitrogen 243·4 mg. %. Ammonia: urea nitrogen ratio 0·207. NaCl 1073 mg. %. Albumen 0. Acetone bodies 0.

* Further details in Table IV.

In the above experiment the titration of alkaline urine was done by the method previously described by Davies, Haldane and Kennaway (16). Acid urines were titrated to *p*H 7·43 in Cole's comparator using phenol red as indicator.

The percentage of chlorides in the cells (Table IV) is increased while that in the plasma remains unmodified. The analysis of the urines is in itself very instructive. Before commencing the experiment the urine was alkaline (a saline purgative having inadvertently been taken earlier

in the day) and the subsequent changes exhibited in a marked degree the production of acid, and the change in ammonia-urea ratio during heavy muscular work. The oliguria during and even one hour after the exercise was very striking. Also the urinary chlorides remained at exactly the same level as before the experiment and were increased only one hour afterwards when, as has been shown by Ryffel(13) and by Douglas and Haldane(14), the lactic acid has been completely oxidised or eliminated. Unfortunately the quantity of urine was insufficient to allow of phosphate estimations being made.

(2) Experiments *in vitro* with β-oxybutyric acid were carried out with exactly the same technique as with lactic acid, and the results were identical. We used 7·6 normal acid, kindly prepared for us by Mr Harington. When it was added to the blood in the manner described above no destruction of blood cells occurred. The results (Exps. 13 and 14) show that when all the "bicarbonate reserve" is not neutralised the transference of chlorides does not take place. In Exps. 13 *a*, 13 *b* there is an increase of chlorides in the corpuscles and decrease in that of the plasma with acidified blood, but the carbon dioxide pressure is much higher and probably the chlorine ion interchange is due to this. The experiment when repeated with 0·15 c.c. of acid for 25 c.c. of blood, in order to neutralise all the bicarbonate, shows a considerable chlorine shift.

Discussion of Results.

It is not necessary to discuss extensively the results for the experiments with carbon dioxide alone. Our work has merely confirmed the well-known results of previous workers, but is slightly more complete in that we have estimated the chlorine percentage in the corpuscular mass as well as in the plasma after centrifugation. We found it impossible to obtain a consistent curve for percentage chlorides in plasma or percentage of total chlorides in plasma with varying carbon dioxide pressure, or varying *p*H. This can partly be explained by the fact that the experiments were performed on different subjects on different days and with different percentages of chlorides in whole blood. The variation in total chlorides may be very large, thus in the case of H. W. D. there was a change of over 50 mg. on two consecutive days (Exps. 8 and 9), while in Exp. 15 after exercise his total chlorides were 545 mg. p.c. and in Exp. 14 a year later they were 402 mg. p.c. At present we are unable to suggest any explanation for this variation. In the case of L.D. the maximum difference was 53 mg. These factors should be partly elimi-

nated by taking hæmatocrit readings and, knowing the percentage of NaCl in plasma and corpuscles respectively, calculating the percentage of total chlorides present in the plasma. Unfortunately our hæmatocrit readings were not always reliable—duplicate readings often failed to agree within 2 p.c. so that we lack accurate quantitative data for this calculation. It appears further that there may be some other factors involved. Possibly there may be changes in the corpuscular membrane as regards permeability to salts and to water, or variations in the hydrophilic and hydrophobic characters of the various colloids. All our experiments have shown that on a given sample of blood an increased partial pressure of carbon dioxide causes passage of chlorine from plasma to corpuscles but we have been unable to obtain consistent quantitative results similar to those of Fridericia who performed all his experiments on the one sample of blood.

For the other types of acidosis *in vitro* within physiological limits no measurable chlorine transference occurs, even when the bicarbonate reserve is greatly diminished. This effect comes into play only when all the bicarbonate has been neutralized. The following seems to us to be the most probable explanation. When any acid is added to the blood it attacks the alkaline salts of the proteins as well as the bicarbonate, the alkaline proteinate being the first attacked and the ratio $\frac{H_2CO_3}{NaHCO_3}$ being maintained, at least with small quantities of acid. Only when the greater part of the alkaline proteinate is neutralized does the acid combine with the bicarbonate, and after that with the sodium of neutral salts, the latter being the most stable. This seems true as well for H_2CO_3 as for stronger acids, the H_2CO_3 having combined with all the Na of the proteins it cannot further combine with $NaHCO_3$, so that it then attacks the basic radicles of the neutral salts. This hypothesis is proved by the fact that on adding a little more than ·12 p.c. of lactic acid to the blood (Exps. 10 *a*, 10 *b*) all the bicarbonate would be neutralized if the acid combined first with bicarbonate before attacking the Na proteinate. Instead of this being the case, the carbon dioxide content of blood treated in this way was only 10 and 11·4 vols. p.c. below normal, the carbon dioxide pressure being respectively 7·6 and 36·8 mm. of Hg. Moreover if we add ·12 p.c. of lactic acid to a sample of blood at a very low pressure of carbon dioxide (Exps. 11 *a*, 11 *b*) and thus with a low bicarbonate content, the bicarbonate would be immediately neutralized if the fixed acid combined first with it. This is not so, for at these low carbon dioxide pressures the acidulated blood still contains relatively slightly more carbon dioxide than at higher carbon dioxide pressures.

That is, at low pressures of carbon dioxide the "bicarbonate reserve" is less diminished than at high carbon dioxide pressures on the addition of the same amount of fixed acid. This shows conclusively that the proteins are weaker acids than is H_2CO_3, and are the first to be turned out on the addition of a stronger acid. Although no chlorine transference occurs *in vitro* until all the bicarbonate reserve is neutralized, yet with an acidosis *in vivo* it is obvious that reduction of bicarbonate reserve would cause the transference to occur more readily when the blood becomes venous, just as in Exp. 10 *b*, because under such conditions the available alkali combined with proteins is diminished.

As regards our experiments with reduced hæmoglobin no further comment is necessary. They show definitely that at constant carbon dioxide pressure no chlorine shift occurs as a result of oxygenation or reduction of hæmoglobin.

The experiments in which severe exercise was performed show many interesting results. The absence of diminution of plasma chlorides is the same as occurs with small quantities of lactic acid added *in vitro*. The increased percentage of chlorides in the cells is a phenomenon which is difficult to explain. The following facts are certain:

(1) In the first experiment where the exercise was of long duration the chloride percentage of the whole blood was increased.

(2) The hæmoglobin percentage is increased, but the increase is greater than that of the chlorides. This is probably due to the increased capillary circulation washing out corpuscles from stagnant capillaries. It seems that, apart from their rôle in the regulation of blood alkalinity, the chlorides may also play a part in the regulation of osmotic pressure in the plasma, and may by some unknown means be retained in the corpuscles in order to maintain the normal osmotic pressure of the plasma. We are unable to offer any elucidation of the mechanism of this retention. Nolf(15) considered that in the maintenance of the normal osmotic tension of the plasma, the chlorine shift could be sufficiently accounted for by the varying carbon dioxide pressure, but our experiments appear to indicate that some further factor is involved. In our second experiment with exercise there was no increased chloride percentage in whole blood. This was due probably to the shorter duration of the exercise and smaller loss of water. The increased hæmoglobin would be due probably to the flushing out of the capillaries.

SUMMARY.

1. Our results with carbon dioxide are in accordance with those of Zuntz, Gürber, Hamburger, Van Slyke and Cullen, Fridericia and other workers, except that under the conditions of our experiments we were unable to obtain quantitative results similar to those of Fridericia.

2. The carbon dioxide dissociation curve for one of us (L. D.) is published. Results for H. W. D. show that his dissociation curve already published remains unaltered.

3. With constant partial pressure of carbon dioxide, reduction of hæmoglobin produces no chlorine transference.

4. Acids other than carbonic acid whether added to the blood *in vitro* or produced *in vivo* do not of themselves cause any chlorine transference unless they be present in quantities sufficient to neutralize the whole of the "bicarbonate reserve."

5. Experiments with severe muscular work appear to indicate that chlorine transference may occur not only for the regulation of the blood alkalinity, but also possibly to maintain the normal osmotic pressure in the plasma.

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Note. In a Paper now in the Press (Heart) we have shown that with the extreme local carbon dioxide acidosis, the chlorine transference occurs *in vivo* in exactly the same manner as would be expected from the experiments *in vitro*.