CXC. ON THE PRESENCE OF A VOLATILE ORGANIC CHLORINE COMPOUND IN BLOOD.

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It is generally assumed that chlorine exists in the blood solely as inorganic chloride. While this hypothesis is supported by a great deal of the classical work of L. J. Henderson, Van Slyke and others on the interchange of ions during disturbance of acid-base equilibrium, it is nevertheless true that some problems are not solved by such a conception. During a prolonged investigation into changes in the chemistry of the blood in pyloric stenosis of infancy, it has become evident that the loss of chlorine from the blood is not necessarily balanced by the gain in carbonic acid or other known acid [Graham and Morris, 1929]. Further as Hanke and Donovan [1927] state "The current theories (of the formation of gastric hydrochloric acid) involve the unreasonable assumption of the separation of the acid locally from an equivalent of alkali." These authors maintain that 10–50 % of the total chlorine is present in organic form in the blood and tissues. They suggest that it is by the action of a special chloro-esterase on this organic chlorine compound that the free HCl of the gastric juice is produced.

The work reported in this paper was commenced at the suggestion of Prof. Leonard Findlay in an endeavour to throw some light on the nature of the chlorine combination in the blood. The method of chlorine analysis employed was that of Van Slyke [1923]. The subjects from whom the blood was obtained were healthy adults or children apparently completely recovered from such conditions as rheumatism. In all cases the subject had ingested nothing for a period of at least 12 hours prior to the withdrawal of the blood.

Effect of drying on the chlorine content of blood-serum.

After a preliminary drying of serum it was found that the amount of chlorine recovered was less than when no drying was employed. In each case to obtain the effect of drying the serum was heated in an Erlenmeyer flask in an oven at 105° for 45 minutes. Table I shows the results obtained from the sera of normal adults.

Table I. *Effect of drying on the chlorine content of serum.*

Serum No.	Chlorine milli-eq./litre	
	No drying	Preliminary drying
1	102.11	97.65
2	100.00	95.10
3	102.39	98.24
4	104.76	100.32

The loss of chlorine on drying might be due to one of four causes: (1) the presence of free chlorine in the blood which was liberated by some complex action similar to that of porous clay on NaCl; (2) the formation and volatilisation of HCl; (3) the loss of inorganic chloride by volatilisation; (4) the presence of volatile organic chlorine.

The presence of free volatile Cl or HCl is excluded by the following experiments. The gases from 100 cc. of blood heated to 105° when led into a solution containing KI and starch failed to give a blue colour. The amount of Cl which is lost during the drying of 100 cc. of blood is sufficient immediately to produce a blue colour with starch iodide. The absence of this reaction together with the fact that no precipitate was found on distilling into silver nitrate solution indicates that only mere traces of free Cl or HCl could have been present.

Table II. *Effect of drying on the chlorine and fixed base content of serum.*

Serum No.	Chlorine milli-eq./litre		Fixed base milli-eq./litre	
	No drying	Preliminary drying	No drying	Preliminary drying
5	106.5	102.2	147.7	147.5
6	110.3	106.7	153.1	153.4
7	103.2	100.0	149.9	149.8
8	102.9	98.3	150.1	150.2
9	99.4	95.7	151.9	152.0

In order to determine the possibility of loss of inorganic chlorides the fixed base content of the serum was determined before and after drying by the method of Stadie and Ross [1925]. Table II gives the results. It is evident that the fixed base is unaltered by preliminary drying while definite loss of chlorine results from this treatment. Accordingly, inorganic chloride could not have been lost during the drying. One is therefore forced to the conclusion that the chlorine lost by drying is in the form of a volatile organic chlorine compound.

The effect of drying on the chlorine content of whole blood and serum was then estimated in order to determine the distribution of the volatile organic chlorine between serum and corpuscles. Blood was collected under oil and defibrinated; samples of whole blood, serum and cells were then analysed. The percentage cell content was determined by the haematocrit. Table III gives the results, from which it is evident that the cells contain a much greater amount of volatile organic chlorine than does the serum.

Table III.

Blood No.	Red blood corpuscles %	Chlorine milli-eq./litre								
		Whole blood		Serum		Cells		Organic volatile chlorine milli-eq./litre		
		No drying	Drying	No drying	Drying	No drying	Drying	Blood	Serum	Cells
10	45	78.57	65.47	101.56	96.82	49.77	24.58	13.10	4.74	25.19
11	46	80.11	65.88	100.48	96.04	55.58	25.10	14.23	4.44	30.48
12	46	79.95	66.15	102.15	98.10	54.06	24.82	13.80	4.05	29.24
13	44	78.99	66.55	102.76	97.85	48.43	25.77	12.44	4.91	22.66
14	45	79.15	66.90	101.09	97.14	52.44	28.77	13.25	3.95	23.67

Effect of varying the duration of the drying process on the organic volatile chlorine.

It is important that the drying process should proceed for at least 400 minutes in order to ensure the volatilisation of the chlorine to a maximum. The following results give proof of this.

Duration of drying in minutes	Volatile organic chlorine in milli-eq./litre
3	3.20
15	3.25
30	4.25
45	6.25
60	7.85
75	8.55
90	9.24
105	9.50
210	11.15
400	12.60
600	12.60

The isolation of the volatile organic complex of chlorine has so far been found impossible owing to the volatilisation of various substances at the same time. On distillation acetic acid and ammonia appear in very large amounts. Distillation at 110° accompanied by the passage of an air current through the blood led to the collection of but minimal amounts of chlorine in the receiving flask. By the usual distillation procedure, however, it was possible to recover and estimate by the Carius or Parr method the amount of Cl volatilised; Table IV gives the amounts so recovered. The amounts collected in the distillates compare very satisfactorily with those lost from the blood samples especially when one remembers the practical difficulties which involve a large experimental error such as the possibility of some of the volatile chlorine being held back by the material as it dries.

Table IV. *Chlorine lost from the blood on distillation.*

Blood No.	Chlorine milli-eq./litre		
	Lost from blood	Collected in distillate	
		Parr method	Carius tube
15	172.4	168.0	165.1
16	146.5	142.1	144.3
17	141.8	140.1	140.7
18	138.7	132.4	136.2

Effect of addition of various substances on the volatile organic chlorine content of blood.

Various compounds containing the NH_2 group were used, including urea, histamine, glycine and a few of the simpler primary and secondary amines. In all cases the materials employed were purified before using. In Fig. 1 is

Fig. 1. Effect of urea-concentration on volatile chlorine.

given a graphic representation of the results obtained when urea was added to the blood in progressively increasing amounts.

To a sample of blood a known amount of urea was added: the two portions were then analysed (one with and the other without previous drying) to obtain the value for the volatile chlorine. All analyses were done in duplicate. It will be seen that this concentration curve corresponds with the adsorption formula $y/m = kc^{1/p}$, where y = amount adsorbed by a quantity m of adsorbent, c = concentration at equilibrium and k and p are constants. In the present case y represents the increase in urea concentration, m the original amount of volatile chlorine and c the amount of volatile chlorine after the addition of the urea. Between the concentrations of urea 2.14–24.10 milli-eq./litre the values of $1/p$ and k are -3.4 and -3.16 respectively. There is a marked resemblance between the curve and the coagulator concentration curves. In this case the coagulator is urea and the reaction consists of a coagulation of the volatile chlorine complex up to a range of concentration of urea of 24 milli-eq., when reversal occurs with consequent peptisation.

When an ammonium salt is added, *e.g.* $(\text{NH}_4)_2\text{SO}_4$, a curve similar to that of the NH_2 series is obtained (Fig. 2). The maximum and minimum values (*i.e.* those concentrations at which reversal takes place) are lower than those found with urea. The curve again corresponds with the adsorption equation. Between concentrations of $(\text{NH}_4)_2\text{SO}_4$ of 0.9672–4.8360 milli-eq./litre $1/p = 2.4$ and $k = -12.13$.

When a chloride is added, the results obtained are the inverse of those

obtained with NH_2 or NH_4 groups (Fig. 3). This indicates that the addition of the chlorine ion produces an increase in the amount of volatile chlorine, possibly because the Cl having the opposite electric charge to that of NH_2 breaks the union of NH_2 and volatile chlorine. At a certain concentration reversal sets in with the consequent diminution of volatile chlorine.

Fig. 2. Effect of NH_4 content on volatile chlorine.

Fig. 3. Effect of KCl and NaCl content on volatile chlorine.

Addition of a very dilute acid produces the same effect on volatile chlorine as does NaCl. As the strength of acid is increased, the amount of volatile chlorine is at first raised and then lowered till with higher concentrations free HCl is formed.

Effect of prolonging the time during which blood was allowed to stand at room-temperature.

The mixtures of blood and coagulator substances (urea or NaCl) were allowed to stand at room-temperature before drying. The mixtures had urea 1.605 and NaCl 50 milli-eq./litre respectively. The results are given in Table V. The general equation for both curves is $y = ac^n$. For the urea mixture $\alpha = 0.54$, $n = 1.7$ and for the NaCl mixture $\alpha = 44$, $n = -0.15$. The effect on

Table V. *Effect of time-factor on volatile organic chlorine of blood to which urea or NaCl has been added.*

Time in minutes	Difference in amount of volatile organic chlorine in milli-eq./litre Blood urea	Difference in amount of volatile organic chlorine in milli-eq./litre Blood NaCl
5	-4.1	-5.7
10	-4.2	-5.46
15	-4.88	-4.62
20	-5.76	-3.58
25	-7.08	-2.56
30	-8.05	-1.56
40	-8.90	-0.46

the volatile organic chlorine of allowing untreated blood to stand was to cause a gradual decrease to a value which remained steady about 5 milli-eq. below the original. Thereafter prolonged standing did not appreciably alter the amount of volatile chlorine. This is interesting in view of the results of Havard and Kerridge [1929] on the change of c_H in blood allowed to stand. They found that the c_H increased to a point and there remained steady. It has already been pointed out that the addition of acid in small amount decreases the content of volatile chlorine so that the effect of standing on the volatile chlorine is associated with the increase in c_H . It seems feasible to assume that the liberation of volatile chlorine produces an increase in the c_H . It is possible however that the relationship of cause and effect is reversed or that loss of volatile chlorine and increase in c_H are both due to some other underlying factor.

From these data we may conclude that the amount of volatile chlorine may be altered by the addition of various substances to the blood but that for each blood there is a certain maximum and minimum beyond which the content does not pass. The simplest hypothesis on which to explain these results is to assume that the chlorine in normal blood exists in three forms (a) inorganic, (b) organic non-volatile, and (c) organic volatile. The relative amounts of non-volatile and volatile organic chlorine seem to depend on the concentration of urea, amino-acid etc. Thus in blood I (Fig. 1) the maximum amount of volatile chlorine is 24.79 milli-eq. per litre. Varying amounts of urea lead to the production of an organic non-volatile chlorine compound in amounts that satisfy the adsorption formula. This indicates the presence of a complex organic colloidal system in which there is present a loose union of amino-compounds with chlorine forming a non-volatile organic compound. As the amino-compounds are increased in amount there occurs an increased formation of this non-volatile compound up to the point where reversal of charge sets in when peptisation occurs. The addition of an inorganic chloride breaks this complex up to a certain concentration of the salt beyond which the chlorine once again commences to unite with the amino-group. This is probably also an effect of the electric charge.

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