CCXL. FURTHER OBSERVATIONS ON THE VOLATILE CHLORINE OF BLOOD.

BY SAMUEL MORRIS AND NOAH MORRIS.

*From the Hannah Dairy Research Institute, Ayr, and the Biochemical
Laboratory, Department of Paediatrics, Hospital for Sick Children,
University of Glasgow.*

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IN a previous paper [Morris and Morris, 1930] it was shown that drying of blood at 105° for a period of 4 hours led to a diminution in the chlorine content and it was suggested that the chlorine lost by drying was probably contained in a volatile organic compound. Sunderman and Williams [1931] published figures indicating that while a loss of chlorine occurred on drying blood and tissues it could be prevented by adding water to the dried blood and allowing the mixture to remain for 3 hours prior to the estimation of the chlorine. They further showed that the addition of olive oil to a solution of sodium chloride with subsequent drying led to the loss of a certain amount of chlorine which also could be prevented by the addition of water after drying and prior to the estimation, and concluded that in the case of blood the loss of chlorine was only an apparent one, being probably due to the effect of occlusion of the chlorine by fat. Quagliariello and Mazza [1931], on the other hand, state that the loss of chlorine on the heating of blood is due to drying and loss of HCl and is therefore no indication of the presence of organic chlorine compounds. Hogartz [1931] found no evidence of organic compounds of chlorine by extraction methods involving the use of alcohol and ether.

In our previous paper the results of experiments were given showing that the chlorine lost from the dried blood could be recovered almost quantitatively although not in the form of free chlorine or hydrochloric acid. In view of the results of Sunderman and Williams with olive oil, it was thought necessary to confirm our previous results and study the variations in the amounts of chlorine volatilised under different conditions.

Effect of drying blood at 105°.

Each sample of blood was divided into three equal parts, and the Cl content of the first was determined immediately by the Van Slyke method. The remaining two were dried for 4 hours at 105°; thereafter one was analysed by

the Van Slyke method and the other was completely ashed and its Cl content estimated by the same method. Table I indicates that practically the same diminution was obtained by drying whether the Van Slyke method was used directly or preliminary ashing was employed. It seems clear therefore that the decrease in the Cl value was not the result of occlusion.

Table I.

Blood no.	Chlorine milliequiv./litre.		
	No preliminary drying	Preliminary drying	Preliminary drying and ashing
35	89.00	71.90	68.50
36	89.60	71.90	72.30
37	87.10	77.00	76.00

Effect of oxygen on the volatilisation of the chlorine from blood.

To study the effect of oxygen, samples of the same blood were dried in ordinary air, air deprived of oxygen and in an atmosphere of pure nitrogen. The air was deprived of oxygen by passing it through two flasks of pyrogallol prior to its entry into the drying flask. The atmosphere of nitrogen was supplied by heating NH_4NO_2 . Table II shows that in the absence of oxygen no loss of chlorine is produced by drying at 105° . Furthermore, in the presence of air, addition to the blood of a strong oxidising agent such as potassium persulphate leads to an increased loss of chlorine on drying (Table III). This suggests that the presence of active oxygen facilitates the formation of the volatile chlorine compound.

Table II.

Blood no.	No drying	Preliminary drying			
		Ordinary air	Dry air	Dry air deprived of oxygen	Nitrogen
46	86.2	72.4	72.8	86.2	86.0
47	79.8	70.1	70.0	78.6	79.1
48	84.5	69.9	70.4	82.9	84.8
49	88.9	74.5	73.9	87.9	88.3
50	79.8	64.8	64.8	78.7	79.6
51	86.5	75.6	75.5	86.0	86.1

Table III. *Effect of addition of potassium persulphate to blood on the chlorine content after drying.*

Blood no.	No drying		Preliminary drying	
	Blood	Blood + $\text{K}_2\text{S}_2\text{O}_8$	Blood	Blood + $\text{K}_2\text{S}_2\text{O}_8$
51	85.66	85.60	73.00	62.30
52	85.20	85.25	69.95	59.24
53	87.68	87.72	65.36	57.71
54 (serum)	115.85	115.81	114.32	103.61

Effect of drying blood in a desiccator (Table IV).

Sunderman and Williams showed that drying blood over P_2O_5 also involved a loss of Cl and concluded that the essential factor was the loss of moisture. We studied the effect of drying blood for 48 hours over H_2SO_4 : from some flasks oxygen was removed, others were filled with nitrogen, while others contained sodium cyanide solution. The effect of drying over H_2SO_4 was to produce a

Table IV. *Effect of drying blood in desiccator at room temperature (18°) in atmospheres of (a) air, (b) air deprived of oxygen, (c) nitrogen, (d) air, with NaCN added to the blood and (e) air, with $K_2S_2O_8$ added to the blood.*

Blood no.	No drying	Preliminary drying in oven at 105°	Air	Air deprived of oxygen	Nitrogen	Ordinary air; NaCN added to blood	Ordinary air; $K_2S_2O_8$ added to blood
(a) 46	86.2	72.4	78.8	86.2	86.0	85.5	71.1
(b) 47	79.8	70.1	74.9	79.5	79.8	79.8	71.9
(c) 48	84.5	69.9	74.9	84.0	84.1	84.0	67.3
(d) 49	88.9	74.5	80.6	89.0	87.9	87.8	76.0
(e) 50	79.8	64.8	71.4	78.8	78.9	78.4	60.6

diminution in the amount of chlorine recovered, although the loss was not so great as when the blood was dried at 105° . The addition of potassium persulphate increased the loss to at least that found on drying at 105° . The absence of oxygen from the flask or the presence of cyanide, which inhibits oxidation, prevented any loss of chlorine. It is clear therefore that the withdrawal of moisture in itself is not responsible for the diminution in chlorine content. It should here be stated that if the cyanide-blood mixtures are dried for over 48 hours the cyanide is converted into HCN and completely volatilised and there may occur some oxidative process with consequent loss of chlorine (Table V).

Table V. *Effect of drying blood with NaCN in desiccator for varying periods.*

Blood no.	No drying	Preliminary drying		
		Duration of drying in hours	Blood alone	Blood + NaCN
46	86.2	24	82.4	85.9
—	—	48	80.6	86.0
—	—	72	72.4	85.5
—	—	120	72.9	80.6
—	—	168	72.1	72.8

Table VI. *Effect of drying tissues (a) at 105° , (b) in desiccator and (c) with acetone and ether.*

Tissue (rabbit)	No drying	In oven at 105°	In desiccator	By acetone and ether
Heart	35.4	27.5	32.6	35.8
„	33.8	26.2	30.1	35.0
Kidney	49.2	31.8	40.4	48.8
„	54.1	36.5	46.2	54.0
Muscle	12.2	8.1	10.4	12.2
„	16.4	11.9	14.0	16.0
„	10.5	7.0	8.8	10.5

Effect of drying tissues under various conditions.

Prior to determination of their Cl content tissues were dried either in the oven at 105° or over concentrated H₂SO₄ or by Best and McHenry's acetone and ether method [1930]. The results (Table VI) fully corroborate the blood analyses, indicating that oxygen is essential for the volatilisation of the chlorine and that more chlorine is lost at a temperature of 105° than as a result of drying in a desiccator.

Effect of addition of olive oil to NaCl solution.

It seemed to us possible that the loss of chlorine on drying a mixture of olive oil and NaCl [Sunderman and Williams, 1931] might be due to reaction between the NaCl and unsaturated fatty acids.

This view was supported by the fact that heat is necessary to produce a loss of Cl from the olive oil-NaCl mixture. Further, the use of a fully saturated acid such as palmitic instead of olive oil should lead to no diminution of Cl content on drying the fatty acid-NaCl mixture at 105°. Experiments show that heating of palmitic acid-NaCl mixtures at 105° leads to no decrease in the Cl.

The only difference between blood and the olive oil-NaCl mixture as far as the loss of chlorine is concerned is the fact that the chlorine is lost from the latter mixture only after heating to over 100° whereas it can be driven off from the blood on drying in the desiccator without the aid of heat. This, however, is insufficient evidence that chlorine is present as an organic compound in blood. In order to demonstrate the presence of such a compound in blood it is necessary to extract it.

Organic solvents such as ether, benzene and toluene were found to be useless for the extraction of any chlorine. It was thought that a mixture of alcohol and ether might be efficient. Alcohol, however, mixed with the water present in blood and carried into solution some of the inorganic chlorine. It was therefore necessary to remove water from the blood by some method other than heat. For this purpose a modification of Best and McHenry's method for drying tissues was used. This consisted in the addition of acetone to blood, filtering, and washing four times with acetone in excess until no chlorine appeared in the acetone filtrate and then three or four times with ether. Air was then drawn over the residue by a suction pump, until a dry powder was obtained. The powder was removed to a flask and a mixture of equal parts of absolute alcohol and anhydrous ether was added. The powder was shaken with the alcohol-ether mixture for three periods of 15 minutes and then allowed to stand overnight. The alcohol-ether extract was then filtered off into a pyrex tube, and the alcohol and ether were evaporated off at a low temperature. Chlorine was invariably found to be present in the extract when this was treated with silver nitrate and nitric acid. So far we have been unable to recover more than 80 % of the amount lost on heating blood. With tissues the same method was employed after they were finely minced.

The alcohol-ether mixture does not extract chlorine from inorganic chlorides unless water is present. This we have repeatedly confirmed for the alcohol-ether mixtures we have been using.

It seemed possible, however, that the blood might have been incompletely de-hydrated by the acetone-ether treatment, and that the chlorine extracted was due simply to the inorganic chlorides being dissolved in the alcohol-ether mixture owing to the presence of moisture. To test this possibility a series of experiments was undertaken in which a synthetic mixture analogous to blood was used, containing 16 g. of protein (egg-albumin, serum-albumin or caseinogen), 0.5 g. of olive oil, 0.5 g. of NaCl and 0.1 g. of glucose per 100 cc. water. The mixture was well stirred and allowed to stand for about 4 hours prior to extraction, by the method used for the blood. In no case was chlorine found in the alcohol-ether extract.

Further, it was shown in a previous paper [Morris and Morris, 1930] that the volatile chlorine of blood diminishes on adding acids to the blood, the diminution being dependent on the strength of the acid. The addition of a strong acid should therefore diminish the amount of organic chlorine extracted from blood by means of alcohol and ether. From the same sample of blood two lots of 100 cc. were taken to one of which was added 10 cc. concentrated HCl. Both were allowed to stand for 4 hours and then extracted by acetone and finally by alcohol and ether. In the blood-HCl sample only minimum traces of chlorine (1-2 milliequiv.) could be detected in the alcohol-ether extract. This experiment which was repeated on four occasions affords further confirmation of the fact that inorganic chlorine is not extracted by the method described.

In view of these facts it seems justifiable to conclude that there exists in blood an organic chlorine compound.

SUMMARY.

1. The presence of oxygen is essential for the loss of chlorine to take place.
2. The heating of an olive oil-NaCl mixture at a temperature over 100° leads to loss of chlorine.
3. Evidence is brought to show that there is close similarity between the two systems, blood and olive oil-NaCl, as far as the loss of Cl is concerned, except that in the latter mixture heat is required whereas with blood drying in a desiccator is sufficient to cause volatilisation of the Cl complex.
4. A method is described whereby organic chlorine can be extracted from blood. The method depends on removal of water by acetone and ether and subsequent extraction of the chlorine by an alcohol-ether mixture.

The authors regret that owing to an unfortunate oversight there was omitted from their previous paper an acknowledgment that the work therein described was commenced by one of them (S. M.) while in the Chemical Department of the Pennsylvania Hospital, Philadelphia.

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