Sapporo Med. J. 43 (2) 134~138 (1974)

Studies on the Hemoglobin-Bound Oxygen Using Gas Chromatographic Technique

I. Studies on the Blood of Man and Several Species of Animals in Normal Condition

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Introduction

Estimation of the postmortem lapse of time is one of the most important problems to be solved in almost all cases of medico-legal examination, and this is the most difficult problem for us who are engaged in forensic sciences, especially those who take part in the medico-legal examination of the dead body.

It is quite obvious that many factors exercise influences on the appearance of cadaveric phenomena. Among the factors, the temperature of the environment in which the cadaver lies is regarded as the most important factor and, moreover, environmental conditions such as, whether in air or water, outdoors or indoors, clothed or naked and so on are all important.

Many studies on methods of estimating the postmortem lapse of time have been undertaken[1~12] but none of them seems to be quite a reliable method by itself because of the factors mentioned above by which the phenomena show complex and miscellaneous appearances.

For estimation of the postmortem lapse of time, as a matter of course, subjects which can offer evidence for the estimation must be collected as much as possible and only then should the conclusion be arrived at. We think it is impossible to reach a conclusion by any one piece evidence alone, but the higher the reliance of each piece of evidence, the nearer the estimation to the accurate time of death.

Studies on the cooling of the dead body originated by Mueller[2,10,11] have been accepted as one of the authorized methods for estimating the postmortem lapse of time. Concerning this cooling of the dead body, Saito's study[9] is highly appreciated in Japan.

We are interested in the amount of oxygen which combines with hemoglobin and the postmortem change in the binding capacity of hemoglobin since no study has been reported on the postmortem changes of combinating between oxygen and hemoglobin. This is expected to lead to reliable results because of the specificity of combination between oxygen and hemoglobin and because of the ease of accurate detection by gas chromatography.

Materials and Methods

Standard Curve: First of all in our experiments, a standard curve was made by using aliquots of oxygen of 99.5% purity (NIPPOKU SANSO Co.) and a gas chromatograph

(Shimadzu GC 4 APFT) with the same condition as for the blood of man and animals was used. Because a slight tailing of the curve for each gas emerges, calculation of the area under the curve, the dotted area in Fig. 1, was achieved by means of planimetry (KENT Polar Planimeter) and the number read out on the meter was expressed by an arbitrary unit.

Oxygen Curve of Blood of Man in Normal State: Blood was collected by a syringe from the median cubital vein of men from 22 to 38 years old in normal healthy condition.

Oxygen Curve of Blood of Animals in Normal State: Blood was collected from the femoral or portal vein of rats and the ear vein of rabbits.

The Method for Releasing Oxygen from Hemoglobin: The method used for releasing oxygen (O_2) from hemoglobin (Hb) was Van Slyke's method[13,14]. A gas sampler was used to obtain the oxygen gas which was liberated completely from the hemoglobin, and the total amount of oxygen gas produced was introduced into a detector. 0.5 mℓ of blood was put in a vial with one drop of n-octyl alcohol as antifoam. After the vial was secured tightly with a silicon rubber stopper, the air in the vial was replaced by helium gas, which was used as a carrier gas, stirring gently for 5 minutes. The stirring was done using a vibrator and a piece of glass rod within the vial. Then 0.25 mℓ of saturated potassium ferricyanide solution was put into the vial with a syringe through the rubber stopper as a breaker of the combination of O_2 and Hb. Reaction of the solution with Hb-O_2 was continued for 5 minutes while the vial was shaken, and then the released O_2 gas was induced into a detector through a stainless steel column packed with Molecular Sieve 5 A, 0.3 cm in diameter and 3 meter in length[15,16].

Examination of Blood Coagulation: Examination of coagulation of freshly collected blood was also made preliminarily using blood with and without an addition of anticoagulant. Blood without anticoagulant was applied to the instrument immediately after collection and filtration through two- or three-folded gauze. Since these two kinds of blood did not show any difference in the amount of oxygen, blood without the addition of anticoagulant was examined in almost all cases.

In addition to the above mentioned freshly collected blood of man and animals, preserved blood of man for transfusion was also examined.

Table 1 *SHIMADZU Model GC 4 AP FT*

Sample: Pure Oxygen Blood (0.5 mℓ)	
Reagent: 0.25 mℓ of Saturated Potassium ferricyanide solution n-Octyl alcohol (one drop)	
Carrier Gas: Helium	Column: Steel, 3 m.
Flow Rate 40 mℓ/min.	Temperature 80°C
Inlet Press 1.5 kg/cm^2	Detector Temp. 120°C
Detector: T.C.D.	Packing:
Range: 16 mV, 100 mA.	Molecular Sieve 5 A
Chart Speed: 20 mm/min.	

Gas Chromatography: The operating conditions of the gas chromatograph is described in Table 1.

Results

According to the conditions described in Table 1, gases produced in the blood samples showed the first curve about 105 seconds after the opening of out-flow valve of the gas sampler, and this time point, the retention time, corresponded to those of pure oxygen and air.

A two-peaks pattern was shown in almost all cases in which freshly collected blood of man and animals was examined. The typical pattern in the cases of the blood of man is shown in Fig. 1. The first peak was higher than the second peak in all cases we examined as is shown in Fig. 1.

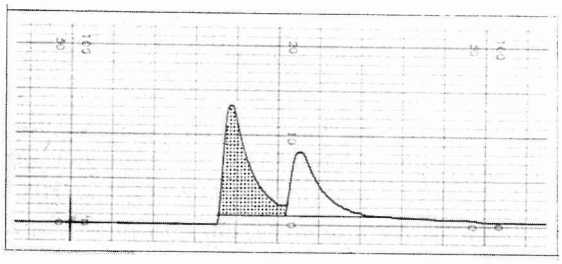

Fig. 1 Gaschromatograph of a Sample of Human Blood.

On the contrary, preserved blood for transfusion which was expected to be useful as the standard for the blood of man at the start of our experiments was observed to show oxygen amounts in a wide range of variation.

We started our experiments with rats, but the results showed a wide range of variation even in the control group fed on a standard diet (Oriental Yeast Industry Co. Ltd.) and in healthy condition.

The oxygen amounts of the blood of man in healthy condition are shown in Table 2.

Table 2 *Oxygen Value of Human Blood in Normal Healthy Condition*

Number	O_2 Value in Arbitrary Unit	Number	O_2 Value in Arbitrary Unit
1	2.0	6	2.0
2	2.0	7	2.1
3	2.0	8	1.9
4	1.9	9	2.0
5	2.0	10	2.1

Discussion

Blood which is obtainable from the dead body for medico-legal examination is in the cardiac cavities, especially in the right atrium and/or right ventricle, and we thus considered that it is of higher and practical significance to study the postmortem changes

of the binding capacity of hemoglobin to oxygen due to the environmental conditions using blood in the cardiac cavities rather than the consumption of oxygen in capillaries by cells or tissues in the early stages after death.

Methods offered by Van Slyke *et al*[13,14] have been utilized for more than forty years as the most popular and useful for volumetrical determination of blood gases. Studies on blood gas analysis using gas chromatographs by Wilson[17] and Lukas *et al*[18] demonstrated that there were almost no statistically significant differences between the two methods.

From the present findings by gas chromatography, we confirmed that the first curve of the gas from blood samples indicates the oxygen which was liberated from hemoglobin. It is not necessary to take mechanical or instrumental error or failure into consideration since the retention time was confirmed as specific for oxygen by using pure oxygen and since the retention time for air and blood corresponded to that for oxygen which showed lineally increasing values corresponding to the increasing volume of oxygen which was injected into the instrument.

Oxygen amounts of freshly collected blood of man showed a highly consistent value (Table 2).

Concerning the determination of the amount of oxygen, there are several other ways of calculation; for example, the method of comparison with some known substrates which act as an internal standard as in ethanol detection, or using the second peak (nitrogen gas peak) which always appears when whole blood is examined.

The oxygen amount (area of the first peak) might be expressed in terms of an absolute unit if the separation of the first and the second peak is completely achieved by controlling the temperature and flow rate of the carrier gas. It is expected that more accurate reproducibility would be obtained by using an integrator connected with the gas chromatograph.

The amount of oxygen bound to hemoglobin is expected to be almost constant so far as freshly collected blood and gas chromatography are used, and thus this method is desirable for prepration of the standard curve.

This paper was presented at the Sixth International Meeting of Forensic Sciences which was held at Edinburgh, Scotland, 1972. (Received March, 27, 1974)

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