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Effect of Heating of Blood on the Quantitative Determination of Carbon Monoxide Haemoglobin

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At routine determination of the carbon monoxide haemoglobin ($HbCO_2$) in subjects who had been burned alive there were sometimes appreciable differences between samples obtained from different regions of the body. It was thought that this variation of the HbCO might be explained by unequal heating of the blood by the fire. In an attempt to check the tenability of this assumption blood from cadavers was treated, with ordinary coal-gas and the effect of heat on its demonstrable HbCO content was studied.

Material and Methods

Uncoagulated human blood was obtained from the heart at autopsies performed within 24 hours of death. Ordinary coal gas from the mains was washed with an aqueous solution of lead acetate and passed through the blood for 1 hour, care being taken to avoid vigorous bubbling. To the blood thus treated and apparently saturated with HbCO, untreated blood was added until the degree of saturation of the sample was about 25, 50 or 75%. The HbCO was determined by the method of WOLFF in tubes containing 4 ml and by the method of MAEHLY in test tubes containing 1 ml. The tubes were corked and placed in a waterbath equipped with a thermostat. The bath temperatures used and the times the samples were left in the bath are given in the figures. Determinations were also made of the HbCO in unheated samples of the same blood. A Zeiss PMQ II photometer with 1 cm deep cuvettes was used for the spectrophotometric determinations. The pH was adjusted with the aid of a Radiometer-pH meter 22.

Results

When heated at 60°C or more the blood changed in colour and consistency. It became chocolate brown. As in SATA's investigation, the colour of the blood containing HbCO was more stable, but the difference was not striking. When heated at 70°C or more the blood was no longer liquid.

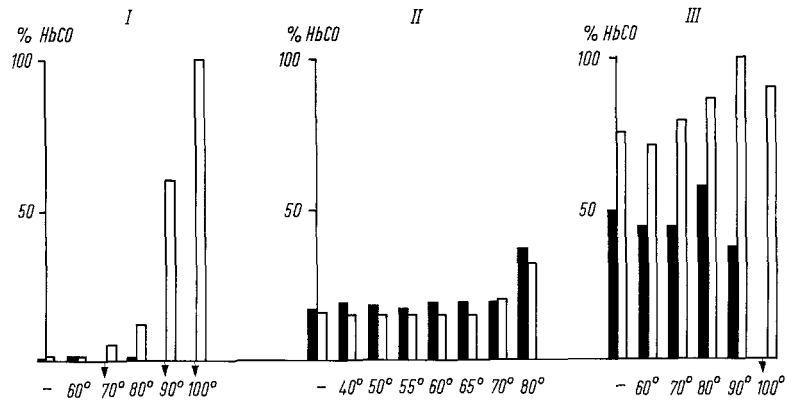

Fig. 1. The demonstrable HbCO-content in blood, unheated and heated for 5 min at different temperatures. I: Blood untreated with coal-gas. II: Blood treated with coal-gas to give about 25% HbCO-content. III: Blood treated with coal-gas to give about 75% HbCO-content. ■ Determination by MAEHLY's method, ↓ negative value, □ Determination by WOLFF's method

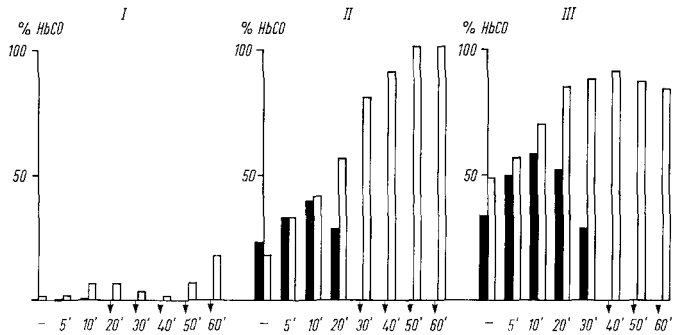

Fig. 2. The demonstrable HbCO-content in blood, unheated and heated at 80° for different times. I: Blood untreated with coal-gas. II: Blood treated with coal-gas to give about 25% HbCO-content. III: Blood treated with coal-gas to give about 50% HbCO-content. ■ Determination by MAEHLY's method, ↓ negative value, □ Determination by WOLFF's method

The intensity of the colour of the filtrates and the supernatants obtained decreased with increasing temperature and duration of exposure of the sample. Before spectrophotometric determinations were made (WOLFF's method) 2 ml of the supernatant was diluted with 10 ml of water. The extinction values noted for blood exposed to temperatures of at least 70°C for more than 5 min were extremely low. According to MAEHLY's method, the samples are diluted to an extinction value of 0.5—0.8 measured against the buffer at 540 μ. Blood heated at 80° for

5 min could thus be diluted about tenfold, while blood heated at 80°C for 60 min could only be diluted with an equal volume if the measurements were to fall within the same range.

The results obtained expressed in per cent HbCO are given in Fig. 1 and 2. Heating of the blood at and over 80°C for 5 min invariably gave unacceptable values. Heating of the blood at 80° for more than 5 min also resulted in false values. The HbCO content, as determined by WOLFF'S method, was always increased; similarly MAEHLY'S method gave increased values after exposure of the sample to 70—80°C for 5 min and to 80°C for at most 20 min. On the other hand, when the samples were heated at higher temperatures or for longer periods the values determined by MAEHLY'S method, were decreased or negative.

Determinations were made in blood partially (75%) saturated with HbCO. The HbCO content of the unheated sample as measured by WOLFF'S method, was 75% and by MAEHLY'S method 49%.

Discussion

As pointed out by MAEHLY, the effect of heating or burning on the demonstrable HbCO content of the blood has received only little space in the litterature. In an *in vitro* investigation SATA [3] studied heated blood for any correlation between the stability of the colour of the blood and the HbCO content. The present investigation showed that both WOLFF'S and MAEHLY'S method give false values of the HbCO content of the heated blood. As expected, heating results in denaturation and precipitation. The critical range of temperature, 70—80°C, agrees well with the coagulation temperature of haemoglobin at about pH 7. Determinations of the HbCO content of heated blood with WOLFF'S method gave increased values (false high values) which may perhaps be explained by haemoglobin not bound to carbon monoxide being less thermostable than HbCO, a possibility also pointed out by MAEHLY. This source of error may also hold for the false high values obtained with MAEHLY'S method. No attempt was made to explain why exposure of the blood to higher temperatures and for a longer time resulted in falling values.

Experiments with blood partially (75%) saturated with HbCO by treating with coal gas were also performed. The HbCO content of the unheated sample was 75%, as determined according to WOLFF against only 49% when measured according to MAEHLY. As mentioned by MAEHLY himself, his method is not reliable for HbCO concentrations above 30%. Moreover, it is apparent from Fig. 1 and 2, though less obviously, that WOLFF'S method is less suitable for measuring low concentrations of HbCO-according to the description of the method, below 10—15%.

The effect of heating on the demonstrable HbCO content of the blood from corpses *in vitro* is of course not fortwith comparable to the effect of heat on blood *in vivo* or in cadavers. In most cases, where subjects have been burned to death alive the deeper parts of the body must have been exposed to higher temperatures and for a longer time than the blood used in the present investigation. Judging from the present results, determination by the spectrophotometric methods of WOLFF and of MAEHLY of the HbCO content of blood from corpses exposed to high temperatures (fire) may not always be acceptable.

Zusammenfassung

Die Bestimmung des Kohlenoxydhämoglobingehaltes im Blut wird routinemäßig bei Todesfällen durch Verbrennung nach den spektrophotometrischen Methoden nach WOLFF oder MAEHLY durchgeführt. Mitunter zeigen die Resultate von Proben verschiedener Körpergegenden erhebliche Unterschiede. Da angenommen werden kann, daß die Erhitzung des Blutes die Untersuchungsergebnisse beeinflußt, wurde eine einfache in vitro-Untersuchung durchgeführt, indem an CO-behandelten und erhitzten Blutproben die Kohlenoxydhämoglobinbestimmung durchgeführt wurde. Nach den Resultaten zu urteilen, ergeben die Methoden von WOLFF und MAEHLY an erhitzten Blut nicht immer sichere Werte.

Summary

Routine determination of the HbCO content of blood from cases burned alive is carried out by WOLFF'S and MAEHLY'S spectrophotometric methods. There are sometimes appreciable differences between samples obtained from various parts of the body. As it could be assumed that the variations of HbCO might be explained by unequal heating of the blood, a simple in vitro investigation was carried out, on blood exposed to coal-gas and heat. Judging from the results obtained, WOLFF'S and MAEHLY'S methods may not always give acceptable values, when carried out on heated blood.

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