In conclusion, it is clear from the present study that anti-AChE pesticides do not exclusively affect activity of the cholinergic system. They may also substantially affect the other, putative neurotransmitter systems and thereby make a multipronged attack on the nervous system of the target insect.

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Reactivity of carbon monoxide with haemoglobin *in vitro* and its spectrophotometric estimation

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Dilution and pH affect the affinity of haemoglobin in blood for carbon monoxide. We show by spectrophotometric estimation of carboxyhaemoglobin that haemoglobin in diluted blood and blood at slightly alkaline pH is carboxylated more quickly than haemoglobin in undiluted blood and blood at slightly acidic pH.

CARBON MONOXIDE (CO) is frequently encountered in forensic toxicology in accidental or intentional victims of fire, exhaust fumes, etc. Haemoglobin (Hb) containing bound CO, carboxyhaemoglobin (HbCO), is relatively stable, the binding constant for CO being 200–300 times that for oxygen[1–5]. CO in chemical combination with Hb in blood prevents oxygenation of cells throughout the human body. In the present study we have determined reactivity of CO with Hb in undiluted and diluted blood samples at different pH's by spectrophotometric estimation of HbCO.

Fresh blood was collected from a healthy adult in a heparinized tube. Two ml of this blood (12 g% Hb) was taken and CO gas was passed for different time periods between one second and 30 min at the rate of 0.5 ml (three bubbles) per second. Again 0.2 ml of fresh blood was taken and made up to 2 ml (10-fold dilution) with 0.4% ammonium hydroxide. Serial dilutions were then prepared, up to 100-fold. CO was passed for different time periods. Undiluted and diluted (with 0.4% NH_4OH at different pH's between 6.5 and 8.0) post-mortem blood was subjected to carboxylation in the same way.

Carbon monoxide was prepared from oxalic acid and sulphuric acid. Oxalic acid was taken in a round-bottom flask fitted with a cork with two inlets. One had a plain glass tube dipped into the solution of oxalic acid and sulphuric acid while other was L-shaped, so as to enable the collection of gases. As carbon dioxide is also formed during this reaction, the evolved gases were passed through potassium hydroxide solution to absorb carbon dioxide.

Carboxyhaemoglobin in the test samples was determined spectrophotometrically in a simulated double-beam spectrophotometer (Beckman DU-64) following the method of Siek and Rieders[6]. The formula used for calculating %HbCO was:

$$\%\text{HbCO} = \left[\frac{A_{530} - A_{583}}{4.2} \times \frac{E^*}{A_{max}}\right] \times 100,$$

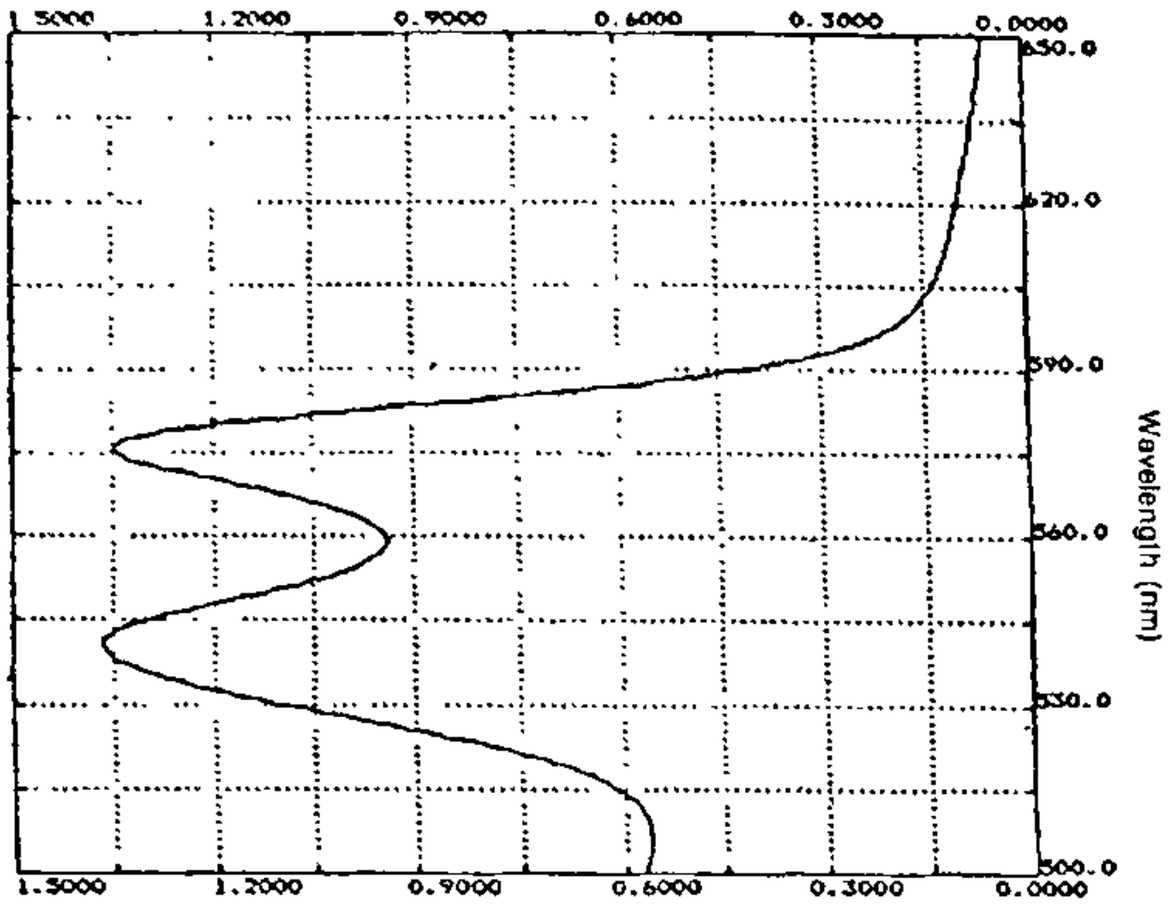

Figure 1. Absorbance spectrum of oxyhaemoglobin in the range 500 to 600 nm.

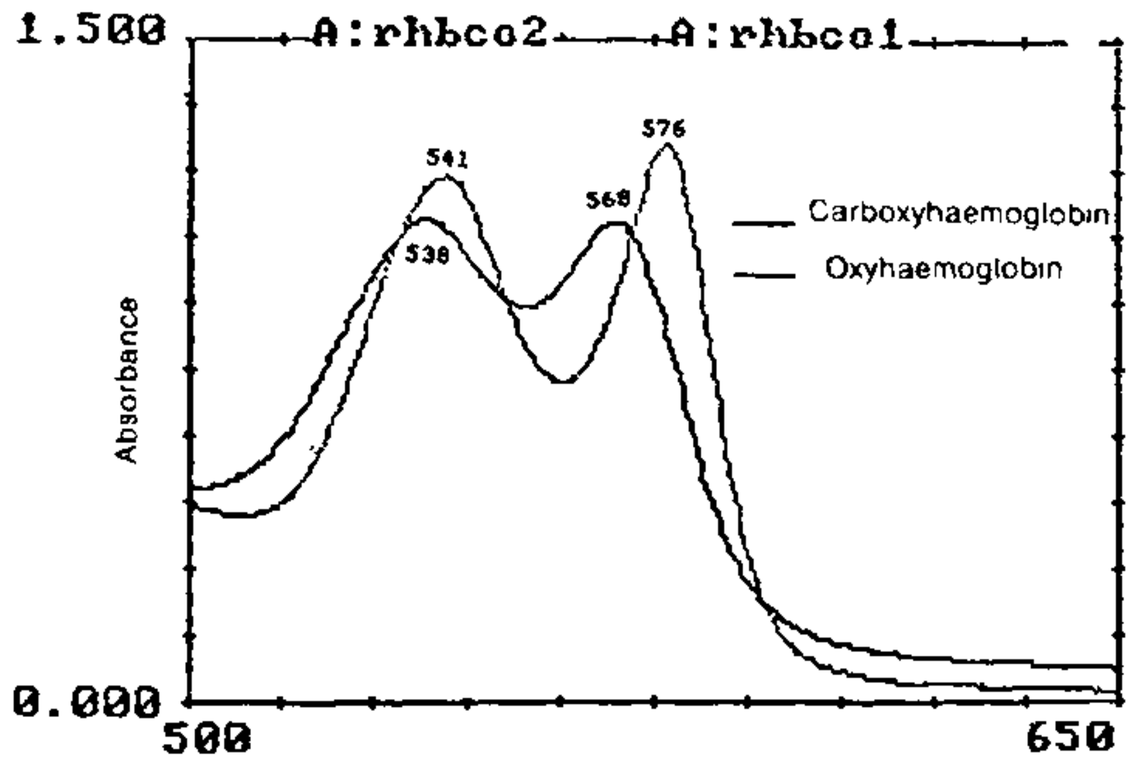

Figure 2. Shifts in absorbance spectrum of haemoglobin with the formation of carboxyhaemoglobin.

where A_{max} is absorbance at 576 nm and E^* is a composite constant that changes with carboxylation of blood, i.e. goes from 9.0 to 8.6 when HbCO goes from 10% to 100%.

Blood was diluted 100-fold with 0.4% NH_4OH and taken in a silica cuvette, and 5 mg of sodium dithionite was added. Absorbance over the range 500 to 600 nm was recorded against a 0.4%-NH_4OH blank (Figures 1 and 2).

The percentage of HbCO in blood was found to increase in direct proportion to the volume of CO passed. It was also found to vary with dilution of the blood.

Table 1. Effect of dilution of blood on formation of carboxyhaemoglobin.

	HbCO formed (%) after passage of CO for							
Dilution	1 Sec.	2 Sec.	3 Sec.	6 Sec.	10 Sec.	20 Sec.	30 Sec.	33 Sec.
0	2	5	10	18	30	75	95	100
10	12	30	45	72	100			
50	20	46	78	100				
100	28	52	100					

Table 2. Effect of pH on formation of carboxyhaemoglobin.

CO passed, in bubbles (in ml)	HbCO formed (%) at pH				
	6.5	7.0	7.2	7.7	8 0
	7	8	8	8	8
50(8 3)	42	72	75	85	87
100(16.6)	55	92	99	100	100
150(25.0)	56	92	99	100	100
200(33.3)	54	90	100	100	100
250(41.6)	56	96	100	100	100
300(50.0)	58	98	100	100	100

Hb in blood diluted 100-fold was completely carboxylated within 3 sec when carbon monoxide was passed at the rate of 0.5 ml per second (Table 1). In 10-times diluted blood, only 45% HbCO could be formed in 3 sec, while it took 10 sec for formation of 100% HbCO. In undiluted blood it took 33 sec for formation of 100% HbCO. This confirms the view of earlier workers that it takes a few seconds for blood to get 100% HbCO and not 30 min or so when carbon monoxide is passed through a 0.4% NH_4OH solution of blood[7].

Table 2 shows the effect of pH on rate of carboxylation of Hb. The results clearly indicate the role of pH in the reactivity of CO with Hb.

The affinity of Hb for CO is highest in the lung, where it is only the basement membrane that intervenes between inspired CO and circulating blood.

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