J. Pharm. Pharmac., 1978, **30**, 20–26 *Received June* 28, 1977

Red cell cyanide

CYRIL J. VESEY† AND JOHN WILSON*

*Anaesthetics Research Laboratory, The Royal Hospital of Saint Bartholomew, West Smithfield, London EC1A 7BE and *The Hospital for Sick Children, Great Ormond Street, London, WC1N 3JH, U.K.*

The amount of cyanide released on acidifying whole blood was much greater than the total from the plasma and red cells assayed separately, and varied directly with plasma thiocyanate concentrations. This artifactual formation of cyanide by whole blood was reduced by exposure to carbon monoxide. Incubation of haemolysates of washed red blood cells showed that optimum cyanide production from thiocyanate occurred at pH 4·5 with none at pH 7·4; spectrophotometric study confirmed the involvement of haemoglobin. It is doubtful whether this activity of haemoglobin is of normal physiological importance and it is probable that the evidence for the enzyme, 'thiocyanate oxidase' was based on this artifact of assay. Plasma cyanide is probably the metabolically relevant measurement, since the red blood cells act as a 'cyanide sink'. Where values for whole blood cyanide are required we recommend separate assay of plasma and saline washed red blood cells.

Cyanide (HCN) is found in traces in plasma, urine and expired air and is derived exogenously from vegetable sources and tobacco smoke. It is converted to thiocyanate (SCN), an anion also present in body fluids, by the irreversible action of rhodanese (E.C. 2811) a mitochondrial enzyme most abundant in liver and kidney (Baumeister, Schievelbein & Zickgraf-Rüdel, 1975). Employing ^{14}C labelled SCN, Boxer & Rickards (1952) demonstrated its endogenous conversion to HCN to produce an equilibrium ratio of SCN to HCN, in body fluids, of 1000:1. The toxic effects of large doses of SCN were attributed to its conversion by an erythrocytic enzyme, 'thiocyanate oxidase' (Goldstein & Reiders, 1951; Pines & Crymble, 1952; Goldstein & Reiders, 1953), which may be identical with oxyhaemoglobin (Chung & Wood, 1971). The physiological significance of 'thiocyanate oxidase' is as uncertain as the *in vivo* formation of HCN from SCN by a number of better characterized eyzmes - salivary lactoperoxidase (Oram & Reiter, 1966; Chung & Wood, 1970), myeloperoxidase in neutrophils (Sorbo & Llungren, 1958), thyroid peroxidase (Maloof & Soodak, 1964) and glutathione transulphurase, which produces HCN from organic thiocynates (Ohkawa, Ohkawa & others, 1972). An observation that whole blood cyanide values, determined by acidifying the sample and aerating, were greater than the sum of the plasma and red blood cell concentrations measured separately, led to re-investigation of so-called 'thiocyanate oxidase' in red cells.

† Correspondence.

MATERIALS AND METHODS

Analar grade chemicals were used and all solutions were prepared with deionized water. Potassium thiocyanate (KSCN) solutions were free of HCN. Stock trichloroacetic acid (TCA, 100 g in 100 ml solution) was kept at 4° and a 10% working solution prepared as required. Buffer solutions were iso-osmolar phosphate pH 7·4 (Hendry, 1961) and citrate-phosphate over the range pH 2·3–7·4 (McIlvaine, 1921). Freshly taken heparinized blood, obtained by standard techniques, was used for all experiments but one, when out-dated transfusion blood was used.

HCN and SCN determination (Vesey, Cole & Simpson, 1976)

Plasma SCN was determined in 10% TCA extracts by the method of Aldridge (1945). HCN was separated according to Boxer & Rickards (1951). Plasma, haemolysed cells or whole blood were acidified (3:1 v/v acid:sample) and the liberated HCN collected in 0·2 N NaOH by aeration with oxygen-free N_2. A droplet trap containing dilute H_2SO_4 was interposed between the sample and the sodium hydroxide to prevent contamination, and frothing was controlled using silicone oil. Trapped HCN was determined as for SCN according to Aldridge (1945).

Red cell HCN

Separated red cells were usually washed six times with refrigerated 0·9% NaCl and centrifuged at 0°. The saline, together with the buffy layer, was removed by means of a Pasteur pipette attached to

a water vacuum pump, and the packed red cell volume determined by micro-haematocrit. Although the washed cells could be stored for up to a week at 4° or −20° without significant losses of HCN, they were usually analysed immediately after saline washing. An aliquot of the washed cells was transferred to an aeration tube and haemolysed with an equal volume of water. Three volumes of 10% TCA were rapidly added and the tube well shaken, stood for 10 min and then aerated with N_2 to separate HCN. Whole blood was treated similarly. Haemoglobin was determined by conversion to cyanmethaemoglobin with Kampen and Zylstra's reagent (Dacie & Lewis, 1968).

Experimental procedures

Recovery of HCN added to whole blood. (Tables 1 and 2)

Weighed amounts of potassium cyanide (KCN) were dissolved in saline and analysed directly by the Aldridge method. The saline KCN was added to whole blood in a stoppered glass tube, mixed and incubated at 37° or at room temperature. The whole mixture or aliquots were then centrifuged at 0°, the cells washed with saline and the plasma and red cells separately assayed for cyanide.

KCN solution was adjusted to pH 7–7·4 with dilute acetic acid, the concentration accurately determined and 1–1·5 ml (i.p.) given to rats. The animals were then anaesthetized with pentobarbitone and blood withdrawn by cardiac puncture 12 min after cyanide administration.

In dogs, anaesthetized with pentobarbitone and N_2O, 2 ml of a saline KCN solution of accurately known concentration was given as a bolus dose via a cannula in the femoral vein and blood samples were obtained from a superficial femoral artery.

Production of HCN on acidification of whole blood (Table 3 and Fig. 1)

Either saline or a saline solution of KSCN was added to samples of whole blood in the proportions of 1 vol of saline to between 10–50 volumes of blood. After thorough mixing some plasma was separated for SCN determination. HCN in aliquots of the whole blood mixtures or in the isolated and saline washed red cells was measured after acidification with 10% TCA (or 0·5 N H_2SO_4, see Fig. 1). Conversion of the haemoglobin to carboxyhaemoglobin was achieved by mixing 5 ml of blood in a syringe successively with three 15 ml volumes of CO under anaerobic conditions. The sample was then acidified by introducing 3 volumes of 10% TCA into the syringe via a 3-way tap, under an atmosphere of CO. After thorough mixing, the contents of the syringe were aerated in the normal way.

Incubation of red cells with SCN at pH 7·4 (Tables 4 and 5)

Freshly obtained saline washed red cells were mixed with an equal volume of isotonic phosphate buffer containing a known amount of KSCN. After incubation of the mixture at 37°, HCN was determined in the mixture and in the isolated and saline-washed red cells using 10% TCA. In one experiment the red cell-buffer mixture was exposed to CO and acidified with TCA as described for blood.

Incubation of haemolysates with SCN at various pH levels (Fig. 2)

Haemolysates were prepared from fresh saline washed cells and adjusted to give a haemoglobin concentration of 100 g litre^{-1}. 0·2 ml of this solution was added to a mixture of 9·5 ml citrate-phosphate buffer (McIlvaine, 1921) and 0·3 ml of 0·3 M KSCN in a stoppered aeration tube and incubated for 30 min at 37°. After incubation the mixtures were aerated without acidification.

Studies of the changes in the absorption spectrum of haemoglobin incubated with SCN (Fig. 3)

Haemolysates of washed red cells were shaken with a small amount of toluene and centrifuged. The toluene and interface were removed, the extraction repeated and the solution adjusted to give a concentration of 100 g Hb litre^{-1}. In two quartz cells of 10 mm pathlength were placed 3 ml of citrate-phosphate buffer pH 4·5 and 0·1 ml of 0·3 M KSCN. In the sample cell 50 μl of haemolysate was added. The solutions before mixing and the cuvettes used were maintained at 37°. The effect of the addition of 0·2 ml, 0·6 M potassium ferricyanide was compared with that of SCN.

RESULTS

Recovery of HCN from blood

80–90% of the HCN added to whole blood was recovered from the separated plasma and red cells after incubation for 5–180 min (Table 1). At low HCN concentrations most (93–99% of that recovered) was present in the red cells but at higher concentrations the proportion (depending on the period of incubation) remaining in the plasma was increased. Similarly *in vivo*, after bolus doses of KCN, most HCN was found in the red cells. In adult rats given 0·15–3 mg KCN kg^{-1} (i.p.), 92–

Table 1. *Recovery of HCN from blood incubated with KCN.*

KCN added (μmol)	Incubation time (min)	Incubation temp. (°C)	% of added HCN recovered from RBC	% of added HCN recovered from plasma
10 ml whole blood				
0·004	30	37	71	5
0·029	30	37	84	2
0·029	5	20	84	1
0·210	30	20	78	3
0·235	15	20	93	5
0·235	15	20	99	4
5 ml saline washed RBC in 5 ml saline				
0·029	30	37	83	—
0·032	60	37	84	—
50 ml whole blood, samples taken at intervals				
11·0	0	37	54	31
	60		70	21
	120		95	11
40 ml whole blood, samples taken at intervals				
31·0	0	37	32	53
	60		40	40
	120		54	31
55 ml whole blood, samples taken at intervals				
55·0	0	37	17	73
	60		32	54
	120		46	39
	180		61	30

97% of the total blood HCN was in the saline washed red cells of blood samples taken 12 min later by heart puncture. In anaesthetized dogs, given approximately 1 mg KCN kg^{-1} (i.v.), 75–99% of the HCN recovered from the blood was in the saline-washed red cells, the proportion present increasing with time after the KCN dose. Representative results are shown in Table 2.

The artifactual formation of HCN from SCN

On acidification, samples of whole blood gave larger amounts of HCN than the total obtained when the plasma and red cells were separately assayed. Washing the red cells free of plasma with saline, a procedure that was demonstrated not to produce any loss in red cell HCN (see above), further reduced the yield of HCN on acidification of the separated red cells (Table 3). Since plasma contains only very minute amounts of HCN these observations suggested that a constituent of plasma was converted to HCN on acidification of whole blood. The data in Table 3 showed that the highest whole blood HCN yields were obtained in the samples with the highest plasma SCN concentrations. Thus the HCN obtained from whole blood

Table 2. *Distribution of HCN in the blood of rats and dogs following bolus doses of KCN.*

Animal (wt kg)	KCN given (μmol kg^{-1})	Time of blood sample (min after KCN)	HCN content of blood RBC (μmol $litre^{-1}$)	RBC (% blood HCN)	plasma (μmol $litre^{-1}$)
Rat, control	—	—	0·4	80	0·1
Rat, control	—	—	0·46	82	0·1
Rat (0·337)	2·17	12	1·33	97	0·04
Rat (0·386)	4·46	12	1·76	92	0·16
Rat (0·355)	16·1	12	24·2	93·5	1·69
Rat (0·434)	13·4	60	4·2	100	0
Rat (0·500)	45·4	12	95·0	95·5	4·75
Dog (21)	—	pre	0·56	85	0·1
	14·77	2	92·7	88	12·8
		5	85·7	92	7·6
		10	81·4	97	2·14
		20	67·8	98·4	1·1
Dog (16·75)	—	pre	0·27	100	0
	16·45	2	123·53	94	8·17
		5	121·39	97	4·33
		10	115·82	99	1·38
		20	99·43	99	1·03
Dog (25·9)	—	pre	0·24	86	0·04
	16·77	2	134·97	75	45·7
		5	141·67	86	23·65
		10	112·05	97·5	2·86
		20	71·47	99·1	0·63

appears to be directly related to the plasma SCN concentration. Exposure of the blood to CO, before acidification, resulted in a reduction in the amount of HCN obtained from the whole blood of *ca* 70% (Table 3), confirming the artifactual origin of the major part of the whole blood HCN and the involvement of the red cell in the mechanism.

Table 3. *The effect of the separation and washing of red cells (RBC), or exposure to carbon monoxide, on the cyanide yield of blood.*

Sample analysed	Plasma SCN (μmol $litre^{-1}$)	Plasma HCN (μmol $litre^{-1}$)	Whole blood HCN (μmol $litre^{-1}$)	RBC HCN (μmol $litre^{-1}$)
Fresh human blood	44	0·2	4·88	
Separated unwashed cells				3·04
Cells washed twice				0·88
Cells washed 6 times				0·20
Fresh human blood	21	0	0·96	
Cells washed 6 times				0·16
Transfusion blood	14	0	1·52	
Cells washed 6 times				0·20
Fresh rat blood	50	0·1	4·1	
Cells washed 6 times				0·46
Fresh human blood	37	0·05	2·15	
Cells washed 6 times				0·20
Blood exposed to CO			0·30	
With added SCN	600	0·05	12·0	
Cells washed 6 times				0·68
Blood exposed to CO			3·3	
Fresh human blood	28	0·2	0·8	
Cells washed 6 times				0·36
Blood exposed to CO			0·76	
With added SCN	631	0·2	16·8	
Cells washed				0·43
Blood exposed to CO			5·7	

Addition of KSCN to whole blood confirmed the direct relation between the HCN yield and the plasma SCN concentration (Fig. 1). This effect was independent of the time between the addition of KSCN and acidification and occurred with either 0·5 N H_2SO_4 or 10% TCA. The effect was also obtained with acid solutions of higher concentration. TCA gave higher yields of HCN than the H_2SO_4. The amounts of HCN obtained from the same sample of blood was variable and the variation seemed to be related to the speed of addition of the acid and subsequent mixing.

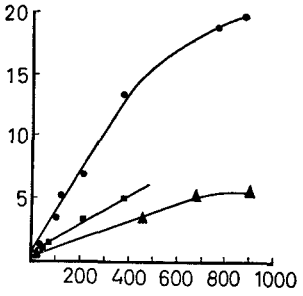

FIG. 1. HCN formation on adding acid to blood containing thiocyanate. Varying amounts of KSCN in physiological saline were added to aliquots of fresh rat or human blood and immediately acidified with 3 volumes 0·5 N H_2SO_4 or 10% TCA. Cyanide formed was measured by combining the aeration technique of Boxer & Rickards (1951) with the spectrophotometric method of Aldridge (1945), ■ rat blood, ▲ human blood, acidified with 0·5 N H_2SO_4, ● human blood acidified with 10% TCA. Ordinate: Whole blood HCN (μmol litre^{-1}). Abscissa: Plasma SCN (μmol litre^{-1}).

After incubation of red cells with KSCN in isotonic pH 7·4 buffer, and subsequent acidification of the entire mixture with TCA, HCN was obtained but showed no linear increase with incubation time (Table 4). When the cells were separated from the buffer mixture and washed with saline the yield of cyanide was reduced to near pre-incubation values. However, when very high concentrations of KSCN were used the HCN obtained on acidification of the whole mixture showed a rise after incubation for 1 h. In addition, significant amounts of HCN were obtained from the cells after their separation and washing with saline in the usual way. Further washing of the cells reduced the HCN yield in parallel with the decrease of SCN concentration in the saline washings (Table 5).

The effect of pH on the formation of HCN from SCN by haemolysates

The higher yield of HCN from whole blood acidified with TCA compared with blood acidified with H_2SO_4 suggested that there might be a pH effect. Although the pH of the mixtures obtained with both TCA and H_2SO_4 showed little difference (pH 1·4 and 1·2 respectively) it was possible that there was a slower fall in pH within the particles of coagulated protein produced by the TCA.

Table 4. *HCN obtained on incubation of washed red cells with SCN in isotonic phosphate buffer pH 7·4 at 37°.*

	Incubation time (min)	SCN concentration (μmol litre^{-1} of buffer)	HCN obtained (nmol g^{-1} Hb)
	0	30	100
	10		101
	20		115
	30		85
	270		107
	0	0	0·7
	20	30	108
	40		118
Cells separated and washed	40		3·7
	0	0	0·8
	0	30	78
	10		84
	20		76
	30		98
Mixture exposed to CO	30		7·7
	0	0	1·8
	0	30	119
Cells separated and washed	0		1·2
	360	0	0·8
	360	30	105
Cells separated and washed	360		2·4
Cells separated and washed	0	15 000	3·3
Cells separated and washed	60		10·0
	60		2240

Table 5. *The effect of extra saline washing on the HCN obtained from red cells (RBC) incubated with high concentrations of SCN (20 mM) at pH 7·4.*

	Incubation time at 37° (min)	SCN concentration of last saline washing (μmol litre^{-1})	HCN obtained (nmol g^{-1} Hb)
Fresh saline washed RBC before incubation with SCN	—	—	1·2
Buffer RBC/SCN mixture	0	—	1925
RBC, saline washed 6 times	0	35·4	16
Buffer RBC/SCN mixture	60	—	2288
RBC, saline washed 6 times	60	38·0	20·3
RBC, saline washed 10 times	60	3·1	6·3
RBC, saline washed 12 times	60	1·4	3·5

Incubation of haemolysates of washed human, rat and horse red cells with KSCN in citrate-phosphate buffer over a pH range of 2·3–7·4 for 30 min and isolation of any HCN formed by aeration without adding acid, indicated that optimum production of HCN took place at pH 4·5. There was also another pH optimum below 2·3 for horse haemolysates (Fig. 2). Freshly prepared haemoglobin gave similar results. In four control experiments in which KSCN was replaced by 0·3 ml of 100 μM KCN recovery of HCN ranged from mean 78 s.d. 8·7% at pH 7·4 to 92 s.d. 12·5% at pH 2·3.

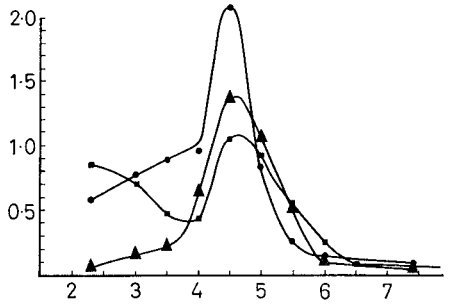

FIG. 2. HCN formation by haemolysates incubated at 37° with KSCN at various pHs. In aeration tubes 9·5 ml phosphate-citrate buffer of various pHs (McIlvaine, 1921) were mixed with 0·3 ml 0·3 M KSCN. 0·2 ml haemolysate (containing 10 g Hb/100 ml) was then added to each, the tubes stoppered and the mixtures incubated for 60 min at 37°. The HCN formed was removed without acidification in a stream of nitrogen. Haemolysate prepared from fresh, saline washed: human cells●; rat cells ▲; horse cells ■. Ordinate: μmol HCN g^{-1} haemoglobin. Abscissa: pH.

Under anaerobic conditions and also after exposure of the haemolysates to CO there was decreased formation of HCN, whilst preincubation of haemolysates at pH 4·5 for 30 min decreased or destroyed the activity. At pH 7·4 no HCN was generated even with raised SCN and haemoglobin concentrations, and prolonged incubation times. Methaemoglobin prepared by the action of ferricyanide and incubated with KSCN in buffer showed no net production of HCN.

Changes in the absorption spectrum of oxyhaemoglobin on incubation with SCN at pH 4·5

The effect of CO on the formation of HCN from SCN by blood on acidification and by haemolysates at pH 4·5 suggested that haemoglobin was involved in the reaction.

On incubation of haemolysates with KSCN at pH 4·5, it was observed from the absorption spectrum of the haemoglobin in the visible region that changes occurred similar to those produced by ferricyanide under the same conditions (Fig. 3). Incubation without KSCN at this pH for 30 min produced a slow but incomplete loss of oxygen with no formation of methaemoglobin.

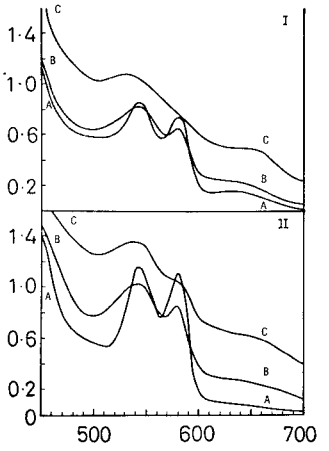

FIG. 3. Absorption spectrum of oxyhaemoglobin incubated in the presence of SCN or ferricyanide at pH 4·5 and 37°. Cell free haemolysates were prepared from freshly separated and saline washed rat or human cells (10 g Hb/100 ml). 10 mm pathlength quartz cells were used at 37°. The reference cell and sample cell both contained 3 ml phosphate-citrate buffer and either 0·1 ml 0·3 M KSCN or 0·2 ml 0·6 M $K_3Fe(CN)_6$. 0·05 ml of haemolysate (100 g Hb $litre^{-1}$) was added to the sample cell. Trace I $K_3Fe(CN)_6$ scanning at 0, 3 and 15 min (A, B, C). Trace II KSCN scanning at 0, 10 and 15 min (A, B, C). Ordinate: Absorbance. Abscissa: Wavelength (nm).

DISCUSSION

Goldstein & Reiders (1951) reported HCN in the blood of dogs poisoned with sodium thiocyanate and also in the blood of patients receiving SCN for the measurement of extracellular fluid space. The HCN appeared to be associated with the red cells. Pines & Crymble (1952) likewise found HCN in the blood of patients given SCN and demonstrated its association with the red cells of rabbit or human blood mixed with SCN and analysed 'within 10 minutes'.

After experiments in which washed red cells or their haemolysates were incubated with high concentrations of SCN (0·1 M) Goldstein & Reiders (1953) concluded that there was an enzyme present in the cells that converted thiocyanate to HCN at a pH optimum of 7·4 and named it 'thiocyanate oxidase'. Both Goldstein & Reiders (1951; 1953) and Pines & Crymble (1952) used the method of

Gettler & Goldbaum (1947) for the assay of HCN. In this method blood samples are acidified with 20% TCA, heated to 90° and aerated and the HCN measured by formation of Prussian blue.

On the basis of our findings that HCN is generated on acidification of whole blood, red cells or haemolysates, containing SCN, we suggest that these workers were measuring artifactually produced HCN, particularly since high concentrations of SCN were used and cells were not isolated and washed before assay. This is confirmed by our observations that incubation of red cells with SCN at pH 7·4 and acidification of the entire incubation mixture gave HCN but when the cells were isolated and washed free of SCN, a procedure in which no significant losses of HCN from cells occurred, little HCN was present.

When high concentrations of SCN were used, incubation of red cells at pH 7·4 resulted in an increase in HCN with time. In addition the saline-washed cells appeared to contain HCN. Further washing of the cells with saline resulted in a fall in the HCN obtained as the amount of SCN in the washings decreased. This suggests that at high SCN concentrations the anion enters or becomes attached to the cells during incubation, giving an increase in artifactually formed HCN on adding TCA, and is removed from the cell only with prolonged saline washing. The fact that haemolysates of washed cells incubated at pH 7·4 with SCN and aerated without prior acidification gave no HCN seems to confirm this interpretation. The more recent and anomalous findings of Smith & Kruszyna (1976) may also be similarly explained (Vesey, Cole & Simpson, 1977). These workers reported that in mice dosed with SCN (6·2 mmol kg^{-1}) blood HCN concentrations of 190 ± 148 μM were obtained. These values were double the blood HCN concentrations for animals given a lethal dose of KCN and yet HCN antidotes such as thiosulphate or nitrite had no effect on the mortality of the SCN-dosed animals. The explanation was that the HCN arising *in vivo* from the SCN was bound in a biologically inactive form. Since their assay procedure for HCN involved acidification of the blood samples with TCA (Ohkawa & others, 1972; Smith & Kryszyna, 1974) their findings are more readily explained by the artifactual formation of the HCN on assay. This would also explain the large standard deviation in their results, since the amounts of HCN generated by whole blood containing high concentrations of SCN will vary with the rate of addition of the acid and efficiency of mixing after acidification.

Although HCN was not produced on incubation of red cells, or their haemolysates, with SCN at pH 7·4, we found that haemolysates produced HCN at lower pH values with an optimum at pH 4·5. This confirms an earlier finding of Brin (1955). That anaerobic conditions or the exposure of blood or haemolysates to CO reduced the amounts of HCN produced led us to suspect that haemoglobin was involved in the reaction. The spectrophotometric changes observed during incubation with SCN seemed to confirm this. Similar findings were reported by Chung & Wood (1971) who showed that both haemoglobin and methaemoglobin, in the presence of hydrogen peroxide produced HCN from SCN. In the absence of added hydrogen peroxide we found that haemoglobin produced HCN although there was no net production of HCN by methaemoglobin. The spectrometric study of the changes in oxyhaemoglobin on incubation with SCN at pH 4·5 shows that it is converted into methaemoglobin in the process of HCN formation. This is apparent on comparing the absorption spectrum in the visible region with that obtained on incubation with ferricyanide.

Since both the formation of carboxyhaemoglobin and incubation under anaerobic conditions reduce but do not completely prevent the formation of HCN from SCN by haemoglobin it is possible that two processes are involved.

With a pH optimum of 4·5 and no significant activity between pH 6–7·4, together with the seemingly low permeability of the red cells to SCN, it is doubtful whether this activity of haemoglobin has any physiological significance except perhaps at abnormally high SCN concentrations and/or abnormal red cell permeability. At pH 6 haemoglobin begins to dissociate into its individual chains. This process is complete at pH 4·8 (Schroeder, 1963) which is close to the optimum for HCN formation. Thus the 'thiocyanate oxidase' activity of haemoglobin may well be associated with its monomeric form. If this is the case then myoglobin should show this property at a higher pH. Preliminary experiments with saline extracts of blood-free rat and bovine ventricular muscle demonstrated a 'thiocyanate oxidase' activity with a narrow pH optimum at 5·6. At this pH the bovine extract incubated with SCN in the same way as haemolysates, produced 2·2 μmol HCN g^{-1} protein. It remains to be shown whether haemoglobin, and possibly myoglobin, contribute to the formation of HCN under normal physiological conditions but their action may explain the production of HCN in

post-mortem material (Curry, Price & Rutter, 1967).

Since red cell HCN concentrations are normally low (smokers 0·6 s.d. 0·3 μmol HCN litre^{-1}, n = 10 and non-smokers 0·9 s.d. 1·0 μmol HCN litre^{-1}, n = 16, Vesey & others, 1976) whole blood HCN measurements, that entail acidification of the sample, will give misleadingly high and variable values which may mask any subtle differences due to a fault in HCN metabolism. This is particularly important where plasma SCN concentrations are elevated as in smokers (Pettigrew & Fell, 1973) in chronic HCN exposure (Osuntokun, Monekosso & Wilson, 1969; Osuntokun, Aladetoyinbo & Adeuja, 1970) and long-term infusion with sodium nitroprusside (Vesey, Cole & others, 1974).

In any determination of HCN concentrations after exposure, plasma values, because of their metabolic significance, are of major importance. Where whole blood concentrations are required we suggest a separate determination of the HCN in the plasma and in the washed red cells to avoid artifactual formation of HCN from plasma SCN. Where plasma SCN is high it is necessary to wash the cells with saline until the washings are free of the ion. It should be noted that any method for determination of SCN which employs a halogenation stage will give a positive result if there is protein present in solution, as may be the case if haemolysis occurs during the saline washing of the cells.

The separate determination of plasma and red cell HCN was first introduced in an investigation of blood HCN concentrations after infusions of the hypotensive agent sodium nitroprusside (Vesey & others, 1974). This subsequently demonstrated that most of the HCN present in the blood was located in the red cells (Vesey & others, 1976, 1977). This present study has also shown that HCN, which is virtually unionized at physiological pH (Izatt, Christensen & others, 1962) and lipid soluble, rapidly enters and accumulates in the red cells. Here it appears to have no toxic action and the red cells act as a 'cyanide sink' (Vesey & others, 1976).

Acknowledgements

This research was supported by a grant from the Wellcome Trust (C.J.V.) in the M.R.C. Clinical Genetics Research Unit, The National Hospital for Nervous Diseases and by the Joint Research Board of St Bartholomew's Hospital.

REFERENCES

ALDRIDGE, W. N. (1945). *Analyst*, **70**, 474–475.
BAUMEISTER, R. G., SCHIEVELBEIN, H. & ZICKGRAF-RÜDEL, G. (1975). *Arzneimittel-Forsch*, **25**, 4056–1064.
BOXER, G. E. & RICKARDS, J. C. (1951). *Archs Biochem. Biophys.*, **30**, 372–380.
BOXER, G. E. & RICKARDS, J. C. (1952). *Ibid.*, **39**, 7–26.
BRIN, M. (1955). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **14**, 186.
CHUNG, J. & WOOD, J. L. (1970). *Archs Biochem. Biophys.*, **141**, 73–78.
CHUNG, J. & WOOD, J. L. (1971). *J. biol. Chem.*, **246**, 555–560.
CURRY, A. S., PRICE, D. E. & RUTTER, E. R. (1967). *Acta pharmac. tox.*, **25**, 339–344.
DACIE, J. V. & LEWIS, S. M. (1968). *Practical Haematology*, p. 37. London: Churchill.
GETTLER, A. O. & GOLDBAUM, L. (1947). *Analyt. Chem.*, **19**, 270–271.
GOLDSTEIN, F. & REIDERS, F. (1951). *Am. J. Physiol.*, **167**, 47–51.
GOLDSTEIN, F. & REIDERS, F. (1953). *Ibid.*, **173**, 287–290.
HENDRY, E. B. (1961). *Clin. Chem.*, **7**, 154–164.
IZATT, R., CHRISTENSEN, J., PACK, R. & BENCH, R. (1962). *Inorg. Chem.*, **3**, 828–831.
MALOOF, F. & SOODAK, M. J. (1964). *J. biol. Chem.*, **239**, 1995–2001.
McILVAINE, J. C. (1921). *Ibid.*, **49**, 183–186.
OHKAWA, H., OHKAWA, R., YAMAMOTO, I. & CASIDA, J. E. (1972). *Pest. Biochem. Physiol.*, **2**, 95–112.
ORAM, J. D. & REITER, B. (1966). *Biochem. J.*, **100**, 373–388.
OSUNTOKUN, B. O., MONEKOSSO, G. L. & WILSON, J. (1969). *Br. med. J.*, **1**, 547–550.
OSUNTOKUN, B. O., ALADETOYINBO, A. & ADEUJA, A. O. G. (1970). *Lancet*, **2**, 372.
PETTIGREW, A. R. & FELL, G. S. (1973). *Clin. Chem.*, **19**, 466–471.
PINES, K. L. & CRYMBLE, M. M. (1952). *Proc. Soc. exp. Biol. Med.*, **81**, 160–163.
SCHROEDER, W. A. (1963). *Rev. Biochem.*, **32**, 301–320.
SMITH, R. P. & KRUSZYNA, H. (1974). *J. Pharmac. exp. Ther.*, **191**, 557–563.
SMITH, R. P. & KRUSZYNA, H. (1976). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **35**, 69–72.
SORBO, B. & LLUNGREN, J. G. (1958). *Acta chem. scand.*, **12**, 470–476.
VESEY, C. J., COLE, P. V., LINNELL, J. C. & WILSON, J. (1974). *Br. med. J.*, **2**, 140–142.
VESEY, C. J , COLE, P. V. & SIMPSON, P. J. (1976). *Br. J. Anaesth.*, **48**, 651–660.
VESEY, C. J., COLE, P. V. & SIMPSON, P. J. (1977). *Ibid.*, **49**, 395–396.