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# The effects of sodium nitroprusside and cyanide on haemoglobin function

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Incubation of human blood with 0·3 mM sodium nitroprusside (SNP) or 1 mM potassium cyanide (KCN) for 180 min produced a 600-fold increase in red cell cyanide concentration, a 4% decrease in oxygen capacity and a 6% increase in oxygen affinity. These effects were not reproduced in patients receiving SNP by infusion probably because in the clinical situation much smaller amounts of the drug were used and red cell cyanide levels were lower. The in vitro observations could be explained by cyanide either combining directly with the haemoglobin or encouraging its spontaneous oxidation to methaemoglobin during the incubation. Differences in distribution of cyanide between red cell and plasma following incubation with SNP or KCN and the initial lag phase in accumulation of cyanide in the red cell with SNP provide further evidence that nitroprusside breaks down principally within the red cell.

It has been demonstrated both in vitro and in vivo that cyanide (HCN) readily enters and accumulates in the red cell (Vesey & Wilson 1978). Also, following infusion of sodium nitroprusside (SNP) in man some 98% of the blood cyanide is to be found in the erythrocytes (Vesey et al 1976). The form in which the red cell cyanide exists and its effects on haemoglobin and red cell function are largely unknown although it has been suggested that cyanide may affect oxygen transport (Vesey et al 1974). A raised concentration of methaemoglobin in a patient was attributed to the action of SNP (Bower & Peterson 1975) while Smith & Kruszyna (1974) showed that cyanide is released when haemoglobin reacts with nitroprusside, the haemoglobin being converted to cyanmethaemoglobin in the process. Thus apart from the possible effect of cyanide itself the formation of cyanmethaemoglobin would obviously reduce the amount of haemoglobin available for oxygen transport, and could also affect oxygen affinity. A study was therefore carried out to assess the effect of SNP and KCN on the oxygen capacity and oxygen affinity of human blood in vitro, and to determine the effect of nitroprusside on these indices of haemoglobin function following SNP infusion in patients (Krapez et al 1977).

## METHODS

Heparinized blood samples were obtained from healthy volunteers immediately before the start of each in vitro experiment. After thorough mixing, 100 ml was dispensed equally between two stoppered foil-wrapped glass tubes. One ml of a saline solution of either SNP or KCN was then added to one of the tubes to give a final concentration of 0·3 mM and 1 mM respectively. An equal volume of 0·9% NaCl (saline) was added to the second tube as a control. The SNP and KCN concentrations were chosen to produce red cell cyanide concentrations 3 times greater than the highest found in a previous study of patients receiving SNP infusions (Vesey et al 1976). Each pair of tubes was continually shaken while immersed in a water bath at 37 °C. Aliquots were withdrawn after 0, 30, 60, 90, 120 and 180 min incubation and oxygen capacity, total haemoglobin and oxygen affinity were determined. Red cell cyanide concentrations were measured in each sample from the experimental blood, but since there was virtually no change in the low HCN concentrations in the control blood, cyanide estimations were confined to the 0 and 180 min samples. In the patient study heparinized blood samples were taken immediately before and at the end of SNP (Nipride) infusion, and the same determinations were carried out. None of the patients were transfused with stored blood.

A portion of each blood sample was immediately centrifuged at 0 °C for 10 min. The red cells were separated, washed with saline, and red cell cyanide determined as previously described (Vesey et al 1976; Vesey & Wilson 1978). Haemoglobin concentration was measured using a standard cyanmethaemoglobin technique. Oxygen capacity was determined using a Lex-$O_2$-Con oxygen content analyser (Lexington Instruments Inc., Adams & Cole 1975) following tonometry with room air, and oxygen

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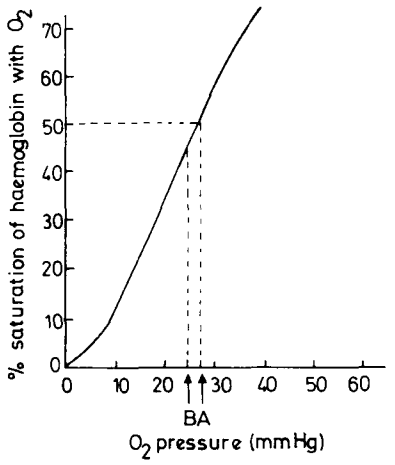


FIG. 1. Oxyhaemoglobin dissociation curve. A indicates the accepted normal value for P50 (the $Po_2$ at which the haemoglobin in normal adult blood is 50% saturated at 37 °C and pH 7·4 is 26·5 mm Hg). B marks the mean change in P50 produced in our experiments after incubating blood with either 300 μmol SNP litre$^{-1}$ or 1 mM KCN litre$^{-1}$ for 180 min and when rbc HCN reached 600 μmol litre$^{-1}$. This change contrasts with the reported normal range of P50 of 22·5–30 (Wilmslow et al 1978).

affinity was quantified by determining P50 (the partial pressure of oxygen required to produce 50% saturation of haemoglobin with oxygen at a pH of 7·4 and a temperature of 37 °C—see Fig. 1). A two point interpolation technique was employed (Astrup et al 1965) using paired 1L237 tonometers (Instrumentation Laboratories, U.K.), control and experimental samples being equilibrated simultaneously. A transmission oximeter (OSM.2 Radiometer Ltd.) was used to determine oxyhaemoglobin and an IL 413 blood gas analyser was used to measure pH.

## RESULTS

The results of the in vitro work are set out in Tables 1 and 2. Compared with the control, there was an overall fall in oxygen capacity and in P50 as incubation time and cyanide concentrations increased in the experimental samples. There was however an initial rise in P50, although not in oxygen capacity, in the 30 min sample during incubation with KCN. Statistical analysis was performed using a paired *t*-test to compare control and experimental results.

The difference in P50 (Δ P50) was found to increase with red cell cyanide concentration to the same degree for both SNP and KCN (Fig. 2A and B, r = 0·58 and 0·6 respectively $P < 0·001$). A similar relationship was found between the differences in oxygen capacity (Δ $O_2$ capacity) and red cell cyanide (Fig. 3 A and B, r = 0·55 and 0·69 respectively $P < 0·002$).

The results of the in vivo study are set out in Table 3. There was no significant change in P50 following SNP infusion, nor was there any alteration in oxygen capacity.

The mean increases (± s.e.m.) in red cell cyanide concentration with time during incubation with the SNP or KCN are shown in Fig. 4. Apart from the initial 30 min there was a parallel increase in rbc cyanide concentrations, reaching mean values of over 600 μmol litre$^{-1}$ in both cases after 180 min

Table 1. Mean red cell cyanide concentration, oxygen capacity and P50 results for blood samples incubated with sodium nitroprusside (0·3 mM).

| Incubation time (min) | Red cell cyanide conc. (μmol litre$^{-1}$ ± s.e.m., n = 7) SNP | Control | Oxygen capacity (vols % ± s.e.m., n = 7) SNP | Control | *t** | *P* | P50 (mm Hg ± s.e.m., n = 7) SNP | Control | *t** | *P* |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 59·2 ±9·3 | 0·72 ±0·15 | 21·3 ±0·63 | 21·3 ±0·62 | 0 | N.S. | 26·8 ±0·35 | 26·8 ±0·36 | 0·5 | N.S. |
| 30 | 266·0 ±20·9 | — | 20·9 ±0·58 | 21·0 ±0·58 | 2·46 | < 0·05 | 26·3 ±0·49 | 26·9 ±0·52 | 2·42 | N.S. |
| 60 | 369·6 ±29·7 | — | 20·6 ±0·64 | 20·9 ±0·68 | 3·42 | < 0·02 | 26·4 ±0·37 | 26·6 ±0·36 | 1·84 | N.S. |
| 90 | 416·6 ±27·7 | — | 20·6 ±0·62 | 21·0 ±0·66 | 4·70 | < 0·01 | 25·8 ±0·31 | 26·5 ±0·36 | 4·55 | < 0·01 |
| 120 | 493·7 ±40·4 | — | 20·5 ±0·62 | 21·0 ±0·68 | 3·20 | < 0·02 | 26·0 ±0·29 | 27·0 ±0·20 | 5·21 | < 0·01 |
| 180 | 622·9 ±43·0 | 1·25 ±0·25 | 20·2 ±0·61 | 21·1 ±0·68 | 7·75 | < 0·001 | 25·8 ±0·49 | 27·3 ±0·20 | 3·90 | < 0·01 |

* paired *t*-test

Table 2. Mean red cell cyanide concentration, oxygen capacity and P50 results for blood samples incubated with potassium cyanide (1 mM).

| Incubation time (min) | Red cell cyanide conc. μmol litre$^{-1}$ ± s.e.m., n = 7) KCN | Control | Oxygen capacity (vols % ± s.e.m., n = 6) KCN | Control | $t$* | $P$ | P50 (mm Hg ± s.e.m., n = 7) KCN | Control | $t$* | $P$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 185·0 ±8·2 | 0·58 ±0·1 | 20·8 ±0·77 | 20·7 ±0·68 | 1·28 | N.S. | 27·8 ±0·23 | 27·6 ±0·23 | 1·80 | N.S. |
| 30 | 271·0 ±9·7 | — | 20·6 ±0·64 | 20·5 ±0·59 | 1·31 | N.S. | 28·3 ±0·34 | 27·6 ±0·28 | 2·97 | < 0·05 |
| 60 | 331·0 ±8·2 | — | 20·5 ±0·62 | 20·9 ±0·68 | 3·3 | < 0·05 | 27·8 ±0·40 | 27·9 ±0·36 | 0·8 | N.S. |
| 90 | 397·0 ±12·7 | — | 20·2 ±0·60 | 20·8 ±0·75 | 2·23 | N.S. | 27·6 ±0·24 | 27·9 ±0·32 | 2·41 | N.S. |
| 120 | 475·0 ±18·1 | — | 20·0 ±0·56 | 20·7 ±0·69 | 3·95 | < 0·02 | 27·0 ±0·40 | 27·9 ±0·49 | 4·80 | < 0·01 |
| 180 | 596·0 ±41·2 | 1·24 ±0·23 | 19·8 ±0·51 | 20·7 ±0·63 | 5·75 | < 0·01 | 26·3 ±0·24 | 28·2 ±0·41 | 4·17 | < 0·01 |

* paired $t$-test

incubation (the nonparametric correlation coefficient $r_s = 0{\cdot}89$ and 0·96 respectively, $P < 0{\cdot}001$).

The means of the ratios of red cell to plasma cyanide concentrations (± s.e.m.) for the in vitro study are given in Fig. 5. The ratios are far higher for blood incubated with SNP.

## DISCUSSION

Incubation of whole blood with SNP (0·3 mM) at 37 °C reduces its oxygen capacity and increases its oxygen affinity (Table 1). Most, if not all of this effect seems to be due to cyanide within the red cell, since similar changes were produced when blood was incubated with KCN (Table 2). In addition red cell cyanide concentrations correlated well with these changes in both cases (Figs 2 and 3). KCN and SNP did not, however, produce completely identical effects. A significant rise in P50, although not in oxygen capacity, was seen in the first thirty minutes of incubation with KCN but the subsequent fall was essentially similar to that seen with SNP (Fig. 2). We have no satisfactory explanation for this increase apart from the possibility that it may be related to the higher initial concentration of red cell cyanide in blood incubated with KCN (Fig. 4).

Decreases in P50 and oxygen capacity are, in general, undesirable since they result in impaired oxygen release and reduced oxygen carriage. The measured reduction in P50 (the mean value fell by less than 2 mm Hg; Fig. 1) must however be compared with the wide normal range for P50 of 22–30 mm Hg (Wilmslow et al 1978). Furthermore the red cell cyanide concentrations associated with this fall were three times higher than any we have found in patients receiving short term infusions of SNP (Vesey et al 1976). Indeed, we calculate that an infusion of a dose of 300 mg SNP over 2–3 h would be required to produce similar cyanide concentrations.

Other workers (Hess et al 1978) have reported no effect of SNP on P50 or on the concentrations of 2,3-diphosphoglycerate (2,3-DPG) and methaemoglobin following in vitro incubation. However, a much lower concentration of the agent was used (10 μmol litre$^{-1}$) and the incubation was carried out for only 60 min. In our own study (using 300 μmol litre$^{-1}$) the alterations in P50 only achieved statistical significance after 90 min incubation.

None of the patients studied showed a significant change in P50 or oxygen capacity—but the highest red cell cyanide concentration measured was only 1/16th of that obtained in vitro. Much higher values would of course be expected during cyanide poisoning due to overdosage with SNP, and here impaired haemoglobin function could play a part. At least three deaths have been reported following SNP infusion where high doses were given (Davies et al 1975 (400 mg); Merrifield & Blundell 1974 (750 mg); Jack 1974 (750 mg)). In the latter two cases we would predict that both oxygen capacity and P50 would be markedly reduced and this may have played a part in the death of these patients.

Red cell cyanide concentrations increased relatively slowly after the initial rapid rise when blood was incubated with KCN (Fig. 4) and the red cell: plasma cyanide ratio remained low (Fig. 5). There-

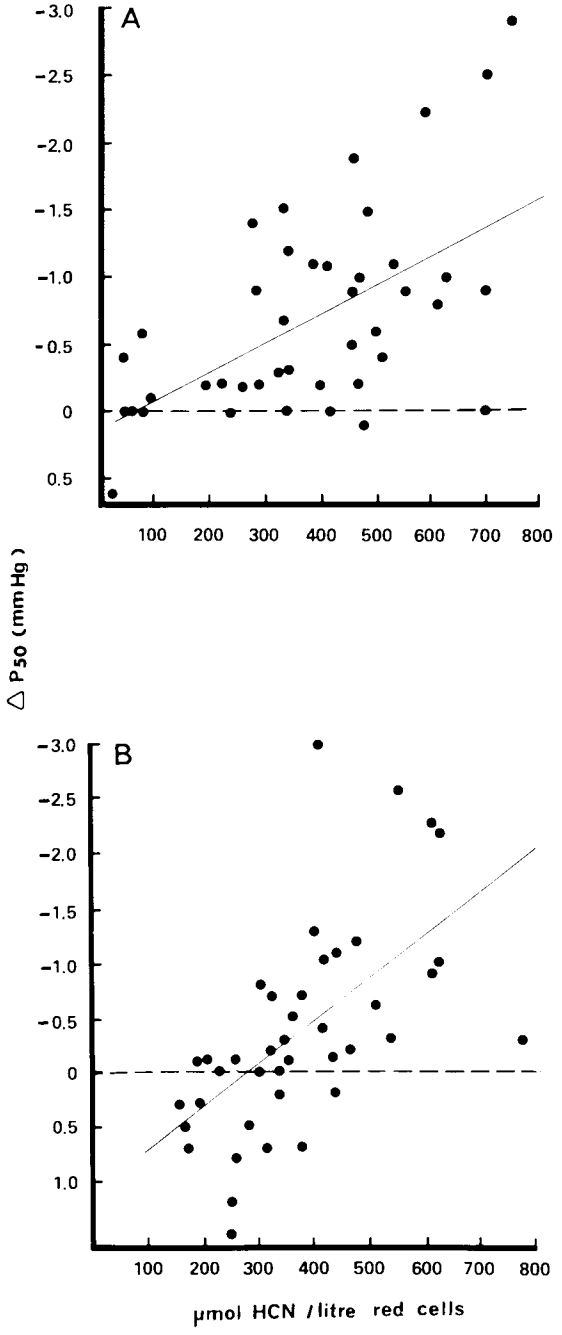


FIG. 2. Differences in P50, (△ P50) between the control blood and blood incubated for 180 min at 37 °C with either SNP (300 µmol litre$^{-1}$) or KCN (1 mM litre$^{-1}$) versus rbc cyanide concentrations. Samples were withdrawn and measurements made at 30 min intervals. A blood incubated with SNP r = 0·58 $P < 0·001$, n = 42, a intercept = −0·13, b slope = 0·0022. B blood incubated with KCN r = 0·6, $P < 0·001$, n = 42, a intercept = −1·0872, b slope = 0·00392.

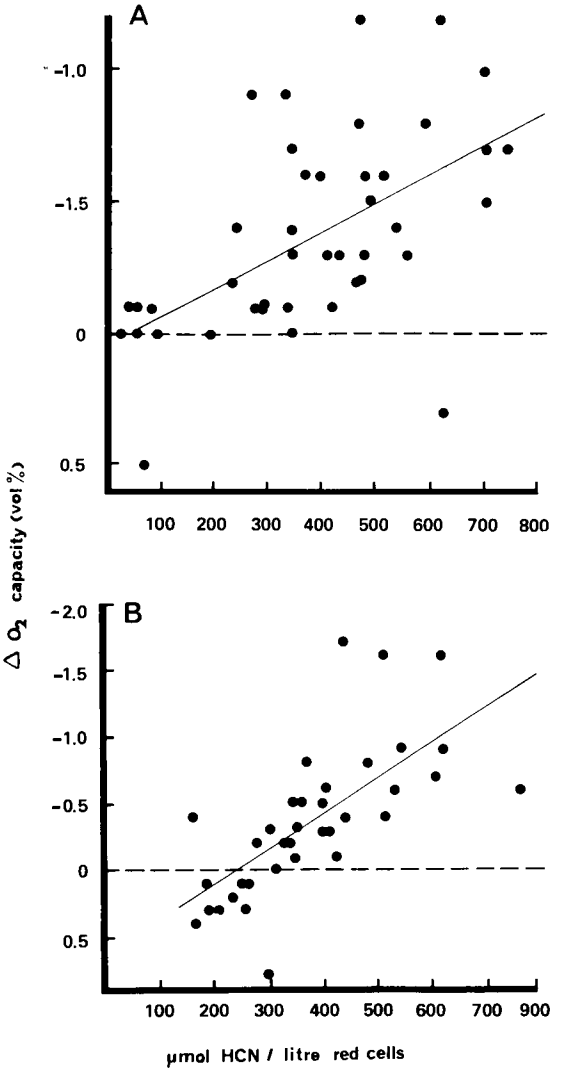


FIG. 3. Differences in oxygen capacity (△ $O_2$ capacity) between control blood and blood to which either 300 µmol SNP litre$^{-1}$ (A) or 1 mM KCN litre$^{-1}$ (B) was added and incubated at 37 °C for 180 min versus rbc cyanide. Samples were withdrawn and measurements made at 30 min intervals. A, r = 0·55, $P < 0·001$, a intercept = −0·0232, b slope = 0·00106, n = 42. B, r = 0·69, $P<0·001$, a intercept = −0·630, b slope = 0·0026, n = 36.

fore in cases of cyanide poisoning the patient probably succumbs to histotoxic effects before there is time for an alteration in haemoglobin function to occur.

The results of our work are also relevant to the study of Smith & Kruszyna (1974). These authors suggest that a reaction with haemoglobin provides the major pathway for SNP breakdown, each mole-

Table 3. P50 and oxygen capacity changes in patients receiving SNP by infusion.

| Patient No. | SNP dose (mg) | Duration of infusion (min) | Pre-infusion sample rbc HCN ($\mu$mol litre$^{-1}$) | P50 (mm Hg) | $O_2$ Cap. (vol %) | Post-infusion sample rbc HCN ($\mu$mol litre$^{-1}$) | P50 (mm Hg) | $O_2$ Cap. (vol %) |
|---|---|---|---|---|---|---|---|---|
| 1 | 14·6 | 70 | 0·47 | 26·0 | 18·1 | 23·8 | 26·0 | 18·3 |
| 2 | 14·7 | 70 | 0·79 | 27·0 | 16·8 | 43·8 | 27·0 | 16·8 |
| 3 | 28·7 | 60 | 0·25 | 23·0 | 19·7 | 45·4 | 24·5 | 19·8 |
| 4 | 6·3 | 101 | 0·45 | 24·5 | 16·1 | 7·36 | 24·0 | 15·6 |
| 5 | 5·0 | 20 | 0·1 | 27·5 | 17·1 | 0·79 | 28·2 | 19·2 |
| 6 | 7·8 | 80 | 0·34 | 26·6 | 21·5 | 5·2 | 27·0 | 20·1 |
| 7 | — | — | 1·6 | 27·8 | 18·0 | 25·1 | 27·1 | 18·0 |
| 8 | 17·6 | 135 | 1·8 | 28·7 | 19·2 | 47·5 | 28·3 | 19·2 |
| 9 | 24·0 | 80 | 0·49 | 29·5 | — | 97·0 | 30·3 | — |

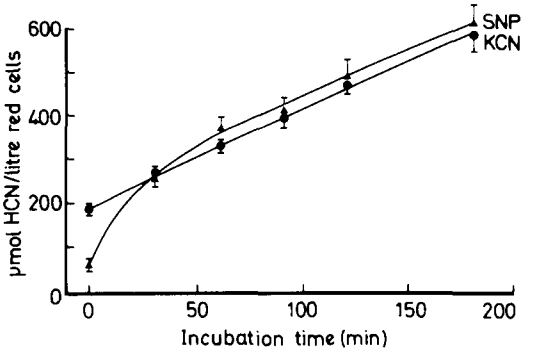


FIG. 4. Increase in red cell cyanide (mean ± s.e.m.) with time when fresh whole blood was incubated with either 300 $\mu$mol SNP litre$^{-1}$ or 1 mM KCN litre$^{-1}$ in foil wrapped tubes at 37 °C for 180 min. Samples were withdrawn at 30 min intervals. Each point represents the mean of 7 determinations. Non-parametric correlation coefficient: (a) for SNP $r_s = 0{\cdot}89$, $P < 0{\cdot}001$ (b) for KCN $r_s = 0{\cdot}96$, $P < 0{\cdot}001$.

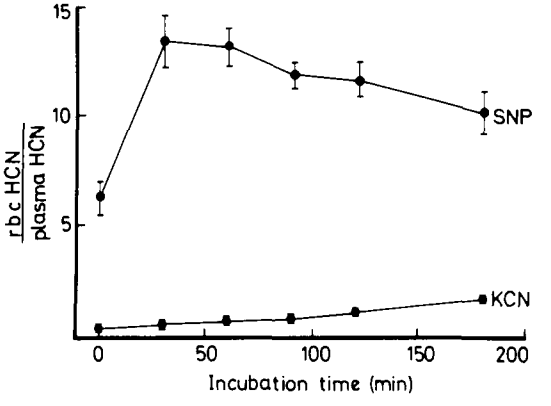


FIG. 5. Mean change in the ratio of red cell HCN: plasma HCN (± s.e.m.) with time in blood incubated with either SNP or KCN.

cule of nitroprusside converting one haemoglobin monomer to cyanmethaemoglobin and releasing four molecules of HCN. We calculate, from the data in Table 1, that 46% of the SNP was broken down after 180 min incubation. This, on the basis of the mechanism suggested by Smith & Kruszyna, should produce a 1·5% fall in oxygen capacity (due to cyanmethaeglobin formation), whereas the measured change was 3·9%. The fact that a similar fall in oxygen capacity (4·4%) occurred in the blood incubated with KCN suggests that the cyanide, rather than changes produced by the SNP molecule, is responsible for the effects on haemoglobin. Since the decrease in P50 was also of this order any explanation should account for both effects. A direct combination of cyanide with one or more amino acid residues in the globin moiety is one possibility. The consequent changes in the allosteric effects which haemoglobin undergoes during oxygenation would certainly alter P50 but interaction between the altered globin and the haem portion could conceivably reduce the oxygen capacity also. A number of other possible factors will alter P50 but without affecting oxygen capacity. An increase in the pH, a decrease in $P_{CO_2}$ and a decrease in the concentration of 2,3-DPG will all increase the oxygen affinity of haemoglobin (i.e. decrease the P50). Our technique employed for the determination of P50 maintains the samples at a constant $P_{CO_2}$ and includes a correction for pH changes (Astrup et al 1965) and these two factors are obviously not responsible. The oxygen affinity of haemoglobin is modulated by the binding of 2,3-DPG to special sites on the $\beta$ chains. It is possible, therefore, that HCN could interfere with this binding or inhibit one or more of the enzymes of the glycolytic pathway which are responsible for the production of 2,3-DPG within the red cell.

The formation of cyanmethaemoglobin, however,

still seems a plausible explanation since it will obviously reduce oxygen capacity and also methaemoglobin (Brewer 1972)—and presumably cyanmethaemoglobin—is known to decrease P50. Methaemoglobin formed by spontaneous oxidation during incubation could be trapped as cyanmethaemoglobin. In the control samples it would be reduced by the enzyme system NADH methaemoglobin reductase present in the red cell.

A report by Bower & Peterson (1975) described a patient who had a blood methaemoglobin concentration of 16% following an infusion of 321 mg SNP. On the basis of the mechanism suggested by Smith & Kruszyna (1974) this quantity of nitroprusside should theoretically produce only 2·3% methaemoglobin. An alternative explanation for this very high methaemoglobin concentration must exist, particularly as the nitroprusside was infused over the course of four days. It is possible that, in the presence of an enzyme defect, prolonged exposure to the low concentrations of red cell cyanide that would have been attained could have encouraged the formation of cyanmethaemoglobin.

More than one of the postulated mechanisms may explain the observed changes in haemoglobin function and invite further investigation to determine which are involved.

Our results also provide evidence for the conjectured site of SNP degradation. The parallel rate of rise in red cell cyanide with both SNP and KCN (Fig. 3) would favour the plasma as the site of breakdown. However the far higher ratio of red cell to plasma cyanide with SNP incubation (Fig. 5) strongly suggests that breakdown occurs inside the red cell (Smith & Kruszyna 1974). The fact that there is an initial delay in the accumulation of cyanide in the red cell, with SNP (Fig. 4), also adds weight to this possibility.

This study shows that at levels of red cell cyanide likely to be attained on short term infusion of well above the maximum recommended dose of SNP (Vesey et al 1976; Michenfelder 1977) the effect on haemoglobin function will be negligible. However, the effect of long term exposure needs investigation. Although the highest red cell cyanide we have noted during long term infusion (Vesey et al 1974) was half the maximum concentration achieved during the in vitro studies, it is possible that continuous exposure to high cyanide concentrations might adversely affect haemoglobin function.

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