Sodium cyanate: from a promising therapeutic agent to a research tool in high altitude physiology

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Sodium cyanate (NaOCN) first appeared on the biomedical scene as a potential therapeutic agent for sickle-cell disease. Although it did not fulfill its early promise in the clinic, it proved to be useful as a pharmacological tool in physiological research, particularly in the physiology of oxygen transport. NaOCN has been especially valuable in the area of investigation which is reviewed here: the study of oxygen transport, both in normoxic and in hypoxic conditions, in experimental models in which NaOCN was used to induce a shift to the left of the oxygen dissociation curve. The classical idea is that a low Hb-O_2 affinity is of adaptive value for life at high altitudes but it has been challenged by several pieces of evidence. One of them is the demonstration of increased survival in hypoxic hypoxia of animals with a high Hb-O_2 affinity induced by NaOCN. We also discuss the advantages and potentially confounding factors which should be taken into consideration when interpreting results of studies in which the oxygen dissociation curve has been modified by administration of NaOCN.

***Key terms**: high altitude physiology; hypoxia; oxygen dissociation curve shifts; oxygen transport; sodium cyanate.*

I. INTRODUCTION

Often scientific disciplines overlap, and methods at first considered as specific for one clearly defined area are later used in another one. When assessing the physiology of organisms, the physiologist often uses a pharmacologically active agent to see whether its administration does or does not modify the function of the whole organism or of some particular organ within it. Sodium cyanate (NaOCN) has been used extensively in the study of high altitude but before that it generated a lot of excitement for a time in clinical pharmacology as a potential therapeutic agent. Therefore we will first give a brief account of the career of NaOCN as such an agent and then we will review the role that it later acquired in physiology, particularly in the physiology of high altitude.

II. SODIUM CYANATE: A POTENTIAL THERAPEUTIC AGENT

Sodium cyanate (NaOCN) is a pharmacological agent with a unique history both in clinical and in physiological areas. Probably the first time it emerged powerfully on the scientific scene was in the early 1970s, when Cerami *et al* (1973) suggested on the basis of *in vitro* studies that it might be the therapeutic agent so badly needed for treatment of sickle-cell disease (Cerami and Manning, 1971; Gillette *et al*, 1971). Earlier it had been found to be a natural product which is formed in the body from urea (Birch and Schutz, 1946; Schutz, 1949).

Patients with sickle cell disease have a lower hemoglobin-oxygen affinity than normal individuals. The early investigations showed that NaOCN carbamylated selectively the NH_2-terminal valine of hemoglobin

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(Kilmartin and Rossi-Bernardi, 1969; Manning *et al*, 1972) and increased O_2 affinity. It was reasoned that the resulting left-shift of the oxygen dissociation curve (ODC) of the erythrocytes of sickle-cell disease would allow a limited oxygen delivery in the microcirculation, and also increasing the saturation of Hb would avoid lysis of erythrocytes and could result in symptomatic improvement due to a reduction of the sickling phenomenon. Also experiments on several species of animals revealed that the toxicity of the drug might be low enough to justify clinical trials (Cerami *et al*, 1973; Graziano *et al*, 1973). Subsequent limited studies with NaOCN in humans with sickle-cell disease showed that it increased the survival time of erythrocytes substantially both *in vitro* and in vivo (Alter *et al*, 1972a; Gillette *et al*, 1971; 1974; Milner and Charache, 1972). These studies also suggested that NaOCN decreased the frequency of hemolytic crises (Gillette *et al*, 1974). It was found that ^{14}C-carbamyl groups accumulated in organs such as bone, skin, liver, and serum proteins, but it was hoped that these non-hemoglobin carbamylations could be neglected from a clinical point of view (Cerami *et al*, 1973). The disease does however need life-long treatment and long-term undesirable effects of the drug emerged as a critical limiting factor, both in clinical and in animal studies (May *et al*, 1972; Rivera-Ch *et al*, 1991).

Perhaps the most consistent and conspicuous effect of NaOCN observed in all these studies was that it led to a shift to the left of the ODC: carbamylation of the hemoglobin molecule increases the affinity of hemoglobin for oxygen. Although NaOCN did not fulfill expectations as a therapeutic agent for sickle-cell disease, its ability to shift the ODC made it an exciting tool for investigating oxygen transport, particularly transport at high altitude.

III. PRELIMINARY CONSIDERATIONS ON THE BIOLOGICAL SIGNIFICANCE OF ODC SHIFTS

The affinity of hemoglobin for oxygen plays an important role in oxygen transport, both at sea level and at high altitude. It is usually represented by an ODC relating PO_2 and O_2 saturation (SO_2) (Fig. 1). The position of the ODC may vary, the change being usually a shift to the right or to the left with all PO_2s increasing or decreasing in the same proportion as in a Bohr shift. For example, the blood ODC is strongly influenced by the concentration of 2,3-DPG in the erythrocytes: it decreases the affinity of hemoglobin for oxygen (Benesch and Benesch, 1969; Chanutin and Curnish, 1967). Lenfant *et al* (1970; 1971) used this property to explain their finding of a decrease in Hb-O_2 affinity in the blood of lowlanders after they had gone up to 4510 m. They proposed that blood pH is the principal factor causing changes in the position of the ODC after arrival at high altitude and that it acts through 2,3-DPG.

The adaptive value of a low or high Hb-O_2 affinity has been extensively studied, both in humans and in animals. On the one hand, animals which are adapted genotypically to altitude show consistently a high Hb-O_2 affinity and cope successfully with the hypoxia of high altitudes. On the other hand, animals which are not so adapted, and man is one of them, have relatively low Hb-O_2 affinities. Thus a high Hb-O_2 affinity appears as an evolutionary trait of animals genotypically adapted to high altitude.

The biological significance of such differences in the position of the ODC in oxygen transport from the lungs to mitochondria in the tissues has also been extensively studied experimentally by shifting the ODC to the left and the shift has usually been produced with NaOCN. The possibility of experimental manipulation of the ODC with NaOCN paved the way for exploring various of the steps of the oxygen transport cascade from lungs to cells.

The physiological consequences of a shift to the left or right of the ODC has long been an issue of intense debate.

Consider first, purely theoretically, an oversimplified model of the problem: what would be the effect of a shift to the left of the ODC, without any change in blood flow on the transport of O_2 between a fixed arterial PO_2 (PaO_2) and a fixed venous PO_2 (PvO_2)?

To indicate the position of the ODC, we use the parameter P_{50}, the affinity constant, which is the PO_2 at which Hb is half saturated with O_2. For example, in the dissociation

curves of Fig 1, which we obtained experimentally in mice (Rivera-Ch *et al*, 1995), P_{50} for half saturation was reduced by NaOCN from 35.8 Torr to 19.4 Torr.

Such a left shift increases SO_2 at all values of PO_2, but the size of the increase varies with PO_2, having a rounded maximum near P_{50} and decreasing sharply to zero at PO_2 = 0, and more gradually as PO_2 increases. Transport of O_2 (S_aO_2 - S_vO_2) is increased by a left shift if the shift increases S_aO_2 by more than it increases S_vO_2, and vice versa. In Fig. 1, the two lines each connecting an arterial and a venous point, show the changes in S_aO_2 and in S_vO_2, with a high P_aO_2 (AB) and with a low P_aO_2 (CD). Clearly at a high P_aO_2 of 100 Torr, there is a greater increase in S_vO_2 (B) than in S_aO_2 (A) and so O_2 transport is reduced by the left shift but if P_aO_2 is as low as P_{50}, the increase in S_aO_2 (C) is greater than in S_vO_2 (D) and so transport increases. A left shift increases O_2 transport in severe hypoxia but reduces it in normoxia or mild hypoxia. This emerges from the experimental data, as will be seen.

An alternative simple model, in which $S(a\text{-}v)O_2$ is constant and changes in P_vO_2 are considered, gives qualitatively the same answer that a left shift is advantageous in marked hypoxia because it raises P_vO_2. How far do such simple models account for observations?

A low Hb-O_2 affinity was long considered an advantage which improved O_2 transport to the tissues in hypoxic hypoxia. Aste-Salazar and Hurtado (1944) found that highlanders showed a slight right shift in the ODC and argued that this may be interpreted as a favourable compensatory adjustment to the low pressure environment at altitude. They stated that under these conditions the basic problem is one of the delivery of oxygen to the tissues, and a right deviation of ODC, even if slight, would be of appreciable benefit to this process, especially when the increased quantity of blood hemoglobin present is considered. Later, Lenfant *et al* (1968) studied the relation between oxygen dissociation and 2,3-DPG in the red cells of subjects moving from low to high altitude

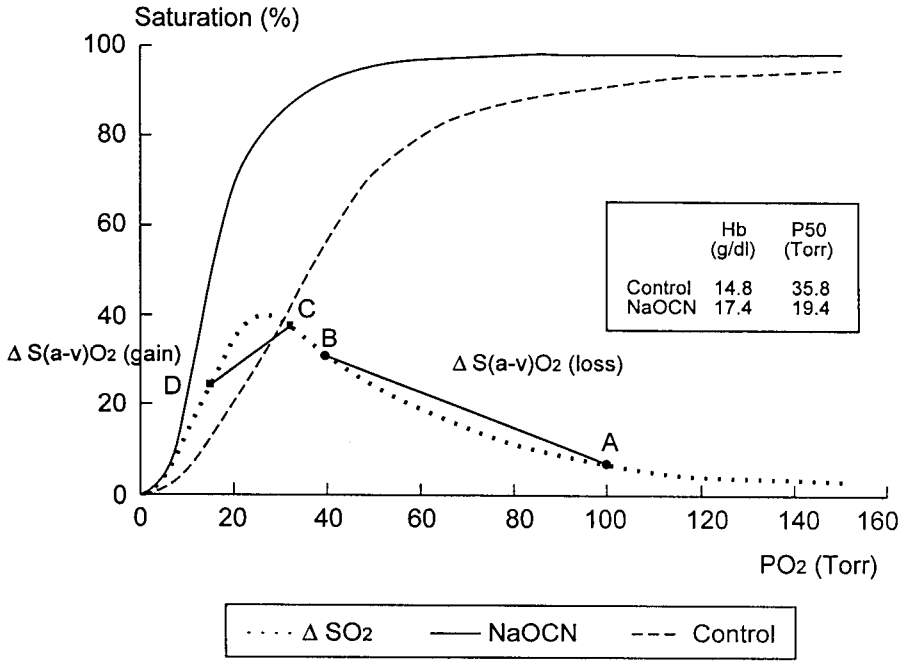

Fig 1. Whole oxygen dissociation curves, one normal and other shifted to the right by NaOCN, with P_{50}s 35.8 and 19.4. Line depicted by small squares, difference between saturation of normal and NaOCN blood as a function of PO_2. Bold straight lines connect A, change in saturation of arterial blood at PO_2 = 100 and B, change in saturation at a venous PO_2 = 40; CD, as for AB, except in marked hypoxia: P_aO_2 = 30 and P_vO_2 = 15. In AB, gain of S_aO_2 is less than gain of S_vO_2, so O_2 transport is reduced; but in severe hypoxia, CD, transport is increased.

and vice versa. They found that, in response to high altitude hypoxia, the ODC shifted to the right within 24 hours and a parallel increase occurred in the 2,3-DPG content of the red blood cells. They concluded that while their study does not provide direct evidence of a cause and effect relationship, the data strongly suggest that, with hypoxia, the observed rise in the 2,3-DPG of the red blood cells reduces the affinity of Hb for O_2 and so is responsible for the right-shift of the ODC, which allows an increased availability of oxygen to tissues. They considered that this change may represent an important rapid adaptive mechanism to high altitude hypoxia. However, in spite of these considerations, the observation is that animals which are genotypically adapted to high altitude show not a low but rather a high Hb-O_2 affinity as their most noteworthy characteristic (Monge-C and León-Velarde, 1991), and our simple model suggests that a high affinity would be valuable in the marked hypoxia of high altitude. NaOCN has been used to test this.

IV. EARLY STUDIES ON LEFT-SHIFTED ODC AND TISSUE OXYGEN TRANSPORT

Changes in erythropoiesis suggest changes in the tissue PO_2 of one particular tissue, which acts as a receptor for the release of erythropoietin. So far as the erythropoietic response is concerned, there may appear to be differences between animals in the extent to which erythrocytosis is induced by administration of NaOCN, and such variable results may be due to various factors such as route of administration, time and intensity of treatment, and biological (intrinsic) differences. In this context, Cerami *et al* (1973) did not find any substantial polycythemia in most of the animals (mice, rats, dogs, monkeys) they treated with cyanate at sea level and that suggests that probably there is good oxygen delivery to the tissues by the carbamylated erythrocytes despite their high Hb-O_2 affinity, and so there is no significant stimulation of erythropoiesis by tissue hypoxia. However, some of the individual mice and dogs did display a moderate 10-15% increase in hematocrit and hemoglobin concentration, when compared to control animals. The authors speculated that this may signify an attempt by the animal to enhance oxygen delivery to tissues through a moderately increased erythropoietic response. Thus they acknowledged that further work needs to be done to rule out the possibility that there is a decreased oxygen supply to some tissues as a result of the high oxygen affinity of the blood produced by NaOCN.

Cerami *et al* (1973) also exposed cyanate-treated and untreated mice to a hypoxic environment in a hypobaric chamber with an oxygen content equivalent to approximately 6 to 7%. They found that the cyanate-fed mice lived and survived for 10 days and so also did the control mice. The hypoxia within the chamber was very close to a lethal range and so this observation was believed to show that there is adequate oxygen transport to tissues even under adverse conditions in animals with a left-shifted ODC. In addition, the authors claimed that their observations also show that, when animals are fed cyanate, they still can respond to hypoxia with increased erythropoiesis. Thus they ruled out the possibility that in vivo there is a substantial inhibition of hemoglobin synthesis by NaOCN such as had previously been shown to occur *in vitro* by Alter *et al* (1972b).

In summary, the papers of Cerami and Manning (1971) and Cerami *et al* (1973) showed that the most striking difference between control and cyanate-treated animals was an increase in the oxygen affinity of the blood of the animals receiving cyanate and this change did not seem to be detrimental to the animals. This opinion seems to be supported by experiments performed by Wolk *et al* (1972) showing that the myocardium of dogs with a left-shifted ODC, induced by treatment with cyanate, was still able to extract the customary 70% of oxygen from the blood. However, it remains to be determined whether an adequate oxygen delivery occurs in other tissues as well.

V. NaOCN AS A TOOL IN HIGH ALTITUDE PHYSIOLOGY

A. Hemoglobin concentration

We have shown, in contrast to Cerami *et al* (1973), that mice given NaOCN 0.5% in drinking water at sea level have a significant

increase in hemoglobin concentration in addition to a marked increase in Hb-O_2 affinity (Rivera-Ch *et al*, 1995). Interestingly, control mice exposed either to chronic or to intermittent hypobaric hypoxia developed a lesser erythrocytosis than did cyanate-fed animals exposed to hypoxia in the same way. The latter animals had already been rendered polycythemic by the NaOCN before they were exposed to hypoxia, and they showed consistently higher hemoglobin values than animals which had been treated with NaOCN at sea level. As to whether there are beneficial or detrimental effects of the high Hb-O_2 affinity induced by NaOCN, we found that at sea level, young mice fed cyanate lost weight, whereas the controls gained weight (Rivera-Ch *et al*, 1991) but when both groups were also exposed to intermittent hypobaric hypoxia the controls lost weight and the cyanate-fed mice regained weight. However, the positive effects of cyanate on the body weight of hypoxic mice were largely outweighed by a cumulative deterioration in mitochondrial function when the animals were assessed after two and three months of treatment with NaOCN. Thus we warned about the need to keep in mind this long-term deleterious effect of cyanate when interpreting the adaptive responses to chronic hypoxia (Rivera-Ch *et al*, 1991). Nevertheless, it remains to be seen whether the deleterious effects of NaOCN shown in liver mitochondria also occur in other tissues. Meanwhile, the administration of NaOCN continues to be the most common strategy for experimentally shifting the ODC to the left.

B. Left-shifted ODC and regulation of erythropoiesis

With regard to changes in the humoral regulation of erythropoiesis in animals treated with cyanate, Lecherman and Jelkman (1985) found in rats that a high Hb-O_2 affinity induced by NaOCN increased plasma titers of erythropoietin (Epo) in normoxia but not in severe hypoxia. They concluded that a high Hb-O_2 affinity reduces the delivery of O_2 to the cells controlling erythropoietin production in normoxia and in moderate hypoxia and that excites them but that this effect is offset during severe hypoxia probably because of an improved O_2 loading of the blood. The animals had their blood oxygen affinity increased by exchange-transfusion with blood from NaOCN treated rats and cyanate had been administered orally to donors in the drinking water for 3 weeks. These differences between moderate and severe hypoxia are consistent with the behaviour of our model.

C. Left-shifted ODC: Survival in hypoxia and other physiological implications

Animals. Eaton *at al* (1974) tested whether an increased Hb-O_2 affinity is of any adaptive value to nonindigenous animals when they are at high altitude. After two weeks of either NaOCN or 0.9% NaCl in the drinking water, the animals were exposed in a hypobaric chamber to an atmospheric pressure equivalent to 9,180 m. All of the treated animals survived the 90-minute trials and their recovery was uneventful, but eight of the ten control animals died and in addition, all the cyanate-fed rats consistently had a much slower heart rate than had the surviving control rats. The authors concluded that increased, rather than decreased, Hb-O_2 affinity makes survival possible in conditions of severe environmental hypoxia. While they recognized that the functional properties of some proteins other than hemoglobin may be altered by cyanate, they proposed that the protective effect of cyanate is due specifically to the increase of Hb-O_2 affinity that cyanate produces. Similar results of increased survival of cyanate-treated rats exposed to extreme hypoxia were obtained by Penney and Thomas (1975).

Man. Hebbel *et al* (1978) studied human subjects who had naturally a hemoglobin of high oxygen affinity, Hb Andrew-Minneapolis. After exposure for one month to a moderately high altitude (3,100 m) and in striking contrast to two of their normal siblings, these high affinity subjects showed lesser increments in resting heart rate, minimal increases in plasma and urinary erythropoietin, no decrement in maximal oxygen consumption and no thrombocytopenia. These results were considered to be evidence that a degree of preadaptation to

altitude had been conferred by the increased oxygen affinity of their Hb Andrew-Minneapolis.

Shortly after the report of Eaton *et al* (1974), another approach to the problem was presented by Monge and Whittembury (1974) that helped to reveal the adaptive significance of changes in Hb-O_2 affinity. They pointed out that in animals native to high altitude, a high rather than a low Hb-O_2 affinity is the rule. That generalization is in conflict with the old idea which supposed that a lowered Hb-O_2 affinity would be of adaptive value in man at high altitudes (Aste-Salazar and Hurtado, 1944; Lenfant *et al*, 1968).

Turek *et al* (1978a) have added weight to the idea that a left-shifted ODC is advantageous by considering blood gases at several levels of oxygenation in rats with their ODC shifted to the left by NaOCN. They found that the calculated arterio-venous CO_2 difference was not changed by a left shift in normoxia but that it was reduced in mild hypoxia. In contrast, in severe hypoxia a left shift gave a higher mixed-venous PO_2 and an increased (a-v)CO_2 difference. The control rats developed a severe acidosis and pronounced hypocapnia on 5.6% O_2 but with a left shift there was a higher arterial and mixed venous pH. These findings suggest that in severe hypoxia the extraction of O_2 in the tissues is higher in rats with a left-shifted ODC. However, the authors considered that their calculations were based on certain assumptions which could be tested by additional experiments. Accordingly, they measured rather than calculated the arterial and mixed-venous oxygen content and the (a-v)CO_2 difference together with the oxygen consumption (VO_2) in rats with their ODC normal or left-shifted by cyanate (Turek *et al*, 1978b), both in normoxia and in hypoxia. They found the same trend in (a-v)CO_2 as in the previous study but the absolute values were somewhat larger, particularly in normoxia. Furthermore, they found that, in severe hypoxia, the nutritional blood flow to spleen, liver, stomach and intestines, skin, and kidney was lower in control rats than in NaOCN-treated rats. These reports show that, relative to normal controls, animals with a left-shifted ODC have an impaired oxygen delivery to tissues in normoxia and in mild hypoxia, but a more efficient O_2 delivery during severe hypoxia, which is what the calculations of Turek *et al* (1973, 1993) and our simpler model predict.

Furthermore, several other experimental studies support this notion that a left-shifted ODC induced by cyanate confers an advantage by allowing an adequate oxygen delivery to tissues in severe hypoxic conditions –when it is most at risk–, but not during moderate hypoxia (Schumaker *et al*, 1985; Teisseire *et al*, 1979; Turek *et al*, 1973; Turek and Rakusan, 1993). But more intense cyanate treatment can induce so low a P_{50} (so great a left-shift of the ODC) that hypoxia-like effects may appear in rats exposed to a normal atmosphere. For example, there may be prevention of normal growth, polycythemia, right ventricular hypertrophy, and pulmonary hypertension (Teisseire *et al*, 1986). The authors did however acknowledge that there was a major problem in deciding whether these effects were due to the great left-shift of the ODC only or rather to direct tissue or cellular effects of cyanate as well. In addition, Juarbe and Sillau (1988) found that two weeks of treatment with NaOCN induced a greater muscle capillary surface area in rats and they considered that this change would greatly facilitate O_2 transfer to muscle tissue in animals in which capillary blood PO_2 had been much reduced by a left-shift of the ODC.

Nevertheless, on the basis of our own work in mice, we believe that results which show beneficial effects of a left-shift of the ODC induced by cyanate in animals acutely exposed to hypoxia should be interpreted cautiously if one wishes to go on to consider whether using NaOCN to produce a left-shift of the ODC would be beneficial in animals chronically exposed to hypoxia. For example, we have found (Rivera-Ch *et al*, 1991) that chronic cyanate treatment leads to progressive and cumulative impairment of mitochondrial respiration in mice. And also, we found that prolonged treatment with cyanate did not prevent the development of further polycythemia in mice that were already chronically hypoxic (Rivera-Ch *et al*, 1995).

Finally, recent *in vitro* studies have assessed the effect of increasing hemoglobin-oxygen affinity on muscle O_2 uptake. The experiments were performed in isolated dog muscles perfused either with left-shifted ODC blood induced by sodium cyanate or with normal blood. In maximally working gastrocnemius muscles at the same O_2 delivery and in similar hemodynamic conditions, Hogan *et al* (1991) found that maximal O_2 uptake and calculated mean capillary PO_2 were lower when the ODC was left shifted. They concluded that these findings are consistent with the hypothesis that O_2 diffusion limitation in the peripheral tissues can be one important determinant of maximal O_2 uptake. To clarify the mechanism underlying the fall in O_2 uptake when O_2 delivery in the arterial blood decreases below a critical point, Kohzuki *et al* (1993) measured the O_2 uptake of isolated dog gracilis muscles which were perfused alternately with normal or with left-shifted ODC blood in which the arterial O_2 content was kept constant, but the blood flow was varied to vary the O_2 delivery. They found that the critical O_2 delivery at which O_2 uptake starts to decrease, the maximal O_2 extraction ratio, and the plateau O_2 uptake were similar with left-shifted ODC blood and with normal blood. The perfusion pressure-blood flow relationship was not influenced by the shift of the ODC. They suggested that the decrease in O_2 uptake in resting skeletal muscle when flow is reduced in hypoxic conditions is not caused by a decrease in PO_2 driving force within the capillaries, but by a decrease in the surface area available for diffusion due to a closure of capillaries induced by the low perfusion pressure and a rise in the distance for diffusion.

D. Pulmonary ventilation

NaOCN has been used also in several studies of ventilatory function and the nervous regulatory mechanisms of ventilation, the significance of which is stressed by the conclusion of West (1982), after extensive experience in humans exposed to extreme altitudes, that ventilatory function is the most important adaptive parameter during ascent to high altitude. The hypoxic ventilatory response (HVR) in those animals which are native to high altitude, and are considered as genotypically adapted to altitude, seems to be different from the HVR of animals which are not genotypically adapted and sodium cyanate has been used in experiments to test whether shifts of the oxygen dissociation curve contribute to changes in the HVR.

In addition, Birchard and Tenney (1986) treated rats with NaOCN and found that treated animals had a lower P_aO_2 and higher hematocrit than control rats. Control ventilatory values were similar to those in other reports. Treatment with NaOCN did not change resting air breathing ventilation. The HVR, expressed as a function of P_aO_2, did not differ between control and NaOCN treated animals and was similar to that observed previously for rats. The fact that the relation of HVR to PO_2 did not change was interpreted by the authors as consistent with there being no change in the PO_2 sensitivity of the chemosensory mechanism. They stated that this supports the hypothesis that the correlation between whole blood oxygen affinity and the interspecies HVR observed is a consequence of natural selection. In addition, they considered that the arterial chemoreceptor response is selected to correlate with P_{50} in such a way as to "protect" arterial saturation. Consequently, Birchard and Tenney (1986) concluded that it is not necessary to postulate any mechanistic role for the whole blood oxygen affinity curve in determining the ventilatory response.

Furthermore, Fukuda *et al* (1988) analyzed the influence of a left-shifted ODC induced by NaOCN and of progesterone on hypoxic ventilatory depression, as well as stimulation in terms of regulation of the breathing pattern. They found in the halothane anesthesized rat that shifting the ODC to the left with NaOCN and treatment with progesterone both increased the ventilatory response to acute hypoxia, whereas they inhibited the occurrence of ventilatory depression in severe hypoxia. They concluded that these beneficial effects probably are the result of reducing the extent to which the deteriorative action of hypoxia on brain tissue damages the central regulatory mechanism for respiratory rate.

We found (Rivera-Ch *et al*, 1994) that chronic cyanate administration left-shifted the ODC less in guinea pigs than in rats and produced a mild erythrocytosis in guinea pigs. At any given level of acute hypoxia, HVR was lower in NaOCN guinea pigs than in NaOCN rats. We thus concluded that animals which are adapted genotypically to high altitude such as the guinea pig seem to display relatively minor ventilatory and Hb-O_2 changes to NaOCN and a relatively minor HVR to acute hypoxia. We proposed that they probably use tissue and biochemical adaptive mechanisms in addition to their limited extracellular responses in order to tolerate ambient hypoxia successfully.

VI. EFFECTS ON THE FETUS

Cyanate has also been used to indirectly manipulate oxygen transport in the animal fetus and has proved of great value. In mammals, fetal blood has naturally a higher oxygen affinity than maternal blood and the molecular mechanisms by which this property of fetal blood is attained are quite well understood (Bunn, 1980; Jelkman and Bauer, 1980; Perutz and Imai, 1980), but the physiological significance of the difference is not clear. Independently both Bauer *et al* (1981) and Hebbel *et al* (1980) performed animal experiments to test whether fetal growth is retarded if the excess of the fetal over the maternal oxygen affinity is reduced by raising maternal O_2 affinity. Pregnant rats were exchange-transfused with high oxygen affinity blood, induced by previous treatment of the donor rats with NaOCN for up to 14 days. Both studies showed that an acute increase in maternal oxygen affinity leads to a reduction of fetal weight. Bauer *et al* (1981) also measured liver and brain weight and found a reduction of fetal liver weight but not of brain weight. From these experiments it was inferred that retardation of fetal intrauterine growth resulted from fetal hypoxia, which in turn was due to abolition of the difference in oxygen affinity between maternal and fetal blood. Also, it was suggested that the increased mortality of newborn animals which were small for their gestational age represented a marked selective pressure that favored the evolution of a fetal oxygen affinity that is high in comparison with the affinity of maternal blood.

VII. OVERVIEW

In several animal species, sodium cyanate has proven to be an extremely useful tool for manipulating the ODC and we have used experiments with it to assess the different steps in the transport of oxygen from ambient air to tissues in a variety of tissues.

Broadly, it is found that NaOCN increases the resistance of animals to intense hypoxia but increases the effect upon them of modest hypoxia and this is the observation predicted by our simple model of the changes in O_2 transport produced by the left shift of ODC that NaOCN produces and by the more complex model of Turek *et al* (1973, 1993).

When interpreting the results of any study in which this drug was used, one must bear in mind such important factors as interspecies differences, the route of administration of the drug, reference to exchange transfusion, for example when a donor animal is used, the duration of treatment, and whether the experiments were performed in normoxia, or in mild, moderate or severe hypoxia.

Genotypically adapted animals such as guinea pigs seem to respond differently to shifts from non-genotypically adapted animals such as rats (Rivera-Ch *et al*, 1994). Interspecies differences may also account for the differences observed in the erythropoietic response, *i.e.* the development or not of significant polycythemia.

Another important point to be borne in mind is that evidence that oxygen delivery to tissues is improved in acute hypoxia when the ODC is left-shifted by cyanate does not indicate that there will also be an adaptive (beneficial) effect of cyanate in conditions of chronic natural or simulated hypoxia. It must be remembered that the great majority of studies reported have been performed in acutely hypoxic animals only.

The life-span of the different animal species is also important when considering the biological meaning of "short-term" or "long-term" treatments with NaOCN.

On the other hand, potentially deleterious effects on the function of organs such as progressive impairement in liver mitochondrial function with prolonged exposure to NaOCN (Rivera-Ch *et al*, 1991) should be borne in mind if the results of the different measurements made are to be interpreted appropriately.

Finally, it is necessary to distinguish between physiological consequences of a left-shifted ODC (high hemoglobin-oxygen affinity) induced experimentally in animals not adapted genotypically to high altitude and the physiological significance of naturally occurring high hemoglobin-oxygen affinity in animals genotypically adapted to high altitude (Monge-C and León-Velarde, 1991).

In recent years, renewed interest has arisen in NaOCN as a potentially useful preventive and therapeutic agent in various neoplastic disorders (Hu *et al*, 1989, 1990; Lazarus and Panasci, 1987; Lea *et al*, 1986, 1987; Shenouda *et al*, 1993; Wattenberg, 1990). If it is eventually demonstrated that it is effective in improving substantially the survival of those affected with such conditions perhaps the toxicity which was thought unacceptable in a treatment of so chronic a condition as sickle-cell anemia could be overlooked in a treatment of malignancies. Meanwhile, sodium cyanate will remain a valuable tool for manipulating the hemoglobin-oxygen affinity in studies of the different steps in the oxygen cascade from inspired air to mitochondria.

REFERENCES

ALTER BP, KAN YW, NATHAN DG (1972a) Reticulocyte survival in sickle cell anemia: effect of cyanate. Blood 40: 733-739

ALTER BP, KAN YW, NATHAN DG (1972b) Inhibition of hemoglobin synthesis by cyanate. J Clin Inv 51: 4a

ASTE-SALAZAR H, HURTADO A (1944) The affinity of hemoglobin for oxygen at sea level and high altitude. Am J Physiol 142: 733-743

BAUER C, JELKMAN W, MOLL W (1981) High oxygen affinity of maternal blood reduces fetal weight in rats. Respir Physiol 43: 169-178

BENESCH R, BENESCH RE (1969) Intracellular organic phosphates as regulators of oxygen release by haemoglobin. Nature 221: 618-622

BIRCH KM, SCHUTZ F (1946) Actions of cyanate. Br J Pharmacol 1: 186-193

BIRCHARD GF, TENNEY SM (1986) The hypoxic ventilatory response of rats with increased blood oxygen affinity. Respir Physiol 66: 225-233

BUNN HF (1980) Regulation of hemoglobin function in mammals. Am Zool 20: 199-201

CERAMI A, MANNING J (1971) Potassium cyanate as an inhibitor of the sickling of erythrocytes *in vitro*. Proc Natl Acad Sci USA 68: 1180-1183

CERAMI A, ALLEN TA, GRAZIANO JH, DE FURIA FG, MANNING JM, GILLETTE PN (1973) Pharmacology of cyanate. I. General effects on experimental animals. J Pharmacol Exp Ther 185: 653-666

CHANUTIN A, CURNISH RR (1967) Effect of organic and inorganic phosphates on the oxygen equilibrium of human erythrocytes. Arch Biochem Biophys 121: 96-102

EATON JW, SKELTON TD, BERGER E (1974) Survival at extreme altitude: protective effect of increased hemoglobin-oxygen affinity. Science 183: 743-744

FUKUDA Y, KIMURA H, KOBAYASHI T, MIKAMI M, KURIYAMA T (1988) Protection of hypoxic ventilatory depression by administration of Na-cyanate or progesterone in the rat. In: UEDA G, KUSAMA S, VOELKEL NF (eds) High Altitude Medical Science. Matsumoto, Japan: Shinsku University. pp 234-238

GILLETTE PN, MANNING JM, CERAMI A (1971) Increased survival of sickle-cell erythrocytes after treatment *in vitro* with sodium cyanate. Proc Natl Acad Sci USA 68: 2791-2793

GILLETTE PN, PETERSON CM, LU YS, CERAMI A (1974) Sodium cyanate as a potential treatment for sickle-cell disease. N Engl J Med 290: 654-660

GRAZIANO JH, THORNTON YS, LEONG JK, CERAMI A (1973) Pharmacology of cyanate. II. Effects on the endocrine system. J Pharmacol Exp Ther 185: 667-675

HEBBEL RP, EATON JW, KRONENBERG RS, ZANJANI ED, MOORE LJ, BERGER EM (1978) Human llamas. Adaptation to altitude in humans with high hemoglobin oxygen affinity. J Clin Inv 62: 593-600

HEBBEL RP, BERGER EM, EATON JW (1980) Effect of increased maternal hemoglobin oxygen affinity on fetal growth in rats. Blood 55: 969-974

HOGAN MC, BEBOUT DE, WAGNER PD (1991) Effect of increased Hb-O_2 affinity on VO_2max at constant O_2 delivery in dog muscle *in situ*. J Appl Physiol 70: 2656-2662

HU HH, DIMAIRA MJ, ZIRVI KA, DIKDAN G, LEA MA (1989) Influence of pH on the modification of thiols by carbamoylating agents and effects of glutathione levels in normal and neoplastic cells. Cancer Chemother Pharmacol 24: 95-101

HU JJ, ZIRVI KA, LEA MA (1990) Combined effect of pH and sodium cyanate on the inhibition of tumor cell proliferation and metabolism by BCNU and hyperthermia. Cancer Chemother Pharmacol 26: 269-272

KOHZUKI H, ENOKI Y, SHIMIZU S, SAKATA S (1993) High blood O_2 affinity and relationship of O_2 uptake and delivery in resting muscle. Respir Physiol 92: 197-208

JELKMAN W, BAUER C (1980) 2,3-DPG levels in relation to red cell enzyme activities in rat fetuses and hypoxic newborns. Pflügers Arch 389: 61-68

JUARBE C, SILLAU AH (1988) Muscle capillarity in rats with increased blood oxygen affinity. Respir Physiol 72: 83-94

KILMARTIN JV, ROSSI-BERNARDI L (1969) Inhibition of CO_2 combination and reduction of the Bohr effect in haemoglobin chemically modified at its alpha-amino groups. Nature 222: 1243-1246

LAZARUS P, PANASCI LC (1987) Mechanisms of decrease of protein synthesis by sodium cyanate in murine P388 leukemia cells. Cancer Res 47: 5102-5107

LEA MA, VELASQUEZ O, CARPENTER L, MARTINSON CF, HILL GJ (1986) Effects of sodium cyanate in

mice bearing B16 melanoma. Cancer Chemother Pharmacol 17: 231-235

LEA MA, LUKE A, HU JJ, VELASQUEZ O (1987) Action of carbamoylating agents on the uptake of metabolites of hepatomas and liver. Biochem Pharmacol 36: 2725-2781

LECHERMAN B, JELKMAN W (1985) Erythropoietin production in normoxic and hypoxic rats with increased blood oxygen affinity. Respir Physiol 60: 1-8

LENFANT C, TORRANCE JD, ENGLISH E, FINCH CA, REYNAFARJE C, FAURA J (1968) Effect of altitude on oxygen binding by hemoglobin and on organic phosphate levels. J Clin Invest 47: 2652-2656

LENFANT CJ, TORRANCE JD, WOODSON RD, JACOBS P, FINCH CA (1970) Role of organic phosphates in the adaptation of man to hypoxia. Fed Proc 29: 1115-1117

LENFANT CJ, TORRANCE JD, REYNAFARJE C (1971) Shift of the O_2-Hb dissociation curve at altitude: mechanism and effect. J Appl Physiol 30: 625-631

MANNING JM, CERAMI A, GILLETTE PN (1972) Chemical and biological aspects of the inhibition of red blood cell sickling by cyanate. In: BREWER GJ (ed) Hemoglobin and Red Cell Structure and Function. New York: Plenum. pp 253-260

MAY A, BELLINGHAM AJ, HUHENS ER, BEAVEN GH (1972) Effect of cyanate on sickling. Lancet 1: 658-661

MILNER PE, CHARACHE S (1972) Effect of carbamylation with varying concentrations of cyanate (NCO) or carbamyl phosphate (CP) on red cell destruction in sickle cell anemia (Hb SS). Blood 40: 928

MONGE-C C, WHITTEMBURY J (1974) Increased hemoglobin-oxygen affinity at extremely high altitudes. (Letter). Science 186: 843

MONGE-C C, LEON-VELARDE F (1991) Physiological adaptation to high altitude: oxygen transport in mammals and birds. Physiol Rev 71: 1135-1172

PENNEY D, THOMAS M (1975) Hematological alterations and response to acute hypobaric stress. J Appl Physiol 39: 1034-1037

PERUTZ MF, IMAI K (1980) Regulation of oxygen affinity of mammalian haemoglobins. J Mol Biol 136: 183-191

RIVERA-CH, DUNIN-BORKOWSKI O, LEON-VELARDE F, HUICHO L, VARGAS M, MONGE-C C (1991) Metabolic effects of cyanate on mice at sea level and in chronic hypobaric hypoxia. Life Sci 49: 439-445

RIVERA-CH M, LEON-VELARDE F, HUICHO L, MONGE-C C (1994) Ventilatory response to severe acute hypoxia in Guinea pigs and rats with high hemoglobin-oxygen affinity induced by cyanate. Comp Biochem Physiol 109A: 675-680

RIVERA-CH M, LEON-VELARDE F, HUICHO L, MONGE-C C (1995) Time-course of the polycythemic response in normoxic and hypoxic mice with high blood oxygen affinity induced by cyanate administration. J Comp Physiol B 164: 659-662

SCHUTZ F (1949) Cyanate. Experientia 5: 133-172

SCHUMAKER PT, SUGGETT AJ, WAGNER PD, WEST JB (1985) Role of hemoglobin P_{50} in O_2 transport during normoxic and hypoxic exercise in the dog. J Appl Physiol 59: 749-757

SHENOUDA G, HUTCHINSON M, NOE A, PANASCI L (1993) Alteration of the systemic antitumor activity of melphalan by sodium cyanate in MOPC-460D myeloma-bearing BALB/c mice. J Surg Oncol 52: 110-114

TEISSEIRE BP, SOULARD CD, HERIGAULT RA, LECLERC LF, LAVER MB (1979) Effects of chronic changes in hemoglobin-oxygen affinity in rats. J Appl Physiol 46: 816-822

TEISSEIRE BP, VIEILLEDENT CC, TEISSEIRE LJ, VALLEZ MO, HERIGAULT RA (1986) Chronic sodium cyanate treatment induces "hypoxia-like" effects in rats. J Appl Physiol 60: 1145-1149

TUREK Z, RAKUSAN K (1993) Computer model analysis of myocardial tissue oxygenation: a comparison of high altitude guinea pig and rat. In: LEON-VELARDE F, ARREGUI A (eds) Hipoxia: Investigaciones Básicas y Clínicas. Homenaje a Carlos Monge Cassinelli. Lima, Perú: Serie Travaux de l'Institut Français d'Études Andines, N° 76, pp 141-153

TUREK Z, KREUZER F, HOOFD LJC (1973) Advantage or disadvantage of a decrease of blood oxygen affinity for tissue oxygen supply at hypoxia. Pflügers Arch 342: 185-189

TUREK Z, KREUZER F, RINGNALDA BEM (1978a) Blood gases at several levels of oxygenation in rats with a left-shifted blood oxygen dissociation curve. Pflügers Arch 376: 7-13

TUREK Z, KREUZER F, TUREK-MAISCHEIDER M, RINGNALDA BEM (1978b) Blood O_2 content, cardiac output, and flow to organs at several levels of oxygenation in rats with a left-shifted blood oxygen dissociation curve. Pflügers Arch 376: 201-207

WATTENBERG LW (1990) Inhibition of carcinogenesis by naturally- occurring and synthetic compounds. Basic Life Sci 52: 155-166

WEST JB (1982) Respiratory and circulatory control at high altitudes. J Exp Biol 100: 147-157

WOLK M, LIEBSON P, BEER N, CERAMI A, KILLIP T (1972) Cardiovascular adjustments to acute and chronic increases in oxyhemoglobin affinity. J Clin Inv 51: 105a