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Hypoxanthine as an Indicator of Hypoxia: Its Role in Health and Disease through Free Radical Production

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Hypoxia is a common insult during the perinatal and neonatal period. New and better ways to evaluate hypoxia are needed. In 1975 we demonstrated high concentrations of the purine metabolite hypoxanthine in umbilical cord plasma after intrauterine hypoxia and proposed that hypoxanthine could be used as an indicator of hypoxia (1). Since then a large number of studies have been published dealing with different aspects of hypoxanthine in hypoxia. However, investigators in this field have encountered several methodologic problems: 1) Hypoxanthine leaks rapidly from erythrocytes (2, 3); if plasma is not separated promptly from red cells, falsely elevated plasma hypoxanthine concentrations will be found. 2) There are large variations in purine metabolism among species. 3) No clear definition of clinical hypoxia is available. Many authors do not distinguish between the terms hypoxemia and hypoxia, which adds to the confusion. We define tissue hypoxia as: "oxygen deficiency resulting in altered or interrupted energy metabolism" (4). It is important to be aware that hypoxia has two stages. In the first stage it is compensated because the cells are able to meet energy demands through anaerobic metabolism and other mechanisms. (We are not dealing with physiologic adaptation to hypoxia.) In the second stage of hypoxia or uncompensated hypoxia, energy demands are not met and cell injury ensues. At present there are no techniques for distinguishing between these two stages in clinical medicine, and such a distinction would be useful. Theoretically hypoxanthine should only be elevated in uncompensated hypoxia, while pH and lactate changes occur in compensated hypoxia. It seems, however, that hypoxanthine is also elevated to some extent in uncompensated hypoxia.

Renewed interest in hypoxanthine developed when it was realized that hypoxanthine is a potential free radical generator (5, 6). Hypoxanthine seems to play a role in posthypoxic reoxygenation cell injury through oxygen radical production (7) and is therefore involved in the pathogenesis of a number of diseases. Hypoxanthine also modulates a number of other processes because it reacts with benzodiazepine receptors (8) and inhibits phosphodiesterase in the brain (9). Hypoxanthine inhibits the effect of several cytotoxic drugs and may therefore influence treatment with such drugs (10).

Herein we summarize the extensive literature on hypoxanthine published during the last decade and try to answer the question: How useful in clinical medicine are hypoxanthine measurements in plasma and other body fluids? Is hypoxanthine a better marker of hypoxia than lactate or pH? Further, we discuss the significance of hypoxanthine as a potential oxygen radical generator and put forward a hypothesis for the pathogenesis of an "oxygen radical disease in neonatology".

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METABOLISM

Hypoxanthine is a breakdown product of ATP. Cells try to maintain a high energy charge even if ATP concentration falls and the energy level decreases (11). The formula for the energy charge (EC): EC = ATP + 0.5 ADP/AMP + ADP + ATP, indicates that EC can be kept high during ATP deficiency by reducing the concentration of AMP; this is exactly what happens. In hypoxia there is an accelerated breakdown of AMP to hypoxanthine. Figure 1 illustrates several important aspects of hypoxanthine metabolism in hypoxia. Normally about 90% of the hypoxanthine formed is reutilized through the salvage pathway and inosine monophosphate (IMP) is formed. Phosphoribosylpyrophosphate (PRPP) and ATP are required for the synthesis of IMP from hypoxanthine by the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT). This is the enzyme missing in the Lesch-Nyhan syndrome (12). The rate of transformation of hypoxanthine to IMP is reduced in hypoxia, as is the further oxidation of hypoxanthine to xanthine and uric acid by xanthine oxidase. This enzyme is mainly found in the liver and small intestine of the human, although there may be low concentrations in other tissues. In other species including the rat, dog, cat, and sheep, xanthine oxidase is also in high concentration in the lungs (13). Experiments measuring hypoxanthine in plasma of animals with lung xanthine oxidase will be difficult to interpret because the lungs have a formidable capacity to clear hypoxanthine from the circulation. It might be difficult to detect hypoxanthine in arterial plasma even during profound hypoxia. Xanthine oxidase is synthesized as xanthine dehydrogenase (type D) and this form accounts for about 90% of the total activity in nonhypoxic tissue (14). During hypoxia and ischemia the dehydrogenase form is converted to the oxidase form (type O). This conversion may result from a protease activated by the high calcium concentration found in the cytosol of hypoxic cells (15).

In most mammals, the main exception being man and higher apes where uric acid is the end product of purine catabolism, uric acid is further oxidized by uricase to allantoin. In adult humans approximately two-thirds of the uric acid is eliminated through the kidneys and one-third through the gastrointestinal tract (16). Renal handling of uric acid seems to involve four steps: glomerular filtration, tubular reabsorption, active secretion, and postsecretory reabsorption (17). The mechanism of xanthine excretion in the kidney is similar to that of uric acid, whereas hypoxanthine is eliminated mainly by filtration along with other purine bases (18, 19). Xanthine excretion is reduced by uricosuric drugs whereas hypoxanthine seems to be unaffected by these agents (19). In adults the hypoxanthine urinary output is on the average 1.0 (range 0.6–1.4) μmol/kg/day (20) as compared with a comparable output of 1.1 (range 0.7–1.6) μmol/kg/day on the 1st day of life. This output slowly decreases over the subsequent days and a mean output of 0.7 (range 0.1–1.5) μmol/

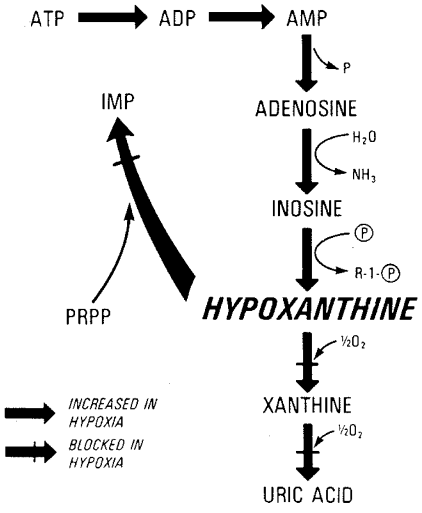

Fig. 1. Schematic outline of adenine nucleotide metabolism. In hypoxia AMP is degraded to hypoxanthine via adenosine and inosine. Salvage of hypoxanthine to IMP is reduced as is the further catabolism to uric acid when lack of oxygen. From Saugstad and Gluck (71) used with permission.

kg/day on the 3rd day of life was measured (21). Hypoxanthine clearance in normal adults range between 40–60 ml/min/1.73 M^2 (19). Plasma half-times in adult pigs and fetal lambs after hypoxia are approximately 40 and 30 min respectively (22, 33). Plasma half-times of hypoxanthine in newborn babies and adults are still not known with certainty.

METHODS

Methods to measure hypoxanthine reliably in plasma were not available until the middle of the last decade, limiting the information about hypoxanthine in pathophysiologic conditions. The first spectrophotometric methods for measurements of plasma hypoxanthine in the late 1940s and early 1950s were inaccurate, laborious, and required large amounts of blood (24, 25). When hypoxanthine was measured for the first time in umbilical cord plasma after uncomplicated deliveries in 1972 (26), 40 ml of blood were required (26). The introduction of the pO_2 method a few years later made it possible to measure hypoxanthine levels in plasma and other body fluids systematically (3). This method is based on the principle that oxygen is consumed when hypoxanthine is oxidized to xanthine and urate. The hypoxanthine concentration can be calculated by measuring the fall in pO_2 in the solution after addition of xanthine oxidase. The method is simple, inexpensive, and rapid, requiring not more than 200 μl plasma. A drawback is that half the xanthine present is measured as hypoxanthine resulting in a somewhat high hypoxanthine value. The xanthine/hypoxanthine ratio in plasma and other body fluids is normally 20–45% (27, 28) and decreases to between 5 and 12% in hypoxia (27, 29). Thus the error becomes almost negligible, at least in the hypoxic state. The pO_2 method is not sensitive at very low hypoxanthine concentrations (less than 3–5 μmol/liter).

Fluorimetry (30, 31) and high-performance liquid chromatography (HPLC) (28, 32–35) provide methods with greater sensitivity. Although several other methods have been described, pO_2, fluorimetric, or HPLC methods are the most generally used. The fluorimetric method also adds half of the xanthine present to the hypoxanthine. In one study where this method was compared with the pO_2 method, the fluorimetric technique had a higher precision and lower blood volumes were required (50 μl) (36). Plasma hypoxanthine measurements have been compared using the pO_2 and HPLC methods in three studies (37–39), and correlation coefficients between the two methods ranged between 0.88 and 0.99. In summary, the pO_2 method is less expensive, simpler, and faster than HPLC method, whereas HPLC is more sensitive, especially at low concentrations. HPLC also separates hypoxanthine and xanthine in addition to other purines.

STUDIES ON ISOLATED ORGANS

The first studies demonstrating an elevation of hypoxanthine in hypoxia were performed on isolated organs about 25 yr ago. The fundamental studies of Berne (40) on the perfusate from isolated hypoxic cat myocardium, and by Gerlach *et al.* (41) assessing the perfusate from isolated hypoxic kidneys showed an increased output of purine metabolites. Berne and Rubio (42) were interested mainly in studying the effect of adenosine on the circulation. Since then other groups have reported high hypoxanthine concentrations in hypoxic tissues including kidney (43), liver (44), brain (44–46), myocardium (44, 47), intestine (48), lungs (49), and placenta (50). In placental tissue the hypoxanthine concentration was correlated with the energy charge and a negative correlation of 0.79 was found (50). This correlation therefore demonstrates that the hypoxanthine concentration accurately reflects the energy status of the cell, which is exactly what we are trying to quantify when diagnosing hypoxia. The hypoxanthine concentration was recently measured in food and used as an index of food quality (51).

ANIMAL STUDIES

We measured plasma hypoxanthine concentrations in hypoxemic and hypotensive dogs (52, 53), not knowing that xanthine oxidase was present in their lungs. There were low hypoxanthine concentrations in arterial plasma even in profound hypoxia whereas high levels were measured in the inferior caval venous plasma. In dogs with endotoxic shock there was a dramatic washing out of hypoxanthine during resuscitation with volume expansion. In the course of 10–15 min venous hypoxanthine levels were raised from zero to more than 100 μmol/liter. In arterial plasma there was also a substantial elevation of hypoxanthine during this procedure (54). In dogs with respiratory arrest hypoxanthine increased linearly in cerebrospinal fluid during the 18 min that the experiments lasted (55).

We studied pigs because pigs and humans lack lung xanthine oxidase. Hypoxia was induced by allowing the pigs to breathe 6 or 7% oxygen in nitrogen. A linear increase of plasma hypoxanthine with duration of hypoxemia was found, and there was no difference between arterial and venous plasma. There were good correlations between hypoxanthine and lactate, base deficit and pH (7, 22). There was also a direct relationship between survival time and increase in plasma hypoxanthine. Survival time correlated negatively with the rate of hypoxanthine increase ($r = -0.62$). All animals died when hypoxanthine exceeded 125 μmol/liter. The increase of hypoxanthine therefore reflected the prognosis of acute hypoxia in contrast to base deficit (7).

In the exteriorized hypoxic fetal lamb Thiringer *et al.* (23) demonstrated a rapid increase in plasma hypoxanthine concentrations. Figure 2 shows the relation between plasma hypoxanthine concentration and duration of hypoxemia. As in adult pigs, there were high correlations with lactate ($r = 0.83$), base deficit ($r = 0.87$), and pH ($r = 0.90$). Hypoxanthine was cleared from plasma after reversal of hypoxia with a half-time of 30 min (23). Subsequent to these experiments it became apparent that the lungs of newborn sheep, by contrast to fetal lambs with minimal pulmonary perfusion, very efficiently clear hypoxanthine, probably because they have high levels of xanthine oxidase. This might explain why one group of investigators found a biphasic increase of plasma hypoxanthine concentration when studying hypoxic newborn lambs (56).

Thiringer *et al.* (57) have shown that the brain of fetal sheep does not release hypoxanthine during either normoxia or even

in mild hypoxia. Hypoxanthine is released only in severe hypoxia, indicating that the fetal brain has a high threshold for degrading its energy-rich intracellular purines. This could be due to the fact that the brain has a high concentration of the enzyme HGPRT which may constitute part of hypoxic defenses of the brain. There was a linear correlation between cerebral arteriovenous differences of hypoxanthine in the fetal lamb and somatosensoric-evoked potential signals. The contribution of hypoxanthine from different organs of the exteriorized fetal lamb was also studied (58). Hypoxanthine was released from the liver even during normoxia which was quite surprising since the bulk of the xanthine oxidase was found in this organ. A substantial output was found in hypoxia from the liver as well as the myocardium. In contrast, the hind leg produced little hypoxanthine in hypoxia. The placenta cleared hypoxanthine efficiently both in normoxia and hypoxia (58). On the basis of this study it was therefore concluded that the liver and myocardium were the main producers of hypoxanthine in fetal hypoxia.

PLASMA HYPOXANTHINE IN MAN

The first clinical study demonstrating augmented hypoxanthine levels in hypoxia was performed with the pO_2 method in umbilical cord plasma (1). In this study the normal hypoxanthine concentration was found to range between 0 and 11 μmol/liter,

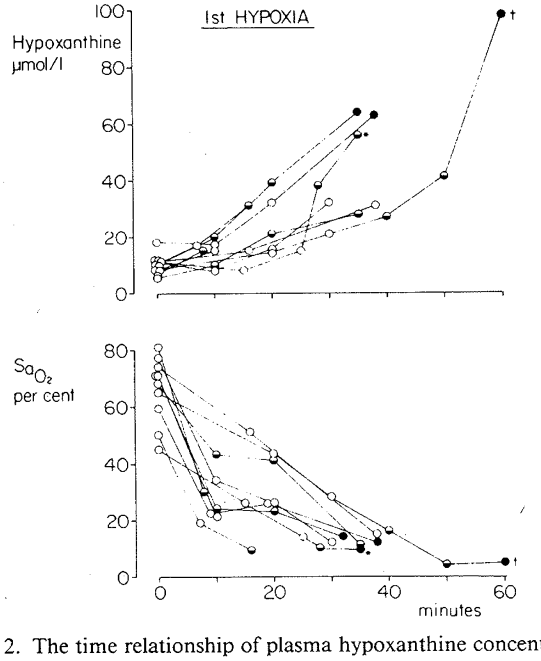

Fig. 2. The time relationship of plasma hypoxanthine concentration and SaO_2 in 10 exteriorized fetal lambs. Hypoxia was induced by ventilation the ewe with 16% O_2. ○, pH > 7.30; ◒, pH 7.15–7.29; ●, pH < 7.15. From Thiringer *et al.* (23) used with permission.

with a mean of 5.8 μmol/liter. The mean level in hypoxic babies was 25 μmol/liter, significantly higher than the nonhypoxic values. Subsequently others have measured the plasma hypoxanthine levels in umbilical cord blood in normal and hypoxic babies (Table 1) (37, 38, 59–64). All investigators found higher hypoxanthine values in hypoxic than in nonhypoxic babies, even though this elevation was not significant in two of the studies (37, 59). There is a remarkable agreement among investigators as to the normal level of umbilical cord hypoxanthine plasma, *e.g.* 4 to 8 μmol/liter, even when different methods for measuring hypoxanthine were used. A positive difference was found between umbilical arterial and venous levels in two studies (37, 59), indicating net transfer of hypoxanthine from the fetus to the mother. However, no significant differences in umbilical venoarterial levels of hypoxanthine and xanthine were reported in another study (65). In these three studies (37, 59, 65) the mean normal hypoxanthine concentrations were rather high, indicating that plasma was not separated from plasma quickly enough. The reliability of these data can therefore be questioned.

In general umbilical cord hypoxanthine levels can be used to distinguish between hypoxia and nonhypoxia. We found a few patients with mild hypoxia having hypoxanthine concentrations in the normal range but we have never found a nonhypoxic patient with elevated values (62). In the most thorough study to date on umbilical cord plasma, hypoxanthine was determined with the pO_2 method in 141 newborn babies (60). Fifty babies suffering from mild or severe intrauterine hypoxia were compared with 29 babies from high risk deliveries but without clinical or biochemical signs of hypoxia and with 62 babies from normal deliveries. The mean hypoxanthine concentration of hypoxic patients was 16.6 μmol/liter, a value significantly higher than that of normal babies (5.7 μmol/liter) or babies at risk (8.7 μmol/liter). From this study a normal upper limit of hypoxanthine in cord blood was defined as 14 μmol/liter.

When correlating hypoxanthine and Apgar score a weak but significant negative correlation of $r = -0.33$ ($p < 0.05$) was found. The correlation between hypoxanthine and lactate concentrations was $r = 0.62$ for hypoxic babies. Two babies in this series developed spastic paresis, both of whom had normal Apgar scores but high hypoxanthine concentrations. One newborn baby with cardiac arrest had a hypoxanthine level after resuscitation exceeding 200 μmol/liter. This study suggests that plasma hypoxanthine concentrations are a better predictor of outcome than Apgar scores or lactate (60). In a recent study, Pietz *et al.* (63) found significantly higher hypoxanthine levels in cord plasma of hypoxic as contrasted with nonhypoxic babies. Five of six babies who died had hypoxanthine levels exceeding 2 SEM above the mean for nonhypoxic controls. These authors conclude that hypoxanthine concentration should be used as the new "gold" standard of hypoxia.

Immediately postpartum, hypoxanthine is washed into the circulation, such that peak values are found 10 to 20 min postpartum (Fig. 3). After this period the hypoxanthine concentration quickly decreases to levels between 0 and 5 μmol/liter.

Table 1. *Hypoxanthine concentration (μmol/liter) in venous, arterial, or mixed umbilical cord plasma (SD)*

Reference	Mean or median concentration		Method	p
	Control	Hypoxia		
Saugstad (1)	5.8 ± 3.0	25.0 ± 18.0	pO_2	<0.01
Guicheney *et al.* (37)	8.5 ± 5.4	15.4 ± 15.5	HPLC	NS
O'Connors *et al.* (38)	4.2 ± 2.9	17.9 ± 28.6	HPLC	<0.025
Bratteby and Swanström (66)	5.3 ± 3.3		pO_2	
Lipp Zwahlen *et al.* (59)	14.4 ± 4.7	16.4 ± 5.1	pO_2	NS
Thiringer (60)	5.7 ± 5.8	16.6 ± 14.9	pO_2	<0.01
Merchurova *et al.* (61)	13.1 ± 4.5	23.0 ± 10.7	pO_2	<0.01
Saugstad *et al.* (62)	6.3 ± 6.6	14.1 ± 10.9	pO_2	<0.005
Pietz *et al.* (63)	8.7 ± 3.4	19.2 ± 5.8	pO_2	<0.005
Issel *et al.* (64)	5.1 ± 1.8	10.0 ± 3.4	Fluorimetric	<0.01

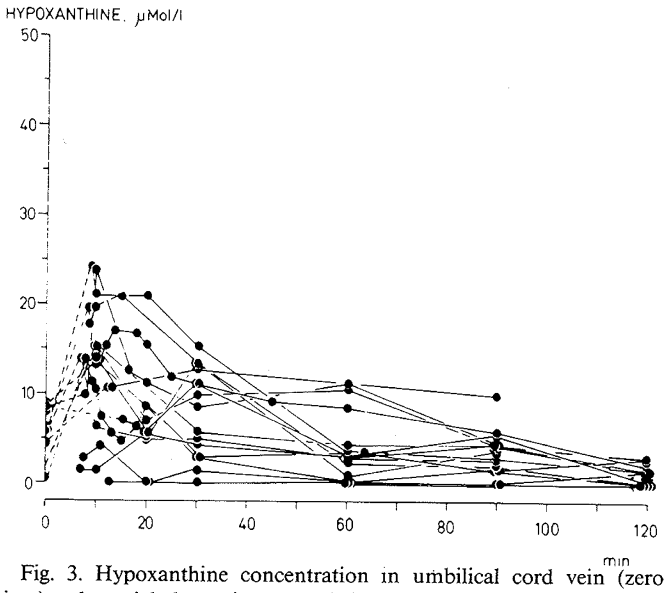

Fig. 3. Hypoxanthine concentration in umbilical cord vein (zero time) and arterial plasma in normal infants during the first 120 min after birth. From Bratteby and Swanström (66) used with permission.

The same pattern was found in nonhypoxic as in hypoxic patients; however, the peak value reached higher levels in the hypoxic patients (66, 67). These data show that the best way to assess intrauterine hypoxia by plasma hypoxanthine determinations is by serial measurements. In both full-term and premature nonhypoxic newborn babies the hypoxanthine level declines during the first days of life. The mean value during the first 12 to 36 h of life in full-term babies is 5.5 µmol/liter with a range of 2.7–11.2 µmol/liter; at 3 and 5 days of life the mean levels were 3.2 and 1.8 µmol/liter, respectively. Premature babies have hypoxanthine values similar to term infants.

The correlation between hypoxanthine and lactate in eight hypoxic babies was $r = 0.64$, $p < 0.001$) (68). High hypoxanthine levels were measured in preterm babies with respiratory distress syndrome (RDS) and with congenital heart lesions (69, 70). In babies subsequently dying of RDS values as high as 100 µmol/liter were found (69). We measured the hypoxanthine concentrations in arterial plasma of sick newborn babies and found correlations between hypoxanthine and pH of $r = -0.80$, between hypoxanthine and base deficit of $r = 0.78$, and between hypoxanthine and paO_2 of $r = -0.64$. The hypoxanthine concentrations were a better predictor of survival than were the other variables. Hypoxanthine also correlated better than pH or base deficit with short-term complications such as intracranial hemorrhage (71).

Plasma hypoxanthine has been used as a marker of hypoxia in adults as well as in newborns. High levels were found in mixed venous blood in a group of critically ill patients, the majority of whom suffered from the adult RDS. By contrast to blood gases, which were not helpful in predicting survival, hypoxanthine and other ATP degradation products proved to be good indicators of survival (72). When aortic flow was interrupted by cross-clamping during surgery, the hypoxanthine concentration increased and correlated well ($r = 0.85$) with clamping time, in contrast to lactate levels and changes in acid-base status (73). Augmented plasma hypoxanthine concentrations have also been found in diabetic ketoacidosis with rhabdolysis (74). In patients undergoing hemodialysis, the plasma hypoxanthine concentration increased simultaneously with decreases in arterial oxygen tension, demonstrating that routine hemodialysis, even in stable patients, is associated with some cellular hypoxia (76). Plasma hypoxanthine is high after exercise (19, 27, 77).

HYPOXANTHINE IN URINE

In newborn infants the urinary hypoxanthine excretion during the first 24 h of life is three to four times higher in babies suffering from intrauterine hypoxia compared with nonhypoxic babies (78). There was also a high correlation between the urinary hypoxanthine excretion and the hypoxanthine/creatinine ratio in the urine ($r = 0.70$) (79). Such a ratio can be determined on random urine samples, simplifying the collection of urine. Even on the 2nd day of life an elevated ratio was found in babies with intrauterine hypoxia. Infants who are neurologically abnormal more than 48 h postpartum have a higher hypoxanthine/creatinine ratio than hypoxic infants who subsequently developed normally (79, 80). In adult patients resuscitated after cardiac arrest, urinary excretion of hypoxanthine increased 6-fold the first 2 h after the cardiac arrest (81). In adult hypotensive patients there was a significant elevation of hypoxanthine + xanthine/creatinine clearance during periods of hypotension as compared with normotensive periods (82).

HYPOXANTHINE IN CEREBROSPINAL FLUID

Elevated levels of hypoxanthine in cerebrospinal fluid have been observed in patients with the Lesch-Nyhan syndrome (83). We found high hypoxanthine concentrations in the cerebrospinal fluid of newborn babies who suffered from hypoxia, as well as in children after convulsions (84). Children with convulsions of several types had elevated cerebrospinal fluid hypoxanthine concentrations. The highest levels were observed 30 to 120 min after the convulsion (85, 86). High hypoxanthine concentrations also have been found in children suffering from bacterial meningitis (84, 86). After hypoxia, a tremendous rise in cerebrospinal fluid hypoxanthine concentrations has been reported in children and adults, and such elevations can be detected for as long as 1 to 2 days after hypoxia (87). The mean normal cerebrospinal fluid hypoxanthine concentration seems to range between 0 and 3–5 µmol/liter in most studies (84, 86–88), whereas after severe intrauterine hypoxia levels up to 700 µmol/liter have been found (87). Mortality tends to be high in patients with high levels, and in one study the surviving babies with cerebrospinal fluid con-

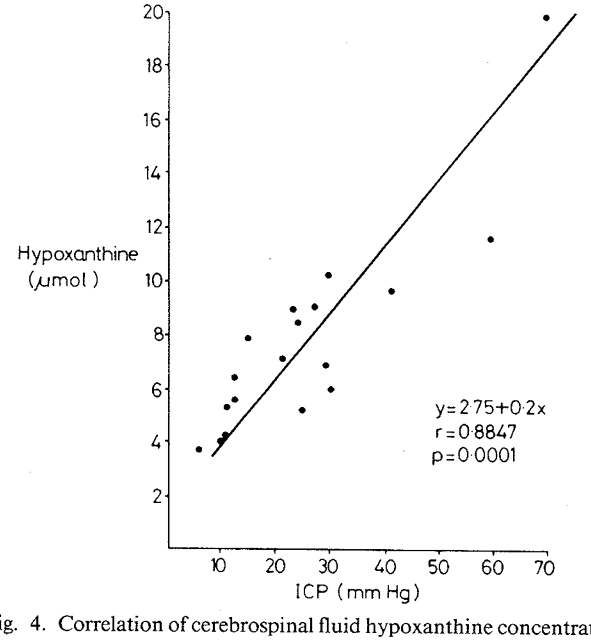

Fig. 4. Correlation of cerebrospinal fluid hypoxanthine concentration against mean intracranial pressure (*ICP*) over a 1-min period during rapid eye movement sleep in 18 hydrocephalic children. From Levin *et al.* (89) used with permission.

centrations of more than 50 μmol/liter had residual cerebral damage (87).

We measured the cerebrospinal fluid hypoxanthine concentration in newborn babies with hydrocephalus. The size of the ventricles was estimated by ultrasound, and high hypoxanthine concentrations were found when the ventricles were large. The concentration then decreased after lumbar tapping or shunting of the ventricles (88). We therefore suggested that the cerebrospinal fluid hypoxanthine concentration might be used as a guide to timing the treatment of hydrocephalus. Levin *et al.* (89) reported a highly significant correlation between the hypoxanthine concentration and the intracranial pressure ($r = 0.88$, $n = 18$) in hydrocephalic children (Fig. 4). This correlation supports the contention that hypoxanthine cerebrospinal fluid concentrations can be used to evaluate intracranial pressure. A third study recently confirmed these data showing a significant decrease in cerebrospinal fluid hypoxanthine concentration values after implantation of a shunt in nine hydrocephalic children with shunt failures (90).

Adult patients requiring cardiac resuscitation had cerebrospinal fluid hypoxanthine concentrations three times as high (18.7 μmol/liter) as the mean level (6.3 μmol/liter) in reference patients. Hypoxanthine was measured 0 to 5 h after resuscitation and decreased to normal levels within 20 h. However, in patients who remained comatose after resuscitation the mean hypoxanthine concentration was as high as 51.7 μmol/liter (91).

HYPOXANTHINE IN OTHER BODY FLUIDS

High hypoxanthine concentrations were found in the amniotic fluid of babies suffering from intrauterine hypoxia (92). In the corpus vitreum humor we found that hypoxanthine concentrations were stable up to 72 h postmortem (93). In adults who died from respiratory depression there were very high concentrations of hypoxanthine in the vitreous humor compared with values after acute death (93). The hypoxanthine concentration in vitreous humor seems to be about 10 times higher than the concentration in plasma even in the absence of hypoxia. In premature babies dying from hypoxia caused by RDS, high levels were found in the vitreous humor, values about eight to nine times higher than in babies dying without preceding hypoxia. Also, in these neonates there was a significant correlation between the vitreous humor hypoxanthine concentration and the duration of PaO_2 values less than 40 mm Hg (5.3 kPa) (29). Hypoxanthine determinations of the vitreous humor might therefore be used to evaluate whether death was preceded by hypoxia.

PLASMA HYPOXANTHINE CONCENTRATIONS IN CANCER

Adult patients with malignant lymphomas pretreated with the xanthine oxidase inhibitor allopurinol had a marked rise in hypoxanthine concentration 24 h after the onset of chemotherapy (Fig. 5). About 1 wk after the start of chemotherapy, values had returned to pretreatment levels (94). High plasma hypoxanthine concentrations recently were found in adults with leukemia (10) and in urine of children with leukemia (95). These findings may be of clinical interest for two reasons: 1) because it has been shown that hypoxanthine inhibits the effect of certain cytotoxic drugs (10, 96), and 2) because hypoxanthine is a potential oxygen radical generator. We know that several cytotoxic drugs exert their action through free radical production. High hypoxanthine concentrations could, in an uncontrolled way, potentiate both beneficial and adverse effects of these drugs.

NEUROCHEMICAL ASPECTS OF HYPOXANTHINE

Hypoxanthine has aroused particular interest because of its putative role as an endogenous ligand of benzodiazepine receptors in the brain and its possible role in termination of epileptic

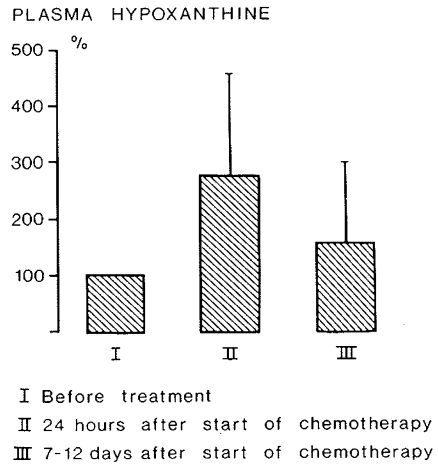

Fig. 5. Plasma hypoxanthine concentration in seven patients with malignant lymphomas pretreated with allopurinol. The initial level of each patient is set to 100%. Twenty-four h after the start of chemotherapy the hypoxanthine level was significantly elevated in mean (273%) compared with initial levels ($p < 0.01$). After approximately 1 wk hypoxanthine concentrations had decreased to a level not significantly different from initial levels (SD is given). From Saugstad (94) used with permission.

activity (8, 97, 98). It is also an inhibitor of cyclic nucleotide phosphodiesterase in bovine brain tissue (9). Recently the cerebrospinal fluid hypoxanthine concentration was measured in patients with major depressive disorders. The hypoxanthine concentration was positively correlated with monoamine metabolites and norepinephrine metabolites (99). The same investigators found that a suicidial tendency was related to low levels of hypoxanthine in the cerebrospinal fluid, whereas there existed a positive relationship between hypoxanthine levels and decreased appetite in such patients (100). There is at present a growing interest in the role of purines in neurotransmission (101).

HYPOXANTHINE AS AN OXYGEN RADICAL GENERATOR

A new and fascinating aspect of hypoxanthine relates to the discovery that it is a potential oxygen-free radical generator (5, 6). Free radicals are unstable compounds with one unpaired electron in their outer orbit. They are highly reactive, injuring cell membranes by peroxidation of unsaturated fatty acids. Oxygen radicals are created in many processes where oxygen is involved. When hypoxanthine (Hx) is oxidized to uric acid in the presence of xanthine oxidase, the superoxide radical O_2^- is formed:

$$Hx + O_2 \rightarrow \text{uric acid} + H_2O_2 + O_2^-$$

The superoxide radical can further react with the hydrogen peroxide produced by this process with the formation of the highly reactive hydroxyl radical:

$$H_2O_2 + O_2^- \rightarrow OH\cdot + OH^-$$

Recently it has been shown that oxygen radicals are involved in a series of disease processes. Possibly oxygen radicals formed through the hypoxanthine-xanthine oxidase system are a cause of the dramatic tissue injury seen after hypoxia in the reoxygenation period. This so-called "oxygen paradox" has been difficult to explain until recently. Because hypoxanthine accumulates in hypoxia and because oxygen is subsequently administered as treatment, large amounts of oxygen radicals can be assumed to be formed (7). Previous authors have suggested that some unknown substance(s) accumulating in hypoxia may be injurious to the tissues after hypoxia. Fridovich (102) stated: "Perhaps, the damage is really not sustained during hypoxia, but rather when

normal oxygenation is subsequently reestablished. Thus we can suppose that reductants accumulate during hypoxia, much as homogentisate accumulates in hepatocytes in hypoxia. The reductants need not, in themselves, be damaging, but since they can autoxidize, they could generate a burst of O_2^- and H_2O_2 in reoxygenation. The damage would then be caused by a temporary production of oxygen radicals, at a rate greater than could be accommodated by the defenses."

We had already suggested that the unknown accumulated metabolite was hypoxanthine (7) and other authors have subsequently confirmed this (15, 103, 104). The term "ischemia-reperfusion injury" has been introduced for this phenomenon (15, 103). It is a term that could be misleading because there are so many uncontrolled factors, in addition to hypoxanthine washout, during the reperfusion process. A better term for this phenomenon may be "posthypoxic-reoxygenation injury."

To test this hypothesis we performed an experiment in which young rats were infused intravenously with hypoxanthine while breathing 100% oxygen for 48 h. Compared with control animals breathing 100% oxygen and infused with glucose, or animals infused with hypoxanthine but breathing room air, we found hemorrhage and edema in the lungs (105). In lung lavage there was a many-fold increase in protein content. Despite a normal surfactant phospholipid profile, surfactant function was destroyed. This could be due to inactivation of surfactant either by direct peroxidation or by the presence of surfactant inhibitor. These data show that during these experimental conditions the combination of hypoxanthine and oxygen is toxic to the lungs by contrast to hypoxanthine or oxygen alone. The instillation of xanthine oxidase into the tracheas of guinea pigs reduces lung thorax compliance quite dramatically, an effect that can partly be prevented by superoxide dismutase (106).

Superoxide dismutase, a superoxide radical scavenger, and allopurinol, a xanthine oxidase inhibitor, protect against reperfusion injury in the intestine of cats and in the isolated rat heart (107, 108). In separated loops of the small intestine of rats where xanthine oxidase and hypoxanthine were instilled in combination, severe hemorrhage and edema ocurred. The hypoxanthine-xanthine oxidase system starts prostaglandin synthesis in the intestinal wall (109). This is in accordance with present knowledge that free radicals can trigger arachidonic acid synthesis (110). In one study where isolated rabbit lungs were perfused with a combination of xanthine oxidase and a purine, large amounts of thromboxane B2 were released and pulmonary vasoconstriction occurred (111).

AN HYPOTHESIS FOR AN OXYGEN RADICAL DISEASE IN NEONATOLOGY

It seems likely that production of oxygen radicals by the hypoxanthine-xanthine oxidase system after reoxygenation after hypoxia contribute importantly to a series of diseases in newborn babies. Animal studies document that the concentrations of the oxygen radical scavengers superoxide dismutase, glutathione peroxidase, and catalase are low before term and reach peak values around term (112–114). The preterm baby therefore has less defense against free radicals produced for example by the hypoxanthine-xanthine oxidase system as compared with the term baby. Bronchopulmonary dysplasia might be caused by such a mechanism. The lungs of preterm babies are probably exposed to higher hypoxanthine concentrations than a normal lung; in addition the former is often exposed to supplemental oxygen. Subsequently a higher flux of oxygen radical production might occur. Necrotizing enterocolitis could be, at least partly, caused by the same mechanism. The intestine contains large amounts of xanthine oxidase (13). Most of the described etiological factors in the development of necrotizing enterocolitis are conditions where oxygen radicals may be produced in excess by the hypoxanthine-xanthine oxidase system. One etiologic factor for necrotizing enterocolitis frequently mentioned is blood transfusion,

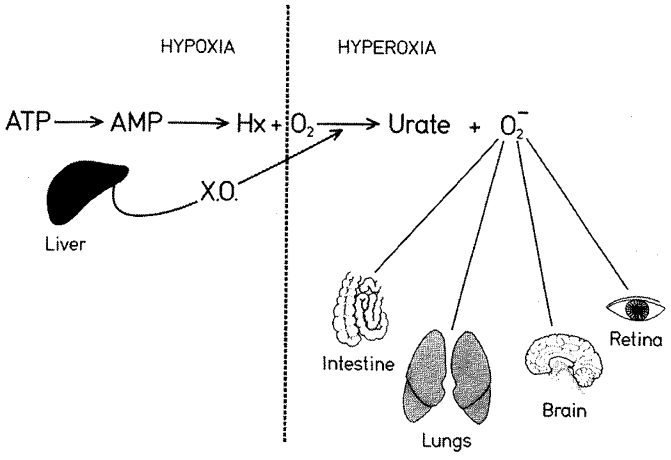

Fig. 6. Proposed mechanism of posthypoxic-reoxygenation injury. During hypoxia hypoxanthine is accumulated and xanthine oxidase (*X.O.*) is released from the liver into the circulation. During reoxygenation oxygen radicals are formed that might injure several organs. From Saugstad (117) used with permission from the author and W. B. Saunders.

and it is of interest that transfused blood has extremly high concentrations of hypoxanthine (2, 3, 115). Retinopathy of prematurity may also be caused by the same mechanism. It has long been known that hyperoxia cannot be the only etiologic factor in the development of this disease (116). One additional factor could be hypoxanthine, which we have found in extremly high concentrations in the eyes of babies dying of RDS (29). Whether there is any xanthine oxidase present in the retinal tissue or in the general circulation during hypoxia, is still unknown.

Figure 6 summarizes the concept (117) that hypoxanthine accumulates in hypoxia. Xanthine dehydrogenase is tranformed to xanthine oxidase in hypoxia and we hypothesize that it is simultaneously released from the liver into the circulation. During reoxygenation, oxygen radicals are formed in excess and different organs are attacked. Thus we may not be dealing with different diseases in bronchopulmonary dysplasia, necrotizing enterocolitis, or retinopathy of prematurity. Therefore, they may be different aspects of one disease; an "oxygen radical disease in neonatology," caused by a common pathogenic mechanism. However, the clinical manifestation of this disease differs according to which organ is most severely affected. Such a general hypothesis for the pathogenesis of these conditions may help us to understand a series of features that have been difficult to explain. However, more experimental and clinical data are required before the hypothesis can be fully accepted.

CONCLUSION

There is now a large body of literature demonstrating that hypoxanthine is a sensitive indicator of hypoxia. In addition, hypoxanthine is a more specific measure of hypoxia than lactate, base deficit, or pH and it reflects the intracellular energy status. One group of investigators recently suggested that hypoxanthine should serve as the new "gold" standard for hypoxia. More importantly, evidence is accumulating that hypoxanthine is a better predictor of hypoxic sequelae than other biochemical variables. More prospective studies are needed to confirm this association.

Because hypoxanthine is a potential generator of oxygen radicals, and may be the mediator of posthypoxic reoxygenation injury, it should be useful to monitor plasma hypoxanthine levels in sick patients. New insights into the possible role of hypoxanthine in neurobiology and cancer treatment are expected in the near future, as well as new knowledge of the modulatory effects of hypoxanthine on circulation and respiration. Ten yr ago hypoxanthine was considered to be an inert metabolite, today it

is acknowledged to play a central role in numerous processes in health and disease.

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