Hypoxanthine, Xanthine, and Uric Acid Concentrations in the Cerebrospinal Fluid, Plasma, and Urine of Hypoxemic Pigs

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ABSTRACT. The concentrations of hypoxanthine, xanthine, and uric acid in plasma and cerebrospinal fluid (CSF), as well as the urinary output of hypoxanthine and xanthine, were measured in four groups of pigs (three groups with different degrees of hypoxemia and one control group). During hypoxemia with arterial O_2 tension between 2.1 and 3.0 kPa [group 1, fractional inspired oxygen (FiO_2) = 0.08], hypoxanthine increased in CSF from a mean basal value of 18.1 to 39.3 μmol/L at death ($p < 0.02$), in plasma from 25.4 to 103.6 μmol/L ($p < 0.05$), and in urine from 21.3 to 87.1 nmol/kg/min ($p < 0.02$). Xanthine changed in a similar way: in CSF from 4.0 to 10.6 μmol/L ($p < 0.02$), in plasma from 0.7 to 48.1 μmol/L ($p < 0.02$), and in urine from 4.0 to 12.6 nmol/kg/min ($p < 0.05$). Uric acid increased in CSF from 2.7 to 11.6 μmol/L ($p < 0.05$), and in plasma from 15.4 to 125.0 μmol/L ($p < 0.02$). During hypoxemia with arterial O_2 tension between 3.0 and 4.0 kPa (group 2, FiO_2 = 0.11), hypoxanthine increased in the CSF from 14.7 to 42.9 μmol/L ($p < 0.02$). Plasma hypoxanthine increased from 20.3 to a maximum of 44.1 μmol/L ($p < 0.02$), but decreased to initial values by the time of death. The urinary excretion of hypoxanthine increased from 13 to 54 nmol/kg/min ($p < 0.02$). Xanthine increased in the CSF from 3.9 to 13.3 μmol/L ($p < 0.02$), in plasma from 0.6 to 36.6 μmol/L ($p < 0.02$), and in urine from 6 to 25 nmol/kg/min ($p < 0.02$). Uric acid increased in CSF from 3.1 to 16.3 μmol/L ($p < 0.02$), and in plasma from 15.3 to 208 μmol/L ($p < 0.02$). During milder hypoxemia with arterial O_2 tension between 4.3 and 5.6 kPa (group 3, FiO_2 = 0.14), or in the control group (group 4, FiO_2 = 0.21), neither of the metabolites changed significantly. (*Pediatr Res* 28: 477–481, 1990)

Abbreviations

CSF, cerebrospinal fluid
FiO_2, fractional inspired oxygen
PaO_2, arterial O_2 tension
$PaCO_2$, arterial CO_2 tension

Hypoxia is a common insult during the perinatal and neonatal period. Several biochemical measurements can be done to eval-

uate hypoxia, but to correlate a biochemically measured value with the outcome of hypoxia is difficult. Measurements of the purine metabolite hypoxanthine can be used as an indicator of hypoxia (1). During tissue hypoxia, elevated hypoxanthine levels have been found in plasma (2–5), CSF (6, 7), and urine (8). However, no clear definition of clinical hypoxia is available, and many authors do not distinguish between the terms hypoxemia and hypoxia, which adds to the confusion. Our meaning of hypoxia has previously been described (1). In our study the pigs were subjected to hypoxemia. We define hypoxemia as deficient oxygenation of the blood. Hypoxemia may, however, not necessarily lead to tissue hypoxia.

To further assess the usefulness of hypoxanthine as a biochemical marker of hypoxia, we report how three degrees of hypoxemia in young pigs influence the level of hypoxanthine, xanthine, and uric acid in the CSF and plasma, as well as the pigs' urinary excretion of hypoxanthine and xanthine.

MATERIALS AND METHODS

Approval. The local hospital's ethical committee for animal studies approved these experiments.

Animal model. The animal model was similar to that previously described (9). Twenty-two young pigs of either sex (the males had been castrated shortly after birth) weighing 17–22 kg (mean 18.1 kg) were used. They were anesthetized with an intraperitoneal injection of sodium pentobarbital (30 mg/kg). An i.v. line was established through an ear vein, and a continuous infusion of Ringer acetate (10 mL/kg/h) was given during the experiment. A further 100 mg of sodium pentobarbital was given i.v. if necessary every 30 min. A cuffed tube was placed in the trachea via a tracheostomy. Artificial ventilation was given by a ventilator (Servo-ventilator 900B, Elema-Schönander, Stockholm, Sweden). Ventilation rate and tidal volume were adjusted until $PaCO_2$ ranged between 4 and 5.3 kPa (30–40 mm Hg). No further adjustments of the ventilator were done during the hypoxemic period.

A midline suprapubic laparotomy was performed and a urinary catheter was placed in the bladder for measuring diuresis and urinary hypoxanthine and xanthine.

Polyethylene catheters were inserted into a femoral artery and a femoral vein. The arterial catheter was used for taking blood samples and for measuring blood pressure continuously with a strain gauge transducer, Gould recorder 2600S (Gould Inc. Recording Systems, Cleveland, OH). The venous catheter was used for giving Ringer acetate plus additional sodium pentobarbital.

The pigs were divided into three hypoxemic and one control group. Seven animals were made hypoxemic by artificial ventilation with 8% oxygen in nitrogen (FiO_2 = 0.08) (group 1). Seven

animals were ventilated with 11% oxygen (FiO₂ = 0.11) (group 2). Five animals were ventilated with 14% oxygen (FiO₂ = 0.14) (group 3), and three animals were ventilated with room air (FiO₂ = 0.21), serving as normoxemic controls (group 4). The oxygen concentrations were measured continuously in all pigs during the experiments with a Dameca OM 831 oxygen monitor (Dameca Inc., Copenhagen, Denmark).

During hypoxemia with FiO₂ = 0.08, the PaO₂ remained between 2.1 and 3.0 kPa (15.8–22.5 mm Hg). This degree of hypoxemia lasted until the animals died after a mean of 170 min (range 120–240 min). During hypoxemia with FiO₂ = 0.11, the PaO₂ remained between 3.0 and 4.0 kPa (22.5–30 mm Hg), and these pigs died after a mean of 283 min (range 165–445 min). During the mildest hypoxemia (FiO₂ = 0.14), the PaO₂ remained between 4.3 and 5.6 kPa (32.5–42 mm Hg). All animals tolerated this degree of hypoxemia for 480 min, after which they were killed with an i.v. overdose of sodium pentobarbital. The pigs in the control group were observed from 185 to 480 min and then killed. During artificial ventilation, 1 mL of pancuronium bromide (Pavulon 2 mg/mL, Organon Teknika, Boxtel, Netherlands) was given i.v. every 30 min to prevent muscular contractions, which may lead to artificially high hypoxanthine levels (10).

Blood samples. Arterial blood samples were taken after a stabilizing period of at least 1 h after surgery but before hypoxemia was initiated (basal values). During hypoxemia, blood samples were withdrawn at 5, 15, and 30 min and then every 30 min as long as the experiment lasted. Blood gases were measured with an AVL 945 Automatic Blood Gas System (AVL Biomedical Instruments, Schaffhausen, Switzerland). Another mL of blood was taken into heparinized tubes before hypoxemia and then every 30 min for hypoxanthine and xanthine analyses. In groups 1 and 2, uric acid was also measured in the blood samples. The withdrawn blood was immediately replaced with an equal volume of sterile NaCl (0.9%).

Sampling of CSF. A lumbar puncture needle was placed in the cisterna magna during the experiment. Two hundred to 400 μL of CSF were drawn from this needle at the same sampling times as previously described for blood hypoxanthine.

The samples of blood and CSF were immediately centrifuged for 10 min at 1800 × g, 4°C, to remove cells. The plasma and clear CSF were transferred into polypropylene tubes and frozen at −20°C until the time of analysis.

Sampling of urine. Urine was continuously collected into a catheter bag during the experiments. Before hypoxemia and then every 30 min as long as the experiment lasted, the amount of urine was measured and a 1-mL sample was frozen for later measurements of hypoxanthine and xanthine. The concentrations were related to diuresis, and the amount excreted per min per kg body weight was calculated.

Analysis of hypoxanthine, xanthine, and uric acid concentrations. The hypoxanthine and xanthine levels in CSF, plasma, and urine were analyzed using an HPLC method (11, 12).

The plasma samples were prepared by centrifugation for 30 min through a filter with 30 000 mol wt cut-off (YMT-filter, MPS-1 System, Amicon Corp., Danvers, MA).

Using a syringe, the other fluids were passed through a 4-mm Millex-HV₄ unit with 0.45-μm pore size (Millipore Corp., Bedford, MA).

The chromatography was performed on a reversed-phase column (0.45 × 15 cm) of 5-μm material (Pecosphere-5C C18, Perkin-Elmer Corp., Norwalk, CT). The mobile phase used for the chromatography was 0.005 M KH₂PO₄, pH 5.7. The eluting compounds were detected at 254 nm.

Identification of the peaks in the HPLC effluents were made on the basis of retention times. The retention times were compared with retention times of known pure standards that were measured routinely after every 4th sample. Primarily, the peak height ratios at 254 *versus* 280 nm were measured for all four body fluids. Small changes in retention times or double peaks

were checked by coelution with known pure standards. This was especially necessary at low xanthine concentrations.

Quantification of peaks was done by peak height ratios. All pure standards used for the HPLC were obtained from Sigma Chemical Co., St. Louis, MO.

Uric acid was measured on an RA-1000 (Technicon Instruments, Tarrytown, NY), automatic blood analyzer.

Statistics. Results are given as mean ± SD. Wilcoxon's two-tailed rank test was applied for testing the significance of differences between comparable groups. A *p* value <0.05 was considered statistically significant.

RESULTS

Blood gases and mean arterial pressure. Table 1 shows the arterial PaO₂, O₂ saturation, and mean arterial blood pressure in the four groups of pigs. There were no differences between the groups before the start of hypoxemia. In the animals with the two most severe degrees of hypoxemia, there was an initial increase in blood pressure at the start of the hypoxemia, lasting for about 1 h. The blood pressure in both these groups then gradually declined toward zero in the terminal phase, as previously described (13). No significant changes in the mean PaCO₂ were found in any group.

Hypoxanthine, xanthine, and uric acid in CSF. The mean concentrations of hypoxanthine in the CSF of the pigs before the start of the hypoxemia was 15.8 ± 4.1 μmol/L, with no difference between the four groups. The changes between prehypoxemic, basal values and the values found during the hypoxemia and at death for groups 1 and 2 are illustrated in Figure 1*a* and *b*, respectively. In these groups, there was a linear increase in CSF hypoxanthine concentrations reaching a maximum at death of 39.3 ± 6.2 μmol/L in group 1 (*p* < 0.02 compared with basal values) and 42.9 ± 20.8 μmol/L (*p* < 0.02) in group 2. There was no difference at the time of death between groups 1 and 2. In the two other groups, there was no significant change in the hypoxanthine concentration in the CSF (results not shown).

The mean xanthine concentration in the CSF of all pigs was 4.0 ± 0.8 μmol/L before hypoxemia. In groups 1 and 2, there were linear increases to 10.6 ± 4.8 μmol/L (*p* < 0.02) and 13.3 ± 5.5 μmol/L (*p* < 0.02), respectively, at the time of death (Fig. 2), whereas the concentrations remained unchanged in the other two groups. There was no difference between the final concentrations of xanthine in groups 1 and 2.

The mean uric acid concentration in the CSF in groups 1 and 2 was 2.9 ± 2.8 μmol/L before hypoxemia, increasing to 11.6 ±

Table 1. *Blood gases, arterial oxygen saturation, and mean arterial blood pressure before and during induced hypoxemia in four groups of pigs ventilated with 8 (group 1), 11 (group 2), 14 (group 3), and 21% oxygen (group 4)**

	O₂%	Before hypoxemia	5 min hypoxemia	60 min hypoxemia	Last sample before death
PaO₂ (kPa)	8	11.6 ± 1.2	2.8 ± 0.4	2.6 ± 0.1	2.9 ± 0.5
	11	10.8 ± 1.0	4.5 ± 1.0	3.5 ± 0.7	3.5 ± 0.4
	14	13.0 ± 0.8	6.2 ± 1.1	5.0 ± 0.8	4.5 ± 0.9
	21	11.5 ± 2.6			10.7 ± 0.6
Sat O₂ (%)	8	97.6 ± 0.9	42.1 ± 10.0	31.4 ± 4.2	25.4 ± 7.6
	11	97.3 ± 1.0	73.5 ± 12.1	53.0 ± 15.4	41.4 ± 9.3
	14	98.1 ± 0.4	85.0 ± 6.3	74.8 ± 7.7	70.1 ± 10.9
	21	96.8 ± 2.2			97.3 ± 0.6
MABP (mm Hg)	8	105 ± 15	135 ± 20	115 ± 20	30 ± 10
	11	120 ± 15	135 ± 20	140 ± 20	50 ± 20
	14	115 ± 30	120 ± 35	105 ± 25	115 ± 30
	21	110 ± 25			115 ± 10

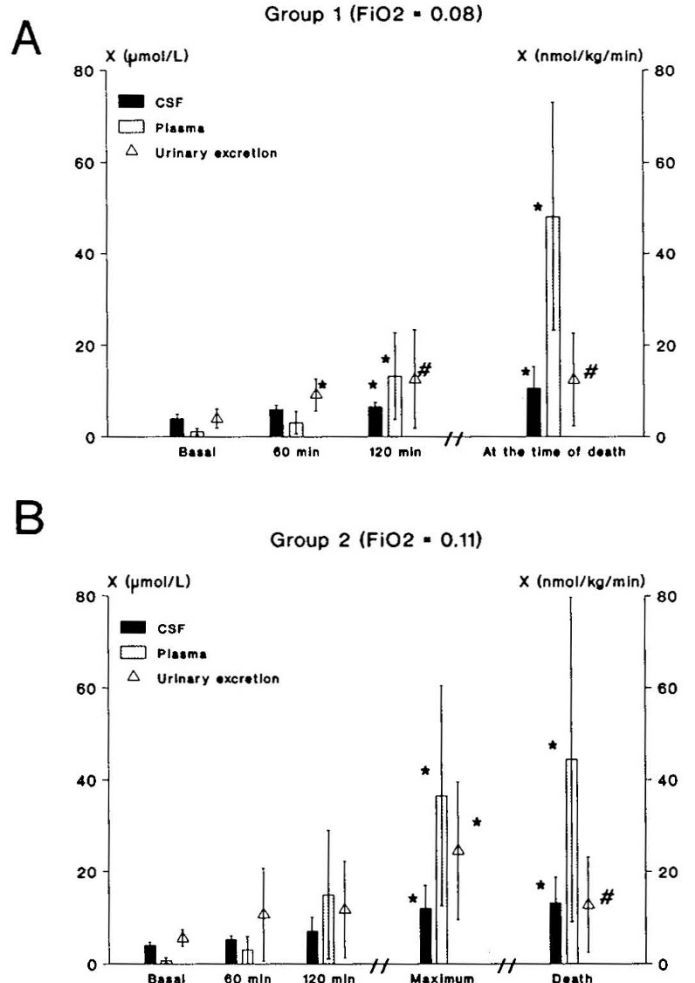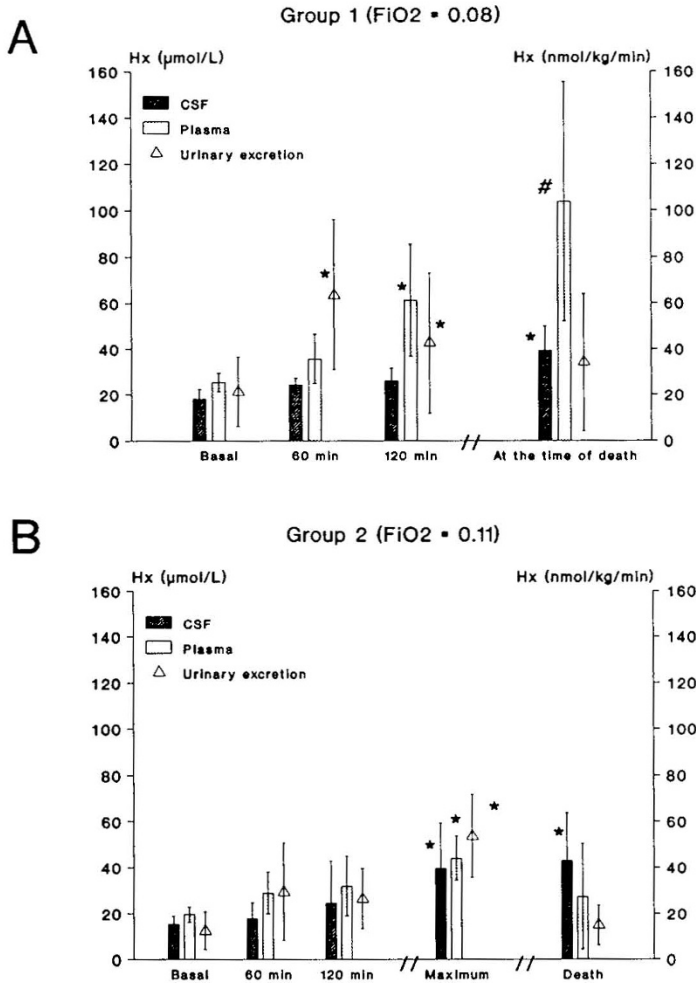* Figures given are mean ± SD. PaO₂, arterial O₂ tension; Sat O₂, arterial O₂ saturation; MABP, mean arterial blood pressure.

Fig. 1. Concentrations in the CSF and plasma and urinary excretion of hypoxanthine. Hypoxanthine (*Hx*) concentrations (μmol/L) and urinary excretion (nmol/kg/min) were measured before hypoxemia (*basal*), after 60 and 120 min of hypoxemia, and at the time of death in hypoxemic pigs. *A*, Group 1, $n = 7$, $FiO_2 = 0.08$; *B*, group 2, $n = 7$, $FiO_2 = 0.11$. The maximum is the highest concentration recorded during the hypoxemic period in the live animal. The maximal concentrations were not necessarily reached at the same time for the three body fluids during the hypoxemic period. $*p < 0.02$ compared with values before hypoxemia. $\#p < 0.05$ compared with values before hypoxemia.

Fig. 2. Concentrations in the CSF and plasma and urinary excretion of xanthine. Xanthine (*X*) concentrations (μmol/L) and urinary excretion (nmol/kg/min) were measured before hypoxemia (*basal*), after 60 and 120 min of hypoxemia, and at the time of death in hypoxemic pigs. *A*, group 1, $n = 7$, $FiO_2 = 0.08$; *B*, group 2, $n = 8$, $FiO_2 = 0.11$. The maximum is the highest concentration recorded during the hypoxemic period in the live animal. The maximal concentrations were not necessarily reached at the same time in the three body fluids during the hypoxemic period. $*p < 0.02$ compared with values before hypoxemia. $\#p < 0.05$ compared with values before hypoxemia.

11.7 μmol/L at the time of death in group 1 ($p < 0.05$), and to 16.3 ± 16.8 μmol/L at the time of death in group 2 ($p < 0.02$). There was no difference between the final concentrations of uric acid in groups 1 and 2. Uric acid was not measured in groups 3 and 4.

Hypoxanthine, xanthine, and uric acid in plasma. The mean plasma concentration of hypoxanthine in the pigs was 21.8 ± 4.4 μmol/L before the start of hypoxemia. There was no difference between the four groups. Figure 1*a* illustrates the variation of plasma hypoxanthine in group 1. In all the pigs in this group, plasma hypoxanthine increased significantly during hypoxemia. However, in one pig, the hypoxanthine concentration toward death fell below the starting value (start, 23 μmol/L; maximum during hypoxemia, 39 μmol/L; at time of death, 11 μmol/L). In the other six pigs, hypoxanthine concentrations continued to increase up to the time of death, resulting in an average terminal value in the seven pigs of 103 ± 51.7 μmol/L ($p < 0.05$). Figure 1*b* shows that the plasma concentration of hypoxanthine increased significantly in pigs breathing 11% oxygen (group 2) as well, reaching a mean maximum of 44.1 ± 9.5 μmol/L ($p < 0.02$). However, also in this group, three of the pigs had a fall in concentrations terminally below the starting value or even to

zero, resulting in an average value at the time of death of 27.0 ± 23 μmol/L (not statistically different from either the prehypoxemic value or the maximum value during hypoxemia). The plasma concentrations of hypoxanthine in the other two groups changed only marginally during the experiment (results not shown).

When relating all our measurements of plasma hypoxanthine to the actual PaO_2 of the pigs, we found that the hypoxanthine concentration rose when the PaO_2 was lower than approximately 4 kPa (30 mm Hg) (Fig. 3).

The mean plasma concentration of xanthine was 0.7 ± 0.7 μmol/L before the start of hypoxemia. There were no significant differences among the four groups. In group 1, plasma concentration of xanthine continued to rise in all seven pigs until death, with a terminal mean level of 48.1 ± 24.9 μmol/L, ($p < 0.02$ compared with prehypoxemic levels) (Fig. 2*a*). In group 2 (Fig. 2*b*), plasma xanthine concentrations increased until death in all but one pig, where the concentration fell to zero terminally, resulting in a mean concentration measured just after death of 44.4 ± 37.0 μmol/L ($p < 0.02$ compared with basal levels). There was no difference between the terminal xanthine concentrations found in groups 1 and 2.
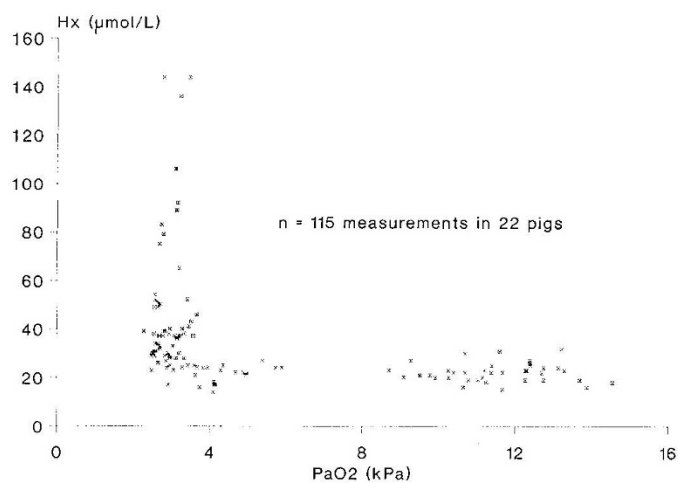
Fig. 3. Hypoxanthine concentrations in plasma in relation to PaO_2 in pigs.

The mean plasma concentration of uric acid in groups 1 and 2 was 15.4 ± 2.7 μmol/L before hypoxemia. The concentration of uric acid continued to rise in all pigs in these groups, even when hypoxanthine or xanthine concentrations fell, resulting in a maximum of 125 ± 32 μmol/L at the time of death in group 1 ($p < 0.02$), and 208 ± 148 μmol/L at the time of death in group 2 ($p < 0.02$). There was no difference between terminal uric acid concentrations found in groups 1 and 2.

The maximum hypoxanthine concentrations were generally reached earlier in the hypoxemic period than the maximum xanthine concentrations.

When considering the total amount of hypoxanthine and xanthine in plasma, we found a starting value of 25.5 ± 3.8 μmol/L and a terminal value of 151.7 ± 50.0 μmol/L in group 1 ($p < 0.02$ compared with basal values) and, in group 2, a basal value of 20.3 ± 3.4 μmol/L, a maximum value during hypoxemia of 80.7 ± 31.3 μmol/L ($p < 0.02$ compared with basal values), and a terminal value of 68.0 ± 41.3 μmol/L, ($p < 0.05$ compared with basal value, but NS compared with maximum values). The terminal total amount of hypoxanthine and xanthine in plasma was significantly higher in group 1 than in group 2 ($p < 0.05$).

In groups 3 and 4, there was no significant increase either of hypoxanthine or of xanthine in plasma during the experiments (results not shown). In these groups, uric acid was not measured.

Hypoxanthine and xanthine in urine. The mean basal excretion of hypoxanthine in the urine was 17 ± 12 nmol/kg/min. There were no significant differences between the groups. In group 1, during hypoxemia there was a nearly 5-fold increase in the amount of hypoxanthine excreted in the urine, reaching a mean maximum of 87 ± 27 nmol/kg/min ($p < 0.02$). In the last measurements before death, the mean values had declined toward basal levels (Fig. 1*a*). The urinary excretion of xanthine in this group followed the same pattern described for hypoxanthine (Fig. 2*a*). Figure 1*b* shows, in addition, the urinary excretion of hypoxanthine in group 2. In this group, there was, compared with basal values, a nearly 3-fold increase in the rate of hypoxanthine excretion, reaching a mean maximum value of 54 ± 18 nmol/kg/min ($p < 0.02$). In the last measurement before death, the mean values had dropped significantly, reaching levels recorded before hypoxemia, 15 ± 9 nmol/kg/min. In the hypoxemic animals breathing 14% oxygen (group 3), there was a 2-fold increase in the rate of hypoxanthine excretion, reaching a mean maximum value of 33 ± 15 nmol/kg/min, but this increase was not statistically significant.

The urinary excretion of xanthine in group 2 followed the same pattern as that of hypoxanthine (Fig. 2*b*).

There was no significant increase in urinary xanthine excretion in the pigs breathing 14% oxygen.

In the control group, neither hypoxanthine nor xanthine excretion changed.

DISCUSSION

We found a higher basal concentration of hypoxanthine in CSF of pigs (15.8 μmol/L) than has been reported in normal human newborns, children, and adults (3.5 μmol/L) (14). During hypoxemia in both groups 1 and 2, the hypoxanthine concentrations increased about 2.5-fold in the CSF. Nevertheless, when comparing CSF concentrations with plasma concentrations, we found a different pattern during the two degrees of hypoxemia. In group 2 (the milder degree), at death the concentrations of hypoxanthine in the CSF and in the plasma were about equal. In comparison, when the pigs died after the most severe hypoxemia (group 1), the plasma level of hypoxanthine was found to be two to three times higher than the CSF level. This indicates that hypoxanthine in the CSF is regulated by a different mechanism than plasma hypoxanthine. An active transport system in the choroid plexus for hypoxanthine has been found (15), and presumed to act to remove hypoxanthine from the CSF. However, Spector (16) stated that the directionality, if any, for transport by that system is not known. From our results, we cannot say whether hypoxanthine is transported from the CSF to blood or vice versa. The differences between the two body fluids during hypoxemia became even more obvious when we looked at the changes in xanthine and uric acid concentrations. In the CSF, xanthine increased about three-fold, whereas in the plasma we found a 60- to 70-fold increase in concentrations. The increases in uric acid concentrations were less marked, about five-fold in the CSF and 14-fold in the plasma.

The concentrations measured in the CSF after hypoxemia correspond well with the findings of Harkness and Lund (14) in children after hypoxia. In their report, however, there is no mention of blood gases or the duration of the hypoxic episodes. Furthermore, their measurements were done 1 to 2 d after the hypoxic episode, so a direct comparison is difficult.

In several pigs from the two most severely hypoxemic groups, a fall in both plasma hypoxanthine and xanthine concentration after an initial peak was found, comparable to data we previously have published from hypoxemic dogs (17). In the dog, there is, however, a large lung metabolism of hypoxanthine and xanthine to uric acid due to the presence of xanthine oxidase (EC 1.1.3.22) (18). Until now, we believed that this enzyme was not present to any extent in the pig lungs (9). If xanthine oxidase is present in the pig lungs, or if xanthine oxidase is liberated into the circulation by a severely hypoxemic liver, this species may have the capacity to clear hypoxanthine from the circulation even during profound hypoxemia. This may also explain the different pattern found between CSF and plasma, with a steady increase in CSF, whereas in some of the pigs hypoxanthine fell in plasma towards the end of the experiments. Another possible explanation may be the presence of the enzyme liver aldehyde oxidase (EC 1.2.3.1). In the rabbit, this enzyme has the capability to catalyze the conversion of hypoxanthine to xanthine, but not the conversion of xanthine to uric acid (19). Concentrations of hypoxanthine and xanthine may also be altered independently, inasmuch as xanthine can arise from breakdown of guanine. The finding of approximately equal xanthine concentrations at the time of death in both CSF and plasma in both group 1 and group 2 may indicate that under hypoxemic conditions only a certain limited amount of hypoxanthine will be converted to xanthine.

Urinary excretion, especially of hypoxanthine, might be a sensitive method for studying adenine nucleotide catabolism in man. There is a high renal clearance of hypoxanthine (20), and acute changes in plasma hypoxanthine concentrations may therefore be reflected in the urine. The renal clearance of xanthine, however, can exceed creatinine clearance, suggesting a tubular origin for at least some of the excreted xanthine (10). Urinary xanthine will therefore not reflect plasma xanthine as sensitively

as urinary hypoxanthine will reflect plasma hypoxanthine. In normal human subjects, there are only a few reports on renal transport/urinary excretion of hypoxanthine and xanthine (10, 21). In the two most severely hypoxemic groups, the pigs increased their urinary excretion of hypoxanthine and xanthine shortly after the hypoxemia was initiated. We found, however, a tremendous drop in urinary hypoxanthine excretion toward death, but these animals had at that time a fall in blood pressure that probably resulted in reduced or even ceased renal perfusion.

In summary, CSF hypoxanthine reflects hypoxemia, but it seems to be partly independent on the degree and duration. The plasma hypoxanthine concentration reflects the degree of hypoxemia more sensitively in severe than in mild hypoxemia, but the duration of hypoxemia seems to play a smaller part than the degree. There is no clear relationship between concentrations of hypoxanthine found in the CSF and concentrations found in plasma. If the hypoxemia leads to death, postmortem measurements of hypoxanthine in the vitreous humor may give an indication of the duration of hypoxemia *ante-mortem* (22). Urinary concentrations of hypoxanthine may be a sensitive method of studying adenine nucleotide catabolism, but a meaningful result can only be obtained if the diuresis is known.

REFERENCES

1. Saugstad OD 1988 Hypoxanthine as an indicator of hypoxia: its role in health and disease through free radical production. Pediatr Res 23:143–150
2. Saugstad OD 1975 Hypoxanthine as a measurement of hypoxia. Pediatr Res 9:158–161
3. Thiringer K 1983 Cord plasma hypoxanthine as a measure of fetal hypoxia. Acta Paediatr Scand 72:231–237
4. Pietz J, Guttenberg N, Gluck I 1988 Hypoxanthine: a marker for asphyxia. Obstet Gynecol 72:762–766
5. Ruth V, Fyhrquist F, Clemons G, Raivio KO 1988 Cord plasma vasopressin, erythropoietin and hypoxanthine as indices of asphyxia at birth. Pediatr Res 24:490–494
6. Meberg A, Saugstad OD 1978 Hypoxanthine in cerebrospinal fluid in children. Scand J Clin Lab Invest 38:437–440
7. Manzke H, Staemmler W 1981 Oxypurine concentrations in CSF in children with different diseases of the nervous sytem. Neuropediatrics 12:209–214
8. Manzke H, Dörner K, Grünitz J 1977 Urinary hypoxanthine, xanthine, and uric acid in newborn infants with perinatal complications. Acta Paediatr Scand 66:713–717
9. Saugstad OD, Aasen AO 1980 Plasma hypoxanthine concentrations in pigs. A prognostic aid in hypoxia. Eur Surg Res 12:123–129
10. Harkness RA 1988 Hypoxanthine, xanthine and uridine in body fluids, indicators of ATP deletion. J Chromatogr 429:255–278
11. Wung WE, Howell SB 1980 Simultaneous liquid chromatography of 5-fluorouracil, uridine, hypoxanthine, xanthine, uric acid, allopurinol and oxypurinol in plasma. Clin Chem 26:1704–1708
12. Simmonds RJ, Harkness RA 1981 High-performance liquid chromatographic methods for base and nucleoside analysis in extracellular fluids and in cells. J Chromatogr 226:369–381
13. Saugstad OD, Aasen AO, Hetland Ø 1978 Plasma hypoxanthine levels in pigs during acute hypoxemia. Eur Surg Res 10:314–321
14. Harkness RA, Lund RJ 1983 Cerebrospinal fluid concentrations of hypoxanthine, xanthine, uridine and inosine: high concentrations of the ATP metabolite, hypoxanthine, after hypoxia. J Clin Pathol 36:1–8
15. Nelson JA, Cserr HF 1976 Transport and metabolism of purines by isolated choroid plexus, liver and brain in the spiny dogfish, *Squalus acanthias.* Comp Biochem Physiol [B]53:371–377
16. Spector R 1987 Hypoxanthine transport through the blood-brain barrier. Neurochem Res 12:791–796
17. Saugstad OD, Bø G, Østrem T, Aasen AO 1977 Hypoxanthine levels of the plasma during hypoxemia in dogs. Eur Surg Res 9:23–33
18. Al-Khalidi UAS, Chaglassian TH 1965 The species distribution of xanthine oxidase. Biochem J 97:318–320
19. Krenitsky TA, Neil SH, Elion GB, Hitchings GH 1972 A comparison of the specificities of xanthine oxidase and aldehyde oxidase. Arch Biochem Biophys 150:585–599
20. Harkness RA, Coade, SB, Walton KR, Wright D 1983 Xanthine oxidase deficiency and "Dalmatian" hypouricaemia: incidence and effect of exercise. J Inherited Metab Dis 6:114–120
21. Kaneko K, Fujimori S, Itoh H, Nakayama Y, Oyama H, Kanbayashi T, Miyashita H, Akaoka I 1988 Renal handling of hypoxanthine and xanthine in normal subjects and in four cases of idiopathic renal hypouricemia. J Rheumatol 15:325–330
22. Poulsen JP, Rognum TO, Øyasæter S, Saugstad OD 1990 Changes in oxypurine concentrations in vitreous humor of pigs during hypoxemia and *post-mortem.* Pediatr Res 28:482–484