Intrapartum hypoxia: the association between neurological assessment of damage and abnormal excretion of ATP metabolites

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SUMMARY A series of 29 newborn infants had been studied after intrapartum hypoxia defined as meconium aspiration, an Apgar score of ≤ 6 at 5 min or a peripheral blood pH of 7.2 or less after resuscitation. Two independent sets of techniques were used; one concerned with the critical system in hypoxic damage, the central nervous system, the other assessing the central biochemical events in hypoxia. Both sets of data were assembled, then graded separately and only then combined. In this way detailed neurological assessment has been combined with measurement of urinary excretion of the ATP metabolites, hypoxanthine and xanthine. The essential metabolic consequence of hypoxia is a reduction in the synthesis of the energy currency of cells, ATP. This is associated with an outflow of ATP metabolites from cells.

The extent of neurological damage was related to the magnitude of the hypoxanthine and xanthine excretion; neither were closely related to the initial blood pH. Infants who were normal neurologically had normal oxypurine excretion. Infants with neurological abnormalities for less than 48 h had lower excretion than those who were abnormal for more than 48 h.

The duration of abnormal oxypurine excretion after an acute episode of hypoxia was studied in two infants with respiratory distress and in two other infants with apnoeic attacks. Severe hypoxia was followed by abnormal oxypurine excretion for at least 40 h after an acute episode. It is justifiable to suggest that abnormalities of oxypurine excretion should indicate intrapartum hypoxia in newborn infants. This excretion should also quantify the metabolic damage.

Intrauterine hypoxia is one of the main contributors to perinatal morbidity and mortality and to persistent neurological impairment.¹ There is also evidence of its possible importance to the pathogenesis of speech and language² and endocrine³ defects in older children.

Despite general agreement on the importance of intrapartum hypoxia, the methods for its diagnosis lack specificity and the methods for its assessment are difficult to quantify especially for comparison.^{4–6} A cumulative measure of the metabolic damage due to hypoxia would therefore be helpful.

The metabolic damage due to hypoxia can be measured by the reduction in intracellular ATP.

Because serial tissue samples are not available in clinical practice, means of estimating ATP breakdown by non-invasive methods are needed. Since some of the metabolic products of ATP breakdown, hypoxanthine and xanthine, can escape from the cell, concentrations of these oxypurines in extracellular fluids can reflect ATP breakdown. These oxypurines can now be estimated specifically by methods of sufficient sensitivity.⁷ A significant negative correlation between hypoxanthine and xanthine concentrations and relative ATP concentration has been shown in human placenta.⁸ Clinically the raised hypoxanthine and xanthine concentrations in meconium-stained amniotic fluid and in the later stage of labour⁹ with the raised urinary hypoxanthine concentrations in newborn associated with birth complications¹⁰ suggested that renal excretion of oxypurines by newborn infants could reflect intrapartum hypoxia.

We report increased hypoxanthine and xanthine

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excretion by infants showing neurological damage in a group defined as suffering from intrapartum hypoxia by one or more of the criteria of meconium aspiration, Apgar score of ≤ 6 at 5 min, or a blood pH of ≤ 7.2 at early postnatal sampling. After an acute period of hypoxia in four newborn infants the prolonged increase in oxypurine excretion suggested that increased excretion can quantify serious metabolic damage at least throughout the period of labour. A possible explanation for the persistence of increased oxypurine excretion 40 or more hours after the return of oxygen supply to normal is suggested.

Materials and methods

CLINICAL DATA

Northwick Park is a large general hospital, with a perinatal department, delivering 3400 infants per year. High-risk deliveries are attended by paediatricians who carry out all the neonatal resuscitation. Babies of more than 34 wk gestation, in the absence of sedation by maternal drugs, were defined as asphyxiated if the Apgar score at 5 min was ≤ 6 , if arterial or well perfused capillary blood pH was ≤ 7.2 after resuscitation or if there was meconium aspiration—that is, meconium seen in the trachea or typical clinical and radiological features of meconium aspiration). During a period of 16 months, 40 infants met these criteria. All significantly asphyxiated infants were admitted to the Special Care Baby Unit and to reduce cerebral oedema, fluids were restricted to 30–40 ml/kg per day for the first 48 h and then 60 ml/kg per day on the third day. Urine was collected from 29 infants, generally in the first 72 h, using adhesive bags. Every 4 to 8 h, the urine sample was removed, labelled with the times of the beginning and end of the collection and frozen at -20°C . It was necessary to discard samples from which leaks were noted. Severely affected infants did not pass collectable volumes of urine for variable periods after birth; in these cases collections were started as soon as practicable.

Babies were carefully monitored for apnoea, arterial or transcutaneous PO_2 , plasma glucose, bilirubin and calcium, blood pressure, renal and cardiac function. Intravenous fluids were given initially to the more seriously ill babies. A daily neurological examination was carried out by one of us (AGLW) using the examination of Dubowitz and Dubowitz¹¹ which used visual and auditory responses as well as posture, active and passive tone in the trunk and limbs, tendon reflexes and neonatal responses such as the Moro and grasp. Neck extensor hypertonia, a good sign of insult to the central nervous system,¹²

was specifically looked for in all babies. The degree of irritability and consolability was noted as well as any sedation given. Seizures were described and timed by the nurses or AGLW. Phenobarbitone and paraldehyde were used as anticonvulsants.

The neurological classification of neonates is a vast and complex subject and it was decided to divide up the babies into groups that any experienced paediatrician could recognise.

- 1 Infants with normal tone, alertness and neonatal responses.
- 2 Infants with diminished or altered neonatal responses and mild variations in tone, lasting less than 48 h.
- 3 Infants with diminished or altered neonatal responses and variations in tone, lasting more than 48 h.
- 4 Pathological irritability requiring sedation.
- 5 Neck extensor hypertonia as well as altered responses and tone lasting under 48 h.
- 6 Neck extensor hypertonia and altered responses and tone lasting more than 48 h.
- 7 Infants having convulsions.

BIOCHEMICAL DATA

Since there were large variations in the results from short periods of collection "pooled" results from periods of about 10 h have been used. Although 24 or even 48 h periods would minimise the effects of errors in collections, such long periods would obscure abnormal trends. Urine was stored at 4°C or -20°C until analysis.

Some preliminary purification of urine was necessary to remove ultraviolet absorbing compounds which could otherwise interfere with identification and measurement of purines and pyrimidines by high pressure liquid chromatography (HPLC). Removal of the majority of ultraviolet absorbing impurities was achieved by the cation exchange resin, Zerolit 225 (BDH). This was suspended in 1 mol/l HCl, and then washed exhaustively with distilled water until the pH of the washings was 4–5. Of this resin, 0.5 ml was introduced into a Pasteur pipette plugged with glass wool. Urine (0.5 ml) was added and the column then eluted with 6 ml distilled water, followed by 5 ml 1 mol/l HCl. The flow rate through the column was 1–2 ml/min. Only the acid fraction was collected, which was adjusted to pH 4–5 with solid potassium dihydrogen phosphate and 10 mol/l sodium hydroxide and filtered through a $0.5\ \mu\text{m}$ Millipore filter prior to HPLC. This procedure removed the majority of ultraviolet absorbing impurities and thymine, inosine, uridine and uric acid, which were all eluted with water.

However, xanthine, hypoxanthine, guanine, 7-methylguanine, cytidine, guanosine and cytosine

were eluted, with good recoveries, by the acid. Since the main objective was to determine the effects of hypoxia on purine excretion the loss of some compounds other than hypoxanthine and xanthine in the water wash was acceptable. The efficiency of our procedure was constantly monitored by the extraction of standard solutions of xanthine and hypoxanthine; mean ($\pm$ SEM) recoveries with one representative batch of Zerolit 225 were $75 \pm 2.2\%$ for hypoxanthine and $87 \pm 4.1\%$ for xanthine ($n = 4$).

The purified extracts were then analysed by HPLC using absorption at 254 and 280 nm for detection. Specificity was achieved by this dual wavelength method and the high plate count of the columns used combined with checks by enzymic removal of peaks. The HPLC part of the method has been described and evaluated in some detail.⁷

Two normal series of infants have been studied using these methods with similar results; one set of such results has been statistically evaluated.¹³ Excretion was calculated per kg body weight per hour and recorded for the relevant age in hours.

The clinical and biochemical data were independently classified into at least three categories—for example, mild, moderate and marked abnormalities then the two sets of data jointly reviewed. In some cases it was possible to achieve approximate ranking of patients within some clinical categories⁴ to compare with the quantitative excretion data.

Results

NEUROLOGICAL ABNORMALITY AND OXYPURINE EXCRETION

In "asphyxiated" infants there was agreement between the degree of neurological abnormality and the magnitude of the oxypurine excretion. The results for different types of neurological abnormalities are shown in Fig. 1–6 in which neurological abnormalities persisting for shorter or longer than 48 h are distinguished. The patterns of results and their heterogeneous nature make the extensive presentation advisable before simplifying assumptions are used to condense some of the data into Tables 1 and 2. More clinical details are included for those infants from whom extensive urine data was obtainable (Table 1).

For those infants who fulfilled the initial criteria of intrapartum hypoxia but were normal neurologically, group 1, the oxypurine excretion (Fig. 1) was normal. Although the initial fall in patient 2 is probably an abnormal trend—none of these four infants showed any persistent abnormality. The mean (SEM) excretion was for hypoxanthine 19.1 (7.5) and for xanthine 18.2 (6.1) nmol/h per kg which were similar to those for normal infants.¹³

The oxypurine excretion of seven infants with mild, altered or incomplete neurological responses for a period of less than 48 h, group 2, is shown in Fig. 2. The results for four similar infants in whom the neurological abnormalities persisted for longer than 48 h, group 3, are shown in Fig. 3. In Fig. 2 the hypoxanthine excretion, often exceeding the 98th centile, was greater than that of xanthine which was largely "normal" although clustered at higher levels than excretion by "normal" infants (Fig. 1). Overall excretion of both oxypurines showed a fall over 20–70 h of age. Patient 6 was neurologically intermediate between groups 2 and 3 that is between the others in Fig. 2 and those in Fig. 3. Since both hypoxanthine and xanthine excretion at about 50 h of age was abnormally raised his oxypurine excretion was also intermediate between the other values in Fig. 2 and those in Fig. 3. All four infants neurologically abnormal for longer than 48 h had "abnormally" high excretion of both xanthine and hypoxanthine. In Fig. 3 only one, 12, provided acceptable samples in the first 20 h of life. In addition the age at which there was a tendency for excretion to fall is possibly greater in Fig. 3 than in Fig. 2.

Two patients 23 and 24 were classified neurologically as cerebral irritability requiring sedation, group 4. Both showed abnormal excretion of hypoxanthine and xanthine. Unfortunately the more severely neurologically affected was only studied between 30–60 h of age but in this time it was clear that his excretion rate was about 20 nmol/h per kg higher than in infant 24 at the same age. It was thus possible to "rank" these two infants by both sets of criteria.

Oxypurine excretion by six infants with neck extensor hypertonia, groups 5 and 6, is shown in Figs. 4 and 5. Those with this finding for less than 48 h are shown in Fig. 4; two of the three showing at least one abnormally high value. In contrast, the sustained high excretion of those showing ≥ 48 h of neck extensor hypertonia is shown in Fig. 5. The one available value in patient 21 since it is high at the age of about 70 h suggests that his excretion might have been similar to that of the other two seriously affected infants. In group 7, three patients with convulsions (for < 24 h) were studied. Infant 26 showed an excretion pattern similar to those in Fig. 5. Infant 25 had only two values which were high, 80 and 82 nmol/h per kg. These were similar to the high values for patient 26, 100 and 65 nmol/h per kg; both 25 and 26 showed these abnormalities at around 20 h of age. The other patient, 27, showed results entirely within the normal range and no consistent trend. This child was not noted to be abnormal neurologically until skilled examination at about 24 h of age but thereafter was found to have persistent neck

Fig. 1

Fig. 2

Fig. 3

Fig. 1 Hypoxanthine (lower) and xanthine (upper) excretion by infants 1 ●, 2 ○, 3 ■ and 4 □ with no neurological abnormalities, group 1

Fig. 2 Hypoxanthine (lower) and xanthine (upper) excretion by infants 5 ●, 6 ○, 7 ■, 8 □, 9 ▲, 10 △, and 11 ◇ with diminished or altered neonatal responses and variations in tone lasting less than 48 h, group 2

Fig. 3 Hypoxanthine (lower) and xanthine (upper) excretion by infants 12 ●, 13 ○, 14 △ and 15 ▲ with diminished or altered neonatal responses lasting more than 48 h, group 3

Fig. 4

Fig. 5

Fig. 6

Fig. 4 Hypoxanthine (lower) and xanthine (upper) excretion by infants 16 ●, 17 ○ and 18 △ with neck extensor hypertonia lasting < 48 h, group 5

Fig. 5 Hypoxanthine (lower) and xanthine (upper) excretion in infants 19 ●, 20 ○ and 21 △ with neck extensor hypertonia, altered responses and tone lasting more than 48 h, group 6

Fig. 6 Hypoxanthine (lower) and xanthine (upper) excretion by an infant 22 ● with marked hypoxic damage, group 7

extensor hypertonia.

Infant 22 in Fig. 6 had the most severe neurological damage of any of our patients including fits for 24 h. The results in Fig. 6 show the most marked delay in starting the study and the most persistently abnormal and highest maximum excretion found.

One patient, 28, had severe neurological abnormalities and hypothyroidism. His hypoxanthine excretions for the periods in hours indicated in brackets from the age of one hour were 42 (31), 45 (11), 8 (21) and 11 (9). His xanthine excretion for the same periods were 43, 50, 12 and 19 (nmol/h per kg). There was therefore a reduction in excretion with age which is probably pathological but absolute excretion was at the upper limits of the normal range for euthyroid infants.

The changing patterns of excretion rates in infants after intrapartum hypoxia (Figs. 1–6) suggested that it would be justifiable to examine the “total” increase in hypoxanthine excretion where enough data were available. In order to avoid normal excretion rates loading the figures, 50 was subtracted

Table 1 Summaries of obstetric, paediatric and biochemical assessments of infants with severe intrapartum hypoxia

Infant	Gest age (wk)	Birth wt (g)	Obstetric complications	Apgar score 1 min, 5 min	Initial pH	Neurological abnormality	Total increase in hypoxanthine excretion (nmol/kg)*
5	36	1930	Pre-eclampsia, antepartum haemorrhage, intrauterine growth retardation	2,5	7.22	Hypotonic for under 48 h	253
6	40	4040	60 min of unsuccessful forceps and ventouse delivery	1,5	7.25	Irritable, sedation for under 48 h	74
12	35	2620	Pre-eclampsia, breech, 2nd twin	4,6	7.17	Respiratory distress, ventilated, pneumothorax, hypotonic for 6 days	473
24	39	3400	Pre-eclampsia, type 2 dips of fetal heart 2½ hours before delivery	4,6	7.14	Pathologically irritable for 3 days	93
19	35	2380	Fetal tachycardia, reduced beat to beat variation and long bradycardia	1,3	7.13	Neck extensor, hypertonia 3 days, irritable 4 days	331
26	41	3610	Pre-eclampsia, loss of beat to beat variation, fetal tachycardia 7 hours before delivery	3,5	7.17	Convulsions day 1, Neck extensor hypertonia for 3 days	153
22	39	2290	Pre-eclampsia. 1st twin. Fetal bradycardia then baseline tachycardia for 3 hours	2,4	7.13	Convulsions for 24 h. Neck extensor hypertonia for 7 days. Acute tubular necrosis.	418

*All positive values > 50. All urine collections were started within four hours of birth except for 22 who started at the age of 100 h.

Table 2 Association between neurological state and increased hypoxanthine excretion after intrapartum hypoxia

Neurological category	No of infants	Initial pH after resuscitation		Increase in hypoxanthine excretion* (nmol/kg)	
		Mean	Range	Mean	Range
1	4	7.21	7.13–7.34	0	0
2 (<48h)	7	7.21	7.02–7.29	16.9	0–51
3 (>48h)	4	7.20	7.13–7.29	70.6	34–106
4	2	7.22	7.14–7.30	29.2	23–35
5 (<48h)	3	7.21	7.04–7.37	3	0–9
6 (>48h)	3	7.13	7.10–7.16	44.4	37–49
7	3	7.20	7.17–7.25	31.2	21–42

*All positive values > 50.

from each 10 hour rate and then all positive data summed. The value of 50 was chosen since the 98th centile had been estimated to lie between 50 and 60 nmol/h per kg for hypoxanthine and not to vary with age.¹³ This total increase in hypoxanthine excretion or “incremental area under the curve” in Figs. 1–6 should allow comparisons to be made between different clinical groups. More complete clinical details for patients for whom such data were available are shown in Table 1. The overall correlation as in Figs. 1–6 was clear starting from 0 values for infants with no neurological abnormalities (not shown in Table 1) to the highest cumulative excretion for infant 22 (Fig. 6), excluding from the comparison infant 12 who required ventilation and therefore was also hypoxic after delivery.

One objective assessment of the infants after intrapartum hypoxia was from their initial capillary blood pH. This showed little or no correlation with

neurological abnormality or with oxypurine excretion (Table 2). The explanation for these findings is suggested by the results in Fig. 7 on an infant with the respiratory distress syndrome who after a normal delivery and good early progress showed a progressive drop in blood pH to very low levels after which he was mechanically ventilated. The prolonged and marked rise in oxypurine excretion persisted for about 60 h after the blood pH had returned to normal. The infant “collapsed” at the point indicated by the arrow although the nature of this collapse could not be defined. Oxypurine excretion and urine volume fell sharply in the succeeding seven hour collection. Figure 8 shows another infant with respiratory distress in whom oxypurine excretion rose markedly about 10 hours after blood pH had returned to normal. Abnormal excretion persisted for about 50 hours.

In addition to the mechanically ventilated infants,

Fig. 7 Hypoxanthine (lower) and xanthine (upper) excretion and blood pH (upper) during mechanical ventilation for the respiratory distress syndrome. The arrow indicates 'collapse'

two infants were studied after apnoeic attacks, another metabolic insult which could be timed. The prolonged and increased oxypurine excretion after two apnoeic attacks is shown in Fig. 9. Another infant was studied after one attack; there was a rise of both hypoxanthine and xanthine excretion to 71 and 75 nmol/h per kg in the 15 hours after the attack. The subsequent 13-hour collection showed a normal excretion of 33 for hypoxanthine and 30 for xanthine (nmol/h per kg). In this subject in whom urine flow was preserved the initial concentrations after the attack were 722 and 274 $\mu\text{mol/l}$ for hypoxanthine and xanthine respectively. Both of these are markedly raised from the normal values of about 50.

One defect of the objective presentation of heterogeneous clinical and biochemical data, even

Fig. 8 Hypoxanthine (lower) and xanthine (upper) excretion and blood pH (upper) during mechanical ventilation for the respiratory distress syndrome.

Fig. 9 Hypoxanthine (lower) and xanthine (upper) excretion by an infant after two attacks of apnoea indicated by the arrows.

as graphs like Figs. 1–6, is the difficulties in allowing the overall pattern between neurological groups to be readily seen. This pattern is visible in Table 2 in which mean increased hypoxanthine excretion is higher in the infants with neurologically defined abnormalities which were present for > 48 h compared to those in whom such abnormalities lasted < 48 h. The hypoxanthine excretion is treated similarly to the results for Table 1.

Discussion

The effect of an acute reduction in ATP concentrations in cells is to increase the outflow of AMP metabolites maintaining the ATP concentrations relative to AMP.¹⁴ In other words, the energy charge,

$$\frac{\{\text{ATP}\} + \frac{1}{2} \{\text{ADP}\}}{\{\text{ATP}\} + \{\text{ADP}\} + \{\text{AMP}\}}$$

falls more slowly than the total adenine nucleotide concentration.⁸ In this way an acute reduction of ATP in man produces an increased excretion of hypoxanthine.¹⁵ Some of our asphyxiated patients showed a high initial excretion (Fig. 1, infant 2; Fig. 3, infant 12). This is the predictable consequence of a marked reduction in cellular ATP concentrations.¹⁴

The little available previous work is consistent with our findings in normal and in pathological conditions. Urinary excretion of urate, the metabolite of hypoxanthine, was previously found to be raised in idiopathic respiratory distress and this was due to an increase in production.¹⁶ These findings were extended to show that urate and xanthine excretion were higher in respiratory distress.¹⁷ Our findings of both raised hypoxanthine and xanthine excretion are probably due to the use of a more sensitive method, HPLC with sensitive ultraviolet absorbance detection. Another difference is that we have mainly studied intrapartum hypoxia. In this way we have avoided the increased oxypurine excretion which occurs after exercise.^{15 18} Respiratory efforts in our two mechanically ventilated infants were minimal.

In our data after intrapartum or postpartum hypoxia the recurring pattern of rising excretion may be simply explained by early renal “failure” associated with a reduction in urine flow since renal failure after intrapartum hypoxia is obvious on the first day of life.¹⁹ Varying degrees of renal damage follow intrapartum hypoxia²⁰ and were seen in our patients generally as initial oliguria or even anuria for up to 72 h. In contrast more than 90% of normal infants pass urine on the first day of life.²¹

The persistence of raised excretion for 40 or more hours after hypoxia was unexpected. In our studies

(Figs. 7 and 8) oxygen supply and the extent of anaerobic glycolysis as reflected by the blood pH were returned to physiological levels rapidly. A similar rapid return of blood pH to normal is seen after delivery.²² The immediate adjustments to maintain relative ATP concentration¹⁴ probably last no longer than about 5 h.²³ It is therefore necessary to search for some other mechanism to explain the raised excretion over 40 or more hours.

There is some experimental evidence which could explain our findings. In the mouse renal ischaemia lasting less than one hour has, after reperfusion, been followed by a rapid return of energy charge to control levels but there was a slower return of total adenine nucleotide concentrations to about 60–70% of control values. Total adenine nucleotide concentrations then returned even more slowly to control values over the next 24 h. If ischaemia lasted more than one hour then, if there was any recovery all the above events were slower.²⁴ These changes may also take place in the human kidney and may be associated with increased oxypurine excretion. Human kidneys after prolonged ischaemia subsequently show an increased outflow of ATP metabolites. The extent of this outflow is related to the viability of these kidneys when grafted.²⁵

The inefficiency of mechanisms maintaining ATP after severe “asphyxia” when substrate supply is completely restored may be due to a series of persistent mitochondrial defects—for example, in succinate oxidase activity, which are correlated with the adenine nucleotide content of such mitochondria.²⁶ These mechanisms may be important in the perinatal period in view of the developmental changes in mitochondria at this time.²⁷

There are two patients in whom initial independent classification by neurological damage and by oxypurine excretion did not produce concordant results. One was explained by hypothyroidism. Since ATP turnover is reduced in hypothyroidism²⁸ and since increased oxypurine excretion is found in exercise^{15 18} linked with ATP “turnover”,²⁹ the normal range of oxypurine excretion in hypothyroidism should be lower than from euthyroid subjects. Unfortunately urine collections were not long enough to ensure that our patient had returned to his own “control” values. It is probable that this infant’s oxypurine excretion was relatively high. It is also possible that the hypothyroidism has contributed, directly or even indirectly, by altering susceptibility to hypoxic damage. Hyperthyroidism has been suggested to predispose to areas of tissue hypoxia.³⁰

There was one unexplained anomaly in our results, patient 27 with the persistent neurological sign of neck extensor hypertonia noted 24 h after

delivery. It is possible that the episode of severe hypoxia occurred well before the onset of labour. Alternatively, an intrapartum episode may have spared the kidneys and the heart³¹ and possibly other systems despite damage to the CNS. Since there is no unequivocal proof of any cause for the neurological damage in infant 27, this discrepancy is unexplained.

The prolonged increase in oxypurine excretion after postpartum hypoxia shows that urinary oxypurine excretion should reflect severe intrapartum hypoxia occurring at any time in labour since this is unlikely to last for more than 40 h. The above data show that a timed collection of a minimum of 10 h preferably 20–50 h duration should distinguish “asphyxiated” infants and quantify the degree of metabolic damage. Existing data suggests this timed urine collection should be started about 24 h after birth or thereafter if urine flow is not yet established. Our results suggest that the assessment of metabolic damage by oxypurine excretion is sensitive and has a large range. However, despite the clinical difficulties, more systematic sampling is needed to determine optimum sampling and data treatment. The somewhat arbitrary procedures in Tables 1 and 2 are probably not optimal. Considerable simplification would appear to be practicable once initial research has provided a sound basis for such decisions. Since little comparable data are available further work is needed to explore the possible applications of our findings.

We are grateful to Sister C Lawrence and SB Coade for their help in the ward and the laboratory.

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