Br. J. Pharmac. (1970), **40**, 310–316.

Histamine and granulocytes in the umbilical cord blood of infants at birth

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Summary

1. Histamine levels in whole blood and plasma from the umbilical arteries and veins, together with circulating basophil and eosinophil counts, were determined in two groups of healthy infants, " term " and " pre-term ".

2. No significant arteriovenous differences were found except in the basophil counts of the " pre-term " group. This result was consistent with the finding of significant arteriovenous correlations for all these sets of estimations except the basophil counts in the " pre-term " group.

3. No significant differences were found between the mean values in either group except for the difference between the mean basophil counts for venous blood.

4. Plasma histamine levels (arterial and venous) were usually less than the lowest limit of the method (1·8 ng/ml). Very small amounts of histamine were detected, however, in one venous and one arterial specimen in the " pre-term " group, but these did not produce a significant difference between the levels for the two groups of infants.

Introduction

It has been established that the amount of histamine in the blood of healthy adult humans is within the range 10 to 100 ng/ml (Lindell & Westling, 1966), but the situation in the newborn infant is less clear.

Mitchell & Cass (1959) found slightly elevated levels of histamine in blood from the umbilical vein (one case in ten was above 100 ng/ml). Subsequently Bjurö, Lindberg & Westling (1961) reported values for both venous and arterial blood from the umbilical cord. The venous values ranged between 33 and 125 ng/ml and the arterial between 40 and 135 ng/ml, with a mean arteriovenous difference of approximately 15%. Dieckhoff & Cobet (1965) also carried out a study of blood histamine levels in the newborn. In a group of eleven infants delivered at term, the arterial and venous blood histamine levels were in the ranges 15 to 67 ng/ml and 5 to 37 ng/ml respectively: again there was a tendency to higher levels in the arterial blood. In a group of nine premature infants, the histamine levels in the umbilical cord blood were 10 to 33 ng/ml (arterial) and 10 to 26 ng/ml (venous) ; there was no significant difference between the arterial and venous ranges.

Wicksell (1949) reported no differences between the plasma histamine levels of mothers and their newborn infants. His work was carried out before the present

accurate methods for the measurement of plasma histamine levels had been developed (Adam, Hardwick & Spencer, 1957; Noah & Brand, 1963). In 1959, Kahlson, Rosengren & White reported that human foetal plasma in early pregnancy contains greater amounts of histamine than does adult plasma. This, together with other evidence, led Kahlson to formulate the hypothesis that the foetus has a high histamine-forming capacity (Kahlson *et al.*, 1959, 1960). Dieckhoff & Cobet (1965) made a study of plasma histamine concentrations in the newborn. Using blood from the umbilical vein they reported a range of plasma histamine levels between 5 and 15 ng/ml, whereas levels in specimens from the umbilical arteries were 5 to 30 ng/ml. These workers obtained similar values with blood from premature infants. Kahlson *et al.* (1959) had previously suggested that the low histamine content of umbilical venous plasma might be due to diffusion between foetal and maternal blood in the placenta.

The earliest reports on the number of basophil leucocytes in the blood used a differential method, the count being made on smears. Wegelius (1948) found average counts of 50 basophils/mm^3 at birth, dropping to 45/mm^3 after 2 h. Mitchell (1955), using a more accurate chamber-counting technique, basically that of Moore & James (1953), confirmed that basophils are plentiful in the peripheral blood in the period immediately after birth, and recorded an increase during the first 24 h with a rapid decrease thereafter. This general pattern contrasted with the eosinophil counts, which were found to fluctuate widely. Later work by Dieckhoff & Cobet (1965) showed similar ranges of basophils in arterial and venous umbilical blood from both term and premature infants, that is, approximately 5 to 50/mm^3. In their infants at term, the upper range was somewhat higher in the arterial specimens, being 58/mm^3 compared with 45/mm^3 in venous blood. Eosinophil counts were also made in umbilical cord blood by Dieckhoff & Cobet (1965), who did not find significantly different values between any of their groups, the range being approximately 5 to 55/mm^3 in all four groups.

The present investigation was undertaken to determine the levels of histamine in the umbilical cord blood of term and pre-term infants, in an attempt to clarify the situation with regard to plasma histamine values using the sensitive and specific techniques of Adam *et al.* (1954, 1957). Basophil, eosinophil and total leucocyte counts were also carried out on specimens from some of the infants.

Methods

Specimens of blood were obtained from two groups of healthy newborn infants of both sexes as follows.

Healthy "term" infants

This group comprised twenty-nine healthy newborn infants with gestational ages between 39 and 44 weeks. The birth weights ranged from 2,630 to 4,000 g. It was possible to estimate the histamine levels in whole blood (arterial and venous) from seventeen subjects, plasma histamine levels in ten arterial and eighteen venous specimens and basophil eosinophil and total leucocyte counts in arterial and venous specimens from thirteen subjects. The small volumes of arterial blood available prevented us from obtaining complete data on all infants.

Healthy " pre-term " infants

This was a group of twenty newborn infants with gestational ages ranging from 26 to 38 weeks and birth weights from 1,630 to 4,000 g. Here, histamine concentrations were estimated in arterial and venous whole blood from eleven infants and plasma histamine levels were estimated in six arterial and eight venous specimens. Basophil, eosinophil and total leucocyte counts were carried out on arterial and venous blood from ten infants.

Withdrawal and partitioning of blood specimens

Blood was withdrawn from clamped sections of umbilical cord. Immediately on delivery the cord was double clamped ; the first clamp was placed at the maternal end, the umbilical arteries were then allowed to fill with blood from the foetal end and the second clamp was placed close to the infant. The blood from the clamped section of cord was withdrawn at once into two disposable polythene syringes (one syringe for the arterial, the other for the venous specimen). Approximately 8 ml of blood was usually obtained from the vein, and a somewhat smaller volume from the arteries. One ml of the specimen was expelled into a tube coated with ethylenediamine tetraacetic acid for the leucocyte count and the remaining 7 ml was added to a chilled siliconed glass centrifuge tube containing 0·7 ml of heparin 100 i.u./ml (prepared in 0·9% NaCl). If the blood remaining in the syringe was less than 7 ml, a correcting factor was applied in the final calculation to compensate for the extra dilution by heparin. A 0·5 ml sample of heparinized blood was removed with a siliconed pipette and transferred to a clean 15 ml capacity centrifuge tube. This was the specimen for the whole blood histamine estimation. The remaining heparinized blood was subjected to a double centrifugation procedure at 4° C to obtain a specimen of plasma.

Estimation of histamine in whole blood and plasma

This was carried out using slightly modified versions of the methods of Adam *et al.* (1954, 1957) and Adam (1961). Plasma and whole blood specimens were extracted with trichloroacetic acid, centrifuged to remove the proteins and further purified on composite cationic resin columns. The eluates, after heating with 6 M HCl and drying, were reconstituted and assayed.

The bio-assay was performed on the superfused guinea-pig ileum preparation, using a semi-automatic apparatus similar to that described by Adam *et al.* (1954). The dried residues were dissolved in a reconstituting solution, the composition of which was calculated to compensate for the concentration of NaCl in the eluates. Thus when the dried eluates were dissolved in 5 ml of the reconstituting solution, the sample test was isotonic with the atropinized Tyrode's solution used to superfuse the preparation.

Frequently, test solutions were re-assayed in the presence of Tyrode's solution containing mepyramine maleate in a concentration of 3 ng/ml.

On several occasions, the potassium content of the eluates from both whole blood and plasma specimens was estimated. This was considered especially important because excess potassium in a test solution can produce anomalous contractions of the preparation and thus give falsely high histamine levels. On no occasion was the

potassium concentration of the test solution high enough to interfere with the histamine assay.

Recovery experiments

When histamine, 2 ng, was added to 1·0 ml of plasma, the percentage recovery was 98 (S.E.M. 6·9, $n=6$) and the mean recovery of histamine from whole blood (25 ng added to 0·5 ml specimens) was 92% (S.E.M. 3·4, $n=11$).

Enumeration of leucocytes

Blood from the EDTA-coated tubes was diluted 10 times with a diluting fluid containing toluidine blue which metachromatically stained the basophil and eosinophil cells. The cells were counted in a Fuchs-Rosenthal haemocytometer using both chambers, according to Mitchell's modification (1955) of the method of Moore & James (1953).

Results

Whole blood and plasma histamine levels (as the free base) are shown in Table 1. When Student's t test was applied to the paired arteriovenous whole blood histamine differences in the " term " group, no significant difference was found ($0·7>P>0·6$), and when the arteriovenous correlation was determined this was found to be significant ($P<0·001$). Similarly, in the " pre-term " group the arteriovenous whole blood histamine differences were not significant ($0·7>P>0·6$) and the arteriovenous correlation was significant at the 5% level ($0·05>P>0·02$).

When the mean whole blood histamine levels for the two groups of infants were compared, the mean arterial levels were found to be not significantly different ($0·6>P>0·5$) and a similar finding was obtained for the mean venous levels ($0·95>P>0·90$).

Plasma histamine levels in the " term " group were all less than the lowest limit of the method (that is, either <1 ng/ml or <1·8 ng/ml, depending on the volume of plasma used for the estimation). Similar values were found in the " pre-term " group except for two specimens. One specimen of arterial blood plasma from a female infant born at 34 weeks gestation (birth weight 2,300 g) was found to contain 3 ng/ml of histamine whereas no histamine was detected in a venous specimen from the same infant (<1·8 ng/ml). Another specimen of plasma from the umbilical vein of a female infant delivered at 32 weeks gestation with a birth weight of 2,320 g was found to contain 4 ng/ml of histamine. Thus no means could be calculated for the " pre-term " group. These differences between the plasma histamine levels for the two groups of infants were not significant ($P=0·3$, arterial and venous—exact test).

TABLE 1. *Mean umbilical cord blood histamine levels in healthy " term " and " pre-term " newborn infants*

	Whole blood histamine (ng/ml)		Plasma histamine (ng/ml)	
	Arterial	Venous	Arterial	Venous
Healthy " term " infants	48 (S.E. of mean 5·6) ($n=17$)	49 (S.E. of mean 4·3) ($n=17$)	<1·8 (Upper limits: <1, <1·8) ($n=10$)	<1·8 (Upper limits: <1, <1·8) ($n=18$)
Healthy " pre-term " infants	54 (S.E. of mean 9·7) ($n=11$)	50 (S.E. of mean 9·7) ($n=11$)	See text (Range: <1–3) ($n=6$)	See text (Range: <1–4) ($n=8$)

The basophil and eosinophil counts are shown in Table 2. Student's t test was applied to the paired basophil arteriovenous differences in the " term " group and no significant difference was found ($0{\cdot}8>P>0{\cdot}7$). This was concomitant with a significant linear arteriovenous correlation ($P<0{\cdot}001$). When this test was applied to the paired basophil arteriovenous differences in the " pre-term " group, an almost significant difference was found ($0{\cdot}025>P>0{\cdot}02$) and the correlation was not significantly linear ($P>0{\cdot}1$).

When the mean basophil counts for the two groups were compared, the mean arterial counts were found not to be significantly different ($0{\cdot}4>P>0{\cdot}3$). An almost significant difference was found, however, between the mean venous levels for the two groups ($0{\cdot}05>P>0{\cdot}025$).

The eosinophil counts were found to be distributed in a skew manner and the use of logarithmic transforms produced a more normal distribution, permitting the application of some statistical analysis to the values. The paired log arteriovenous differences for the " term " group were found not to be significantly different ($0{\cdot}8>P>0{\cdot}7$) and the log correlation was significant ($P<0{\cdot}001$). Similarly, in the " pre-term " group the paired log arteriovenous differences were not significant ($P<0{\cdot}001$).

When the log mean eosinophil counts for the two groups were compared, no significant differences were found ($0{\cdot}7>P>0{\cdot}6$) for both arterial and venous values.

Discussion

All the whole blood histamine levels in the " term " group of infants were within the normal adult range, 10 to 100 ng/ml (Lindell & Westling, 1966), which is somewhat at variance with the earlier finding of occasional higher levels by Mitchell & Cass (1959), who used less refined techniques. Some elevated levels were found in the " pre-term " group, the highest level being 115 ng/ml. The 15% difference between arterial and venous whole blood histamine levels reported by Bjurö *et al.* (1961) was not substantiated in either group. This is in agreement with the finding of Dieckhoff & Cobet (1965), who reported no significant difference between the arterial and venous umbilical cord blood histamine levels for a group of premature infants. They did, however, find a significant arteriovenous difference in a group of infants at term.

The only significant arteriovenous difference found in the present study was for the basophil counts in the " pre-term " infants. This is rather unexpected because

TABLE 2. *Mean counts of basophil and eosinophil leucocytes in umbilical cord blood from healthy " term " and " pre-term " newborn infants*

	Basophils (/mm³)		Eosinophils (/mm³)	
	Arterial	Venous	Arterial	Venous
Healthy " term " infants	45 (S.E. of mean 6·7) ($n=13$)	44 (S.E. of mean 5·3) ($n=13$)	260* ($n=13$)	350* ($n=13$)
Healthy " pre-term " infants	38 (S.E. of mean 3·9) ($n=10$)	29 (S.E. of mean 2·4) ($n=10$)	230* ($n=10$)	210* ($n=10$)

* Antilogarithm of the logarithmic mean.

there was no concomitant significant difference in the whole blood histamine levels in this group, and basophil counts generally parallel histamine content of the blood (Code & Mitchell, 1957).

The range of eosinophil counts in this study was much wider than that found by Dieckhoff & Cobet (1965) in similar groups of infants. Whereas Dieckhoff & Cobet found ranges of approximately 5 to 55/mm^3 in their " term " and " pre-term " (arterial and venous) specimens, in this study the approximate range was 50 to 1,000/mm^3. The German workers gave no details of the methods they used for the partitioning of the blood, but if specimens are stored for prolonged periods at room temperature there can be breakdown of the cells resulting in falsely low counts. The distribution of eosinophils found here was skew and a similar distribution was reported by Cooper, Harvey & Davis (1969).

The minimal plasma histamine levels are in sharp contrast to those of Dieckhoff & Cobet (1965). Their high plasma levels may have been due to the techniques they used: it is thought that the histamine-containing cells (basophils) may be more fragile than normal at birth and these workers gave few details of their methods of collection and plasma separation. Cooper *et al.* (1969) found small amounts of histamine in the plasma of healthy " term " newborn infants. Their specimens were obtained by heel pricks and the histamine estimated spectrophotofluorimetrically. A disadvantage of the heel-prick sampling technique is the risk of contamination of the specimens with tissue juices which may contain histamine.

It has been postulated by Kahlson that the function of plasma histamine is related to maturation of the foetus (Kahlson *et al.*, 1960). The evidence on which this is based seems to be rather scanty, for Kahlson never published a full report of his findings in human foetuses. Lindell & Westling (1966) suggested that, if any histamine appears in the plasma, all that can safely be concluded is that the histamine-containing cells in the condition studied are more fragile than normal. This provides an acceptable explanation of the very low levels found in our study using scrupulously careful collection and separation techniques.

The authors wish to thank Miss Sandra Smith for technical assistance and Dr. G. Hems for advice on statistical methods.

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(*Received April* 20, 1970)