# Enzyme Activities in the Blood of Infants and Adults

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Morphological studies of the blood of infants and adults have been fairly numerous, but functional or chemical comparisons have been rare. The circulating blood at birth has been shown to contain two kinds of haemoglobin, an infantile type which comprises four-fifths of the whole and which is relatively resistant to alkaline denaturation, and the adult type which makes up the remaining fifth (Haurowitz, 1930, 1935; Brinkmann & Jonxis, 1935). Immunological differences between the adult and infant haemoglobins were detected by Darrow, Nowakovsky & Austin (1940). Whitby & Hynes (1935) found that concentrations of sodium chloride, which caused no haemolysis of adult red cells, readily haemolysed a proportion of the cells of a sample of infant blood. A concentration of sodium chloride, however, sufficiently dilute to cause total haemolysis of normal adult cells, did not disrupt all the cells in a sample of infants' blood. Such samples therefore contained some cells which were more and others less fragile than normal adult cells. Mollison (1948) compared the survival time in the infant circulation of red cells taken from cord blood with that of cells taken from an adult vein. He found that cord red cells disappeared at nearly twice the rate of the adult cells during the 10 days following transfusion. Stevenson (1943) estimated the carbonic anhydrase activity of infant and adult red cells and found it to be low in the former, and still lower in red cells obtained from premature infants, and Anselmino & Hoffmann (1931) claimed that the catalase activity of red cells derived from full-term infants was higher than that of maternal red cells. If all the morphological, physiological and chemical evidence is taken together it would appear that the red cells of an infant are a more heterogeneous population than those of an adult.

The present work was undertaken to compare the enzyme activities of the serum and erythrocytes of the newborn and adult human. The true and pseudocholinesterase of the serum have been investigated, and in the erythrocytes true cholinesterase, glyoxalase, carbonic anhydrase, catalase and acid phosphatase.

## METHODS

*Preparation of serum and red cells.* Blood was taken from the placental end of the divided umbilical cord or by puncturing the umbilical vessels with a large bore needle. Two samples were collected in sterile glass tubes or small bottles, one for the preparation of serum and the other, received into a few ml. of a solution containing 1 g. of sodium citrate dissolved in 100 ml. 0·9% (w/v) NaCl, for the separation of red cells. The specimens collected to provide serum were centrifuged as soon as the fibrin clot had separated. Haemolysed sera were not investigated. The citrated sample was centrifuged and the plasma removed. The cells were washed once with an equal vol. of 0·9% (w/v) NaCl, suspended in 0·9% NaCl and covered with a layer of liquid paraffin. In all manipulations care was taken to avoid bacterial contamination. Both the cells and the sera were stored in the dark at +4° until required. Keilin & Wang (1947) have shown that the activity of the enzymes under investigation was reduced by a very small amount when blood was similarly stored for many years, and no evidence of any loss of activity was found in the present work.

*Cholinesterase* was estimated in Warburg manometers at 37°. Two substrates were used: (*a*) 0·135 M-acetylcholine chloride, and (*b*) 0·18 M-acetyl-β-methyl choline. The latter is said to be hydrolysed only by the so-called true cholinesterase (Mendel, Mundle & Rudney, 1943). Since pseudocholinesterase in human blood is thought to be confined to the serum, and the true enzyme almost entirely to the red cells, it seemed permissible to use the unspecific acetylcholine chloride as substrate for the serum enzyme.

The quantities of the reacting substances used to estimate cholinesterase activity are set out in Table 1. In each case

Table 1. *Quantities of reactants used in Warburg flasks to estimate blood cholinesterase activity*

| Enzyme | Side bulb containing substrate | Main compartment | Unit of activity |
|---|---|---|---|
| Serum pseudocholinesterase | 0·135 M-Acetylcholine chloride, 0·5 ml. | Serum, 0·1 ml.; *Ringer-bicarbonate, 0·9 ml. | μl. $CO_2$/min./ml. serum |
| Serum true cholinesterase | 0·18 M-Acetyl-β-methyl choline, 0·5 ml. | Serum, 0·5 ml.; 0·03 M-$NaHCO_3$, 0·5 ml. | μl. $CO_2$/min./ml. serum |
| Red-cell true cholinesterase | 0·18 M-Acetyl-β-methyl choline, 0·5 ml. | Lysed red cells, 0·2 ml.; *Ringer-bicarbonate, 2·3 ml. | μl. $CO_2$/min./mg. Fe |

* Bicarbonate solution of Krebs & Henseleit (1932).

the manometers were gassed with a mixture of 5% $CO_2$ and 95% of $O_2$ (v/v), and equilibrated until two successive readings were constant. With the quantities of reactants given, $CO_2$ evolution was a linear function of time over the period of the experiment. Readings were taken at 2·5 min. intervals for 15 min. except when the true cholinesterase was being estimated in the serum. Readings were then taken at 10 min. intervals for a period of 1 hr.

Column 4, Table 1, defines the unit of activity employed. In the red cells iron was estimated by the method of Lyons (1927), described by McCance, Widdowson & Shackleton (1936), and the activity of the enzymes was calculated/mg. of Fe.

*Glyoxalase* activities were measured in Warburg manometers at 37°. 0·5 ml. of a 0·0139M-solution of methylglyoxal, which had been neutralized in bulk with a few drops of $Na_2CO_3$, was used as substrate. The main chamber of the Warburg flask contained 0·1 ml. of diluted lysed red-cell solution, 0·65 ml. of Ringer bicarbonate (Krebs & Henseleit, 1932) and 0·25 ml. of 0·36% glutathione. The gas space was filled with a mixture of 5% $CO_2$ and 95% $O_2$ (v/v). After equilibration six readings were taken at 2·5 min. intervals over which period $CO_2$ evolution was linear. The results were based on the iron content of the sample and the activity was expressed in $\mu$l. $CO_2$ evolved/min./mg. Fe.

*Carbonic anhydrase* activity was measured by the 'boat' method of Meldrum & Roughton (1933). Experiments were conducted at 16·0±0·2°. The activity was calculated from the formula given by the authors, and the result was expressed in their arbitrary units/mg. Fe in the sample of red cells under investigation.

*Catalase.* George (1947) showed that in the reaction between catalase and $H_2O_2$, $O_2$ evolution takes place in two phases. An initial rapid phase, of about 30 sec. duration, is followed by a slower phase. He claimed that the rate of $O_2$ formation in the rapid phase was proportional to the enzyme concentration. These observations formed the basis of the method employed to compare the activity of catalase in adult and infant red cells. The Meldrum & Roughton (1933) 'boat' apparatus was used. 1·5 ml. of 0·2M-phosphate buffer, pH 6·5, and an equal vol. of diluted lysed red-cell solution were added to one side of the boat, and 1·5 ml. of 0·4M-$H_2O_2$ to the other. After a period for equilibration with temperature and pressure, the 'boat' was shaken violently in the apparatus and the $O_2$ evolution followed to completion manometrically. Experiments were performed at 16·0±0·2°. Experiments in which water was substituted for the red-cell solution showed an insignificant $O_2$ evolution. The blank value was therefore neglected. $O_2$ evolution over the initial rapid phase of the reaction was linear for about 30 sec. All the results were calculated from the pressure difference after 20 sec. The results are expressed/mg. of Fe in the solution of red cells under investigation.

*Acid phosphatase* was estimated in red cells by the technique of King (1947). The unit of activity used is a modification of that defined by King, Wood & Delory (1945) and represents mg. of phenol liberated under the conditions of the experiment in 1 hr./mg. Fe in the sample of red cells under investigation.

*Haemoglobin* was estimated by the method of Gibson & Harrison (1945).

## RESULTS

A summary of the results obtained for the seven enzymes examined is set out in Table 2. The mean activity of each enzyme, with the exception of acid phosphatase, was lower in infancy than in adult life. The differences were statistically significant. In the case of the red-cell acid phosphatase there was no significant difference between the mean values of the adult and infant group.

The values recorded for carbonic anhydrase confirmed the finding of Stevenson (1943), who showed the activity of infant blood to be lower than that of the adult, and although the unit used by him was not identical with that of the present work the difference was in the same ratio (roughly 1/3). We also confirmed his observation that the activity of the red cells in some newborn infants is almost immeasurably low. He also stated that the carbonic anhydrase activity of the erythrocytes of premature babies (1500 g. or less) was low when compared with the corresponding values in full-term infants. In the present series the birth weights varied between 2150 and 4470 g., but there was no evidence that a low

Table 2. *Comparison of enzyme activities in serum and red cells of adult and cord blood*

| Enzyme and unit of activity | Age and no. of samples | Mean activity and S.E. | $t$ | Degree of significance ($P$) |
|---|---|---|---|---|
| Serum pseudocholinesterase. Unit = $\mu$l. $CO_2$/ml./min. | Adult 24<br>Infant 24 | 76·7 ± 2·82<br>50·2 ± 2·94 | 6·43 | <0·001 |
| Serum true cholinesterase. Unit = $\mu$l. $CO_2$/ml./min. | Adult 24<br>Infant 24 | 1·053 ± 0·055<br>0·827 ± 0·043 | 3·22 | <0·005 |
| Red-cell true cholinesterase. Unit = $\mu$l. $CO_2$/mg. Fe/min. | Adult 11<br>Infant 24 | 48·31 ± 5·49<br>32·90 ± 1·94 | 3·3 | <0·005 |
| Red-cell carbonic anhydrase. Arbitrary units/mg. Fe × $10^2$ | Adult 13<br>Infant 26 | 36·65 ± 2·51<br>9·10 ± 2·41 | 14·8 | <0·001 |
| Red-cell catalase. Arbitrary units/mg. Fe | Adult 13<br>Infant 27 | 27·67 ± 2·39<br>19·23 ± 1·58 | 2·89 | <0·01 |
| Red-cell glyoxalase $\mu$l. $CO_2$/mg. Fe/min. | Adult 7<br>Infant 9 | 555·0 ±75·2<br>281·0 ±34·2 | 3·57 | <0·005 |
| Red-cell acid phosphatase. Arbitrary units/mg. Fe | Adult 11<br>Infant 9 | 4·61 ± 0·423<br>4·84 ± 0·517 | 0·375 | Difference not significant |

The difference is considered to be statistically significant when $P<0·05$.

birth weight was associated with low, or for that matter with high, carbonic anhydrase activity.

Estimation of catalase showed that infant red cells had a lower mean activity than adult red cells, and did not confirm the work of Anselmino & Hoffmann (1931), who compared the catalase activity of cord blood with that of maternal blood. However, these authors used a method which involved the estimation of residual hydrogen peroxide by permanganate titration, which cannot be assumed to give results comparable with those given by the present technique. There is, for example, no evidence that the activity measured by permanganate titration is equivalent to that measured over the initial part of George's (1947) biphasic reaction, or that the catalase activity of maternal blood at term is the same as that of the non-pregnant adult.

## DISCUSSION

The average haemoglobin found in twenty-two samples of cord blood was 15·6 g./100 ml. and the average value for the adults was 15·9 g./100 ml. The cord blood, however, contained 54·3% of cells by volume and the adult blood 49·5%. The enzyme activities of the cells are expressed/mg. of iron, but since the percentage of iron in infant and adult haemoglobins is thought to be the same (Best & Taylor, 1945; Haurowitz, 1930, 1935) the activities of the enzymes can be expressed/g. of haemoglobin without altering their relationships. If, however, the enzyme activities had been expressed/unit volume of red cells the differences found would have been magnified because the infant cells contained less haemoglobin/unit volume. Thus the mean activity of the erythrocyte cholinesterase in adults was 52·25 units/ml. of cells, as against 48·31 units/mg. of iron, and in infants 31·8 units/ml. of cells against 32·9 units/mg. of iron.

It is difficult to decide what significance should be attached to the low enzyme activities of the red cell at birth. It is important to recognize that the erythrocyte, even at this time of life, is a highly specialized cell. Any chemical activity which it manifests, therefore, must be either a legacy of the haemopoietic mechanism which formed it, or an expression of the metabolism of a mature cell. If the erythrocyte enzymes investigated are involved in the haemopoietic processes, variations in their activity may be the cause of some of the differences in chemical composition which are known to exist between the adult and the infant red cell. Differences in activity might also affect the rate of haemopoiesis, and Rittenberg & Shemin's (1946) work suggests that the lower activity of the infant enzymes need not necessarily imply a slower rate of haemopoiesis. But until the differences in chemical composition and the factors controlling haemopoiesis in the adult and the infant are better understood such arguments must remain largely speculative. If, on the other hand, the erythrocyte enzymes are involved in the maintenance of the cell while it is in the circulation, it should ultimately be possible to correlate the differences of activity which have been demonstrated in adult and infant erythrocytes with the survival of these cells in the circulation. On this hypothesis, indeed, lower activities in the infant erythrocyte might have been inferred from Mollison's (1948) observations that transfused cord red cells disappeared more rapidly from the circulation than did adult cells. The integrity of the red cell must depend on the preservation of its membrane, and in this connexion the observation of Paleus (1947) is interesting, for he claimed that the erythrocyte cholinesterase was localized in the membrane.

The foregoing discussion should not be considered to embrace carbonic anhydrase, since its function in carbon dioxide transport and in the 'chloride shift' is well established, although the significance of its very low activity in the infant red cell is still obscure.

So little is known of the physiological function of the serum cholinesterases, that attempts to interpret the present findings seem neither profitable nor justifiable.

## SUMMARY

1. The mean cholinesterase activity of sera obtained from cord blood was 50·2 units for the 'pseudo' enzyme and 0·827 unit for the 'true' enzyme. The mean values for normal adult sera were 76·7 units and 1·053 units respectively. The differences were statistically significant.

2. The activities of the erythrocyte enzymes, true cholinesterase, glyoxalase, carbonic anhydrase, and catalase obtained from cord blood were found to be 32·9, 281, $9·1 \times 10^3$ and 19·23 units/mg. iron. The corresponding values for normal adults were 48·3, 555, $36·6 \times 10^3$ and 27·7 units/mg. iron. All differences were statistically significant.

3. The erythrocyte acid phosphatase activity was measured in cord blood and was found to be 4·84 units/mg. iron, the adult control group showed a value of 4·61 units. The difference was not statistically significant.

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# The Fate of Certain Organic Acids and Amides in the Rabbit

## 9. LOWER ALIPHATIC AMIDES

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In an earlier investigation, reported in Part 3 of this series (Bray, James, Ryman & Thorpe, 1948), it was shown that rabbit-liver extracts were only slightly active in hydrolysing formamide, acetamide, propionamide and phenylacetamide. It had previously been shown (Bray, Neale & Thorpe, 1946) that phenylacetamide was apparently completely hydrolysed in the intact rabbit, since its excretion products were qualitatively and quantitatively virtually the same as those of phenylacetic acid. In the case of the aromatic amides for which information is available (Bray, Thorpe & Wood, 1949*a*) there is reasonable agreement between results *in vivo* and *in vitro*, so that it seemed desirable to investigate the metabolism of aliphatic amides in greater detail.

These compounds have, in general, attracted little attention, probably because of the difficulty in determining their fate. Schultzen & Nencki (1872) concluded that acetamide was not hydrolysed in the dog. This was supported by Steinhausen's (1914–20) finding that in the phlorrhizinized dog 72 % of a dose of the amide was excreted unchanged over a period of 6 days. In the rabbit, however, acetamide appeared to be largely hydrolysed, since Salkowski (1877–8) could detect only a small proportion of unchanged amide in the urine, and Rudzski (1876) claimed that a large proportion of a dose was excreted as acetic acid. Pommerrenig (1902) reported that small doses were completely catabolized. Indirect evidence that acetamide is slowly and only partially hydrolysed in the rat has been obtained recently by Anker & Raper (1948) in feeding experiments with acetamide labelled with $^{13}C$. Halsey (1898) found that formamide gave rise to as much urinary formate in the dog as did formic acid itself, and was therefore presumably completely hydrolysed, while Gonnermann (1902) found that both formamide and acetamide were hydrolysed by sheep liver and formamide by sheep kidney. The other amides studied here do not appear to have been investigated previously, except by Fiske (1923), who carried out a study of their hydrolysis by fasting cats, using urinary amide nitrogen estimation as the criterion of splitting. Acetamide was excreted over about 4 days, a total of 70 % of the dose appearing unmodified. With the straight-chain amides up to valeramide, the lag in excretion diminished and the extent of hydrolysis increased progressively with increasing molecular weight, *n*-valeramide being almost completely hydrolysed (95–96 %) within 24 hr. The extent of hydrolysis of propionamide was 55–57 % and of *n*-butyramide 74–80 %. The order of stability *in vivo* was the reverse of that towards alkali, with which reagent the degree of hydrolysis