Amniotic Fluid Alkaline Phosphatase, Gamma-Glutamyltransferase, and 5'-Nucleotidase Activity from 13 to 40 Weeks' Gestation, and Alkaline Phosphatase as an Index of Fetal Lung Maturity

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Reference ranges for amniotic fluid alkaline phosphatase, gamma-glutamyltransferase, and 5'-nucleotidase are described from 13 to 40 weeks' gestation. Gamma-glutamyltransferase and 5'-nucleotidase activities peak early in the second trimester and then decrease to low values. Alkaline phosphatase shows a similar pattern of activity from 13 to 29 weeks' gestation, but thereafter activity increases to term; this late increase is mainly related to the heat-labile particulate form of alkaline phosphatase. Total and heat-labile alkaline phosphatase alone or expressed as a ratio with gamma-glutamyltransferase can be used with or as an alternative to lecithin/sphingomyelin ratios in the investigation of fetal lung maturity. A total alkaline phosphatase activity of 0.36 μkat/L and an alkaline phosphatase/gamma-glutamyltransferase ratio greater than 2 indicate pulmonary maturity.

Additional Keyphrases: fetal status - enzyme activity - reference intervals - respiratory distress syndrome

A number of enzyme activities have been demonstrated in amniotic fluid supernate (1), including alkaline phosphatase (orthophosphoric monoester phosphohydrolase, alkaline phosphatase; EC 3.1.3.1) (2), gamma-glutamyltransferase (5'-glutamyl)-peptide:amino-acid 5-glutamyltransferase; EC 2.3.2.2) (3), and 5'-nucleotidase (5'-ribonucleotide phosphohydrolase; EC 3.1.3.5) (4). Only in the case of alkaline phosphatase does the activity appear to have been measured throughout gestation (2, 5). Beckman et al. (5) also studied isoenzyme patterns, particularly the heat-stable and -labile fractions, and showed that there was only a slight increase in the heat-stable (placental) fraction between 31 weeks' gestation and term, whereas there was a sevenfold increase in the heat-labile fraction during this period. Eighty-five percent of this fraction is particulate (6), a form that in monkeys has been shown to be associated with the lamellar structures from the maturing pneumocytes (7).

Here we report the reference ranges we found for these three enzymes in amniotic fluid throughout gestation; identify and indicate how the primarily heat-labile particulate form of alkaline phosphatase can be used to assess fetal lung maturity; and indicate how assays for gamma-glutamyltransferase may be used alone or in combination with alkaline phosphatase in the clinical assessment of complicated pregnancies.

Materials and Methods

All reagents were supplied by British Drug Houses (BDH), Poole, UK (AR grade), except for the gamma-glutamyltransferase reagent kit supplied by Boehringer Corp., Lewes, U.K.

Each fresh sample of amniotic fluid was centrifuged at 656 × g for 10 min to remove cell debris. The supernatant liquid was stored at 4 °C until the enzyme assay. Any fluid with obvious meconium contamination was excluded from the study because of its high alkaline phosphatase content. Reaction rate analysis with a Gilford 3500 analyzer at 30 °C was used for assays of total and heat-stable alkaline phosphatase and gamma-glutamyltransferase; the buffer substrate conditions were as described in Hausamen et al. (8) and Szasz (9) for the respective enzymes. 5'-Nucleotidase was assayed at 30 °C by endpoint analysis with the BDH kit method based on the method of Persijn et al. (10). Heat-stable alkaline phosphatase was estimated after the amniotic fluid supernate had been denatured by heating to 65 °C for 20 min. To increase sensitivity, the sample volume was quadrupled to 200 μL. Heat-labile alkaline phosphatase was estimated from the difference between the heat-stable and total alkaline phosphatase activities. Alkaline phosphatase starch gel electrophoresis and Sepharose 4B gel filtration were done as described elsewhere (11).

Amniotic fluid for the identification of heat-stable alkaline phosphatase by starch gel electrophoresis was concentrated as described in Brocklehurst and Wilde (12). Lecithin/sphingomyelin ratios were determined by the method of Gluck et al. (13), except that the chromatogram was run in a solvent mixture of chloroform/water/methanol/ethanol (65:4:20/5 by vol) and the developing stain was molybdenum blue. The blue phospholipid spots were measured planimetrically, and the sample was reported as being from an immature fetus if the ratio was less than 4, mature if greater than 4.

Results

Within-batch precision data obtained over the range of total activities of alkaline phosphatase and gamma-glutamyltransferase were as follows: alkaline phosphatase, 0.23 (SD 0.036) μkat/L, CV 15%; and 0.52 (SD 0.029) μkat/L, CV 5.5%. gamma-glutamyltransferase, 0.38 (SD 0.01) μkat/L, CV 2.6%; and 4.2 (SD 0.148) μkat/L, CV 3.5%.

We studied reference intervals (mean, 2 SD) of alkaline phosphatase and gamma-glutamyltransferase in 325 amniotic-fluid samples from normal pregnancies from 13 to 40 weeks' gestation; for 5'-nucleotidase we studied 209 samples (Figure 1). Gamma-glutamyltransferase and 5'-nucleotidase peak early in the second trimester and then decrease markedly. Alkaline phosphatase follows a similar pattern between 13 and 19 weeks' gestation but thereafter increases in activity to term.

Alkaline Phosphatase Isoenzymes

Figure 2 shows the heat-stable and heat-labile alkaline phosphatase results for eight patients who had multiple amniocenteses between 27 and 40 weeks' gestation. All the patients were being investigated for assessment of fetal lung maturity; one case had concomitant hemolytic disease of the newborn. All specimens were screened for alkaline phosphatase isoenzymes by starch gel electrophoresis. Every patient showed an increase in total and heat-labile alkaline phosphatase, mainly in the particulate form, with increasing gestational age. In six of the eight patients there was a slight increase in heat-stable alkaline phosphatase; in the two re-
Fig. 1. Amniotic fluid reference ranges, 13–40 weeks' gestation, (μkat/L at 30 °C) for (a) alkaline phosphatase, (b) gamma-glutamyltransferase, (c) 5'-nucleotidase. Mean (O-----O) and 2SD (●—●). Numbers in parentheses indicate no. of results.

Routine starch-gel electrophoresis was used to assess qualitatively the contributions of the particulate and soluble amniotic fluid enzymes. The particulate and soluble enzymes were quantitated by Sepharose 4B gel filtration. Both enzymes were present at 14–23 and 32–40 weeks' gestation. A typical example from a 38-week mature pregnancy is shown in Figure 3. Sepharose 4B gel filtration showed that 96% of the activity was in the initial void-volume peak in the particulate form and only 5% was in the later, soluble peak. When the amniotic fluid was denatured at 65 °C for 20 min and eluted through the same column, there was no change in the activity in the soluble peak, which was therefore heat stable. However, only 21% of the particulate peak remained after heat treatment, indicating that this peak was mainly heat labile.

Alkaline Phosphatase and Fetal Lung Maturity

The amounts of total and heat-labile alkaline phosphatase activity were related to fetal lung maturity, as judged by the lecithin/sphingomyelin ratio, in 14 immature and 19 mature fetuses. The mean total alkaline phosphatase activity of the immature group was 0.27 (SD 0.1) μkat/L and that of the mature group 0.84 (SD 1.2) μkat/L. The mean heat-labile activity of the immature group was 0.21 (SD 0.19) μkat/L and that of the mature group was 0.72 (SD 1.18) μkat/L. In both
cases the activities of the mature and immature groups were significantly different (p < 0.001) (Figure 4). One patient with hemolytic disease of the newborn at 35 weeks' gestation, however, had a lecithin/sphingomyelin ratio characteristic of an immature fetus, while the total alkaline phosphatase activity suggested maturity. Upon subsequent amniocentesis both the lecithin/sphingomyelin ratio and the alkaline phosphatase values indicated a mature pattern; the baby showed no respiratory distress at birth the day after this amniocentesis. The differentiation of the mature and immature groups of patients was very similar, whether activity of total alkaline phosphatase or the heat-labile alkaline phosphatase was used.

A total alkaline phosphatase activity exceeding 0.36 μkat/L was consistent with pulmonary maturity.

Alkaline Phosphatase/Gamma-Glutamyltransferase Ratios and Fetal Lung Maturity

Because the gamma-glutamyltransferase activity is relatively constant during the last 10 weeks of pregnancy (Figure 1b), the effect of any sudden change in fluid volume on the alkaline phosphatase activity should be minimized by using the ratio of alkaline phosphatase to gamma-glutamyltransferase activity. When we did so, and related the total and heat-labile alkaline phosphatase/gamma-glutamyltransferase ratios to fetal lung maturity as judged by the lecithin/sphingomyelin ratio, there was more overlap between the immature and mature groups of fetuses (Figure 5) than when the activity of alkaline phosphatase alone was considered. The patient with hemolytic disease of the newborn, however, who was previously noted to have a high alkaline phosphatase with lecithin/sphingomyelin ratio indicating immaturity, now was ranked as immature by the alkaline phosphatase/gamma-glutamyltransferase ratio as well. The mean of the total alkaline phosphatase/gamma-glutamyltransferase ratio for the immature group was 1.17 (SD 0.5) and 2.96 (SD 1.7) for the mature group. The mean of the heat-labile alkaline phosphatase/gamma-glutamyltransferase ratio for the immature group was 0.9 (SD 0.45) and 1.36 (SD 1.6) for the mature group. In both cases the ratios for the immature and mature groups were significantly different (p < 0.001) (Figure 5).

An alkaline phosphatase/gamma-glutamyltransferase ratio greater than 2 was always consistent with maturity in this series, but there was a grey area of ratios between 1.2 and 2, in which the fetus may or may not be mature.

Care should be taken in interpreting alkaline phosphatase/gamma-glutamyltransferase ratios when the gamma-glutamyltransferase is greater than the upper limit of the reference range for a particular gestational age. For example, activity of amniotic-fluid gamma-glutamyltransferase was significantly increased in a pregnancy in which the baby had esophageal atresia.

Discussion

We confirmed the biphasic distribution pattern of total alkaline phosphatase in amniotic fluid through gestation (2) and found patterns similar to those described by Beckman et al. (5) for the heat-labile and heat-stable fractions. Gamma-glutamyltransferase and 5'-nucleotidase both show peaks of activity at about 17 weeks' gestation, with distribution patterns similar to that for alpha-fetoprotein. An explanation of the origins of the peak of activity at 17 weeks' gestation for all three enzymes has yet to be established. If an analogy can be drawn from Brock's explanation of the disappearance of amniotic-fluid alpha-fetoprotein (14), the secretion of the

![Diagram](image-url)
enzymes into the fluid may well be via fetal urine, followed by degradation on subsequent fetal swallowing.

The second increase in alkaline phosphatase activity, which appeared after 30 weeks' gestation, was heat-labile (Figure 2b) and mostly particulate (Figure 3). This confirms the observations of Salasfy and Nadler (6) and Beckman et al. (5). The activity of the heat-stable fraction (Figure 2a) was less than that of the labile fraction, although there was also a slight increase in the heat-stable fraction in six of eight patients studied; part of this heat-stable activity was particulate and did not migrate into starch gel. In one patient who was primarily investigated for hemolytic disease of the newborn, activity of this fraction decreased with increasing gestational age. This phenomenon has been reported previously (5, 15).

The amniotic-fluid particulate enzyme is excluded from starch gel electrophoresis and Sepharose 4B gel filtration (Figure 3). It may also be fractionated by differential centrifugation (unpublished work). The phospholipids used to monitor fetal lung maturity are also associated with large (lamellar) structures, which may be harvested by differential centrifugation (16). Because we thought that both alkaline phosphatase activity and the phospholipids might be associated with the same macromolecular complex, we investigated the use of alkaline phosphatase activity as an index of fetal lung maturity.

When we compared activities of total alkaline phosphatase and the heat-labile alkaline phosphatase with lecithin/sphingomyelin ratios, either alone or as ratios to gamma-glutamyltransferase, our approach was most discriminating for fetal lung maturity when total alkaline phosphatase was used. Heat-labile alkaline phosphatase and total and heat-labile alkaline phosphatase expressed as ratios to gamma-glutamyltransferase were slightly less discriminating. Although in the population studied the total alkaline phosphatase activity gave marginally better correlation, the reader should appreciate that there is always the possibility of placental (heat-stable) alkaline phosphatase contamination, for example, in transplacental sampling. In such a case an index based on heat-labile alkaline phosphatase would be more reliable.

Ammiotic-fluid alkaline phosphatase exceeding 0.36 µkat/L and a ratio to gamma-glutamyltransferase greater than 2 are apparently consistent with fetal lung maturity. When results are in the borderline area, determination of the trend by serial assay of fluids from the same patient increases the degree of certainty about fetal maturaion.

The use of ratios in phospholipid measurements and in the enzyme work described here is designed to minimize the effect of changes in fluid volume on the index measurement. However, one should not take for granted the reliability of the denominator; e.g., sphingomyelin can be falsly increased by hemolysis in erythrocyte-contaminated fluids, and we have noted inappropriately high activities of gamma-glutamyltransferase in a case of esophageal atresia. Previous reports of this condition associate it with increases in amniotic-fluid alpha-fetoprotein (17) and bilirubin (18), but we believe this is the first case where increases in gamma-glutamyltransferase have also been recorded.

The increase in total alkaline phosphatase activity in the last 10 weeks of pregnancy is a good index of increasing fetal lung maturity in the absence of meconium staining. It is easier to quantitate than the lecithin/sphingomyelin ratio commonly used for this purpose, and the measurement of the enzyme intimately involved in the specific production of pulmonary lecithin has much to recommend it. Further work, however, is needed to eliminate occasional false results, particularly in the borderline area; serial assays obviously help, but it may be necessary to improve still further the precision of the assay at these low activities. Other possible approaches are the measurement of phosphatidate phosphohydrolase (EC 3.1.3.4) (19) or of the particulate fraction of alkaline phosphatase activity, which will be similar to, but not identical with, the heat-labile fraction investigated here. At present, neither method lends itself readily to routine use: the former requires the synthesis of radioactive substrate, and the latter an isolation procedure by gel filtration or centrifugation before the enzyme assay.

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References