Antenatal Development of Amylase Isoenzymes

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Relatively little is known about the normal physiology of amniotic fluid, its dynamics, and its constituent enzymes and isoenzymes.

The antenatal diagnosis of some metabolic errors is frequently based upon the investigation for the presence or absence of an enzyme in the amniotic fluid or cells. In establishing an antenatal diagnosis of this kind, it is essential that 2 major factors be borne in mind:

(1) During fetal development and, indeed, during the first months of life, an enzyme may not be present in normal circumstances and its absence is of no prognostic or diagnostic value.

(2) If an enzyme is present during fetal development, it may only be represented by some of its isoenzymatic forms, not by its phenotypical appearance and function as recognized later in life. This can influence, both quantitatively and qualitatively, a prenatal diagnosis, possibly in the wrong direction.

The purpose of the present study was to investigate the dynamics of such an enzyme, namely, of alpha-amylase (alpha-1, 4-glucan 4 glucanohydrolase) during fetal development, by subjecting amniotic fluid at various stages of pregnancy to the electrophoretic technique used for amylase detection and separation.

Previous studies have revealed the presence of 2 polymorphisms controlled by 2 (possibly 3) loci—one controlling pancreatic amylase secretion and the other salivary amylase secretion. Other previous investigations, based on the study of 100 infants, followed monthly from birth to 12 months, have shown that, postnatally, pancreatic activity develops more slowly than salivary activity and the complete phenotype, as observed in the electrophoretic pattern of serum or urine of older children and adults, is not usually present until approximately the 12th to 14th month of life. The curve of development of each fraction, with age, is demonstrated in Fig. 1 (Kamarýt and Fintajšlova, 1971).

Both polymorphisms are easily detectable and qualitatively identical in the serum and urine of mature individuals. Since fetal urine probably constitutes a major part of amniotic fluid, it was assumed that it should represent a suitable medium for the antenatal detection of both these polymorphisms.

Materials

Forty specimens of amniotic fluid† (collected for various other reasons) were investigated and the observed isoenzymatic variants compared with corresponding specimens of maternal urine. The stages of pregnancy at which transabdominal amniocentesis (and collection of urine) was performed are shown in Table I. All specimens were investigated, within 4 days of collection, by the standard electrophoretic technique.

Results

(1) No amylolytic activity was detectable in amniotic fluid before, or during, the 12th week of pregnancy, ie, in 7 out of 40 specimens.

FIG. 1. Development of pancreatic, salivary, and total amylolytic activity in 100 infants from 1 to 12 months of age (after Kamarýt and Fintajšlova, 1971).

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TABLE I
NUMBERS OF SPECIMENS OBTAINED FROM TRANSABDOMINAL
AMNIOCENTESIS AND COLLECTION OF URINE AT VARIOUS STAGES
OF PREGNANCY

	Stage of Pregnancy (wk)									Total
	Under 12	12	14-16	18	20	25-26	35	37-38	39-40	
Number of specimens	6	1	5	1	3	1*	2	4	18	40

* The 25-26 weeks specimen and urine were repeated at full term and identical results obtained.

(2) In all specimens where activity was detected (33 out of 40), only one of the polymorphisms was present, namely that representing the salivary locus—migrating more slowly from the anode (Fig. 2).

(3) Two of the 33 specimens (6%) were characterized by the heterozygous 2-band Amy 1^{A1B} variant (see Fig. 3 where the record obtained from the amniotic fluid is compared with that from the maternal urine). The remainder were of the one-band Amy 1^{A1A} homozygous type (as demonstrated in Fig. 2).

(4) No pancreatic amylolytic activity was observed in any of the specimens of amniotic fluid investigated, though all maternal urines had typical findings with both polymorphisms present.

(5) In two of the maternal urine specimens, amylolytic activity was found to be considerably raised.

FIG. 2. Salivary isoamylase (homozygous form) in amniotic fluid compared with presence of both polymorphisms (salivary and pancreatic) in maternal urine.

FIG. 3. Heterozygous form of salivary isoamylase in amniotic fluid compared with same form and additional pancreatic isoamylase in maternal urine.

One specimen had been collected from a diabetic mother, the other from a mother who had a severe antepartum, and subsequent postpartum, haemorrhage.

Discussion and Conclusions

(1) None of the amylase isoenzymes appears to be developed in amniotic fluid before the 12th week of pregnancy.

(2) After the 12th week of pregnancy, amylase activity is represented by the salivary locus only, the pancreatic locus does not seem to be active. This is in agreement with previous observations of the development of both fractions during the first 12 months of life.

(3) Two out of 33 specimens (6%) had the heterozygous, 2-band Amy 1^{A1B} phenotype. The sample is small but this is the expected frequency, corresponding to that in the general population, where this particular phenotype has been observed in 7%.

(4) The absence of pancreatic isoamylase from all 33 specimens seems to suggest that they all consisted of exclusively fetal material, since no healthy, mature individual has as yet been observed to lack pancreatic amylase. Consequently, this may prove

to be a useful technique which would facilitate differentiation between fluid of maternal and fetal origin.

(5) A quantitative increase in amylase activity in prenatal specimens of maternal urine may prove to be of pathological significance (eg, in diabetes).

(6) The pancreatic locus has now been definitely assigned to chromosome No. 1 by the finding of linkage between Amy 2 and the 'uncoiler' phenomenon (Un-1) by Kamarýt, Adámek, and Vrba (1971); by the original discovery of linkage between Duffy and congenital cataract Cae by Renwick and Lawler in 1963; the subsequent report of linkage between Duffy and Un-1 by Donahue *et al* (1968); and the recent confirmation of linkage between Amy 2 and Duffy by Hill, Rowe, and Lovrien (1972). Preliminary studies on data from one family (Laxova, 1968) showed that both amylase loci were probably linked.

Since the salivary locus, which is identifiable antenatally, is also on chromosome No. 1, it may eventually prove useful for future antenatal linkage studies in a limited number of indicated cases, possibly also in connection with the early detection of congenital cataract.

Summary

The two types of the amylase polymorphism, which are controlled by the salivary and pancreatic loci respectively, do not appear in their permanent form, in the serum or urine, until after the 2nd year of life.

The development of the isoenzymes has been investigated electrophoretically in a series of samples of amniotic fluid collected at various stages

of pregnancy and compared with the isoamylases in the maternal urine. It seems that, antenatally, only the salivary polymorphism is active. After the 12th intrauterine week it may be detected in both its homozygous and less frequent heterozygous forms. Since the amylase loci have now been assigned to chromosome No. 1, these findings may eventually prove useful for antenatal linkage studies in indicated cases.

Addendum

Since the preparation of this paper, evidence for close linkage between the two loci has been submitted for publication by A. D. Merritt, Marian L. Rivas, and J. G. Ward (personal communication).

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