Tatarinow (1968) recorded three immunologically distinct foetus-specific serum proteins which behaved, in electrophoresis, as alpha-, beta- and beta-globulins. Of these, alpha-fetoprotein (AFP) has been widely studied. It is a carcinoembryonic antigen which recurs in individuals with hepatomas and some embryonic tumours. AFP normally reaches peak levels in sera of 13 week-old foetuses (Gitlin and Boseman, 1966), with a gradual decrease so that the cord-blood at birth shows a mean of 5.5 mg/100 ml. serum (Furth and Adinolfi, 1969). Its half-life subsequently is 3 to 5 days (Gitlin and Boseman, 1966). AFP also reaches the liquor amnii where it occurs in fairly constant levels which progressively decrease with advancing gestation (Seppala and Ruoslahti, 1972). Maternal blood shows very low levels measurable by radioimmunoassay (RIA) (Purves et al, 1973b). In the last few years many papers have appeared on AFP levels in maternal blood and liquor amnii as a means of monitoring foetal age and detecting foetal abnormalities. Malformations of the central nervous system lead to raised levels of AFP in the liquor (Brock and Scrimgeous 1974; Seller et al, 1974a) and sometimes even maternal blood (Seller et al, 1974b). Individual abnormalities with raised levels of AFP include anencephaly (Brock et al, 1973; Field et al, 1973; Harris et al, 1974; Lorber et al, 1973), spina bifida (Harris et al, 1974; Lorber et al, 1973; Allan et al, 1973; Lawrence et al, 1973) and myelocele (Duravetz and Moore, 1973). The consensus of opinion is that an abnormality of the central nervous system (an "open" defect) which permits escape of cerebro-spinal fluid into the liquor amnii leads to the abnormally-high levels; further absorption causes raised maternal AFP levels, though these are not as reliable. Raised AFP levels provide a means of detecting crippling defects in early stages of pregnancy and permits medical termination. AFP levels, however, are not infallible (Lawrence et al, 1973) particularly in "closed" defects of the neural tube.

Besides malformations of the central nervous system, raised AFP levels have been noted in severe foetal distress (Guibaud et al, 1973; Cohen et al, 1973) twin pregnancy (Ishiguro, 1973) Rh incompatibility (Guibaud et al, 1973) congenital nephrosis (Seppala and Ruoslahti, 1972) and foetal oesophageal atresia (Seppala 1973). Purves et al, (1973a) pointed out that any event which disturbed the foeto-maternal barrier, and these were many,
could lead to changes in AFP in the maternal blood.

We have failed to find any reports of studies of AFP in liquor amnii in the Indian literature. We are therefore recording our findings of the levels of AFP in liquor amnii in normal pregnancies at different stages and in some abnormal pregnancies.

Material and Methods

Cases: Liquor amnii was collected aseptically with a syringe and needle from women lying in at the Medical College Hospital, Aurangabad. This was either from women in early labour or from patients coming for medical termination of pregnancy. The gestational age varied from 8 to 40 weeks and it was determined by careful interrogation with respect to the last menstrual period. All liquor samples were coded and stored frozen. Qualitative detection of AFP was done by agar-gel double-diffusion (AGD). Positive samples were quantitated by radial immunodiffusion (RID). Tests were done without knowledge of the gestational age of the foetus from which the sample had been obtained. In every case the foetus was carefully scrutinised for any abnormality.

AFP Standard: This was serum of a 20 week-old human foetus standardised according to the method of Gitlin and Boseman (1966). The total proteins in this sample were estimated and the fractions, including AFP, quantitated accurately by electrophoresis on cellulose acetate membrane.

AFP Antibody: This was prepared by hyperimmunization of a rabbit with sera from 20 to 24 week-old human foetuses. Initially 5 injections of 0.5 to 1 ml of a foetal serum emulsified with an equal volume of Freund's complete adjuvant (Difco) were administered subcutaneously at monthly intervals. The rabbit was bled and serum absorbed with about half its volume of pooled normal adult human serum and tested in immuno-electrophoresis. This serum yielded a single arc in the alpha1 position indicating its monospecificity and anti-AFP nature (Fig. 1). Eight months later a booster injection was given and the rabbit now showed not one but two foetus specific components—the classical AFP and an additional arc with Beta2 mobility. Both these components were absent in adult sera. All amniotic fluid samples were tested in AGD against both these sera. The monospecific AFP antiserum was used for quantitation in RID.

Agar-gel Double-diffusion test: The buffer was a barbital acetate buffer of pH 8.4 and ionic strength 0.03µ. Gel was made by adding 0.85 per cent of Difco "Bacto" agar. Glass plates 75 mm square were layered with 4.5 ml of hot gel. Nine well patterns made up of a large central well (5 mm diameter) with 8 radial peripheral wells (3 mm diameter) were punched. The central well was filled with antibody and peripheral wells with liquor amnii. The gels were kept in moist chambers at room temperature and read after 24 hours (Fig. 2). All tests were in triplicate. The sensitivity of the technique was established by testing dilutions of the standard serum. Clear-cut arcs were obtained with dilutions containing 0.3 to 0.5 mg/100 ml of AFP.

Radial Immuno-diffusion Test: The mono-specific antibody (containing anti-AFP only) was incorporated in the same gel as used for AGD in a final dilution of 1 in 29. Wells were 2 mm in diameter. They were filled with 3 µL of standards or liquor amnii samples delivered with a Hamilton syringe. A ring of precipitate
indicated presence of AFP. The ring diameters of three standards—1 mg, 5 mg and 10 mg/100 ml—were measured and a standard graph plotted of square of diameter against concentration. Values of unknowns were read from this calibration graph. Tests were done in triplicate and mean values calculated. RID could give results only for values over 0.7 mg/100 ml of AFP. When a value exceeded the upper standard, the concerned sample was tested after dilution so that it fell within the range of the standards.

Results

The text-figure summarises the results of AFP in the liquor amnii. All amniotic fluids in the first trimester contained AFP. In the second trimester 20 of 23 samples contained AFP while in the third trimester, all normal pregnancies except one showed absence of AFP by the AGD technique. The Table shows the occurrence of AFP and Beta_2-fetoprotein in liquor amnii at different gestational periods.

**TABLE 1**

<table>
<thead>
<tr>
<th>Weeks Gestation</th>
<th>No. of Cases</th>
<th>Presence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Both present</td>
</tr>
<tr>
<td>8–12</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>13–16</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>17–20</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>21–24</td>
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<td>33–36</td>
<td>9</td>
<td>2*</td>
</tr>
<tr>
<td>37–40</td>
<td>49</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td>24</td>
</tr>
</tbody>
</table>

* Foetus anencephalic

Discussion

The text-figure shows that there is a decrease in the values of AFP with increasing gestational age. The trend is unmistakably gradual and downwards and, after about 24 weeks gestation, AFP is not detectable in AGD with its limit of sensitivity of 0.3 mg/100 ml of AFP. AGD appears to be quite useful as it effectively screened out liquor amnii samples collected before and after the gestational period of 25–27 weeks. It is quite remarkable that all the positives obtained after the 27 weeks of gestation, except one, were abnormal pregnancies though two abnormal pregnancies gave a negative result.

AFP values ranged from a trace (0.3 to 0.5 mg/100 ml) to 21.3 mg/100 ml during the first two trimesters (24 weeks). The mean during this period was 3.7 mg/100 ml. These findings are comparable to those of other workers. Seller et al, (1973) noted a range of 0.7 to 2.2 mg/100 ml in amniotic fluid from foetuses of gestational age 17 to 18 weeks. Similarly Seller et al, (1974b) recorded values for AFP in liquor amnii of 4 mg/100 ml at 14 weeks which went down to 0.5 mg/100 ml at 26 weeks and disappeared after 28 weeks. The results of Field et al, (1973) were lower; 12 liquor
FETOPROTEIN IN LIQUOR AMNII IN NORMAL samples from foetuses of 13-18 weeks gestation showed a mean of 0.8 mg/100 ml with a maximum value of 2 mg/100 ml. The range given by Seppala and Ruoslahti (1972, 1973) was 0.28 to 2.6 mg/100 ml during the second trimester with considerably reduced values in the third trimester. A word of caution in this respect is necessary because of differences in standards used at different centres (Seppala and Ruoslahti, 1973).

There were a total of 9 abnormal pregnancies. The foetus was anencephalic in 3 cases and in all these the AFP was abnormally high for the gestational period the values being 8.1, 0.7 and about 0.3 mg/100 ml respectively. An abortion with a blighted ovum of 8 weeks gestation showed low levels of AFP as did an aborted foetus of 12 weeks gestation. A twin abortion of 24 weeks gestation was unremarkable as was a case of an accidental haemorrhage of 28 weeks gestation. A macerated foetus of 38 weeks gestation showed abnormally high AFP levels while fluid from a pair of twins of 40 weeks gestation was normal.

Beta-1-fetoprotein remains to be characterised in the literature. While producing antibody the Beta component did not appear in the first, rather rigorous, immunization schedule adopted and was noticed only in the antibody when a booster was given almost a year after the commencement of immunization. Our findings indicate that Beta-2-fetoprotein closely parallels AFP but seems to persist in the liquor amnii a little longer. A perusal of the literature indicates a reference to a protein with a similar electrophoretic mobility in the liquor amnii, specifically in cases of "open" neural tube defects. This, however, is a Beta-trace protein occurring in the cerebrospinal fluid of normals but not found in the serum (Macri et al, 1974).

In advanced countries sophisticated means of measuring AFP levels—namely radioimmunoassay—are available in some centres. This technique permits detection of even the small traces of AFP found in sera of normal people. The equipment needed is costly and difficult to come by. The means we have used are simple, unsophisticated and are such as can be developed in any ordinary laboratory. The AGD test is extremely simple and our findings indicate that a positive test in the third trimester means some foetal abnormality. Quantitation by RID is also not difficult but the test requires a deal of expertise in its performance.

Summary

Alpha-fetoprotein (AFP) was looked for in 89 amniotic fluids by agar-gel double-diffusion (AGD). This test had a sensitivity of up to 0.3 to 0.5 mg/100 ml. Positive samples were quantitated by radial immuno-diffusion (RID). All 4 samples in the first trimester showed AFP. Twenty of 23 samples in the second trimester were positive. The mean AFP concentrations measured in RID in the first two trimesters was 3.7 mg/100 ml (range 0.3 to 21.3). Fifty-seven of 62 samples from the third trimester showed absence of AFP; 4 positive were from abnormal foetuses. AFP levels showed a gradual decline with advancing gestation. AGD effectively screened liquor amnii samples from the first two and the third trimester. Three foetuses with anencephaly showed raised values of 8.1, 0.7 and 0.3 mg/100 ml. Abnormal AFP levels were seen in a macerated foetus (38 weeks). Liquor amnii from an abortion with a blighted ovum (8 weeks), abortion (12 weeks), twin abortion (24 weeks) accidental haemorrhage (28 weeks) and
a twin pregnancy (40 weeks) were in the normal range. A Beta-fetoprotein was also detected in the liquor amnii. This paralleled AFP but persisted in the liquor a little longer than AFP.

References