THE INHIBITORY EFFECT OF AMNIOTIC FLUID ON THE GROWTH OF COMMON MICROORGANISMS

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The incidence of chorioamnionitis varies from 6% (Stroup *et al,* 1962) to 12% (Harwick *et al,* 1969). The absence of clinical infection despite the occasional presence of microorganisms in the amniotic fluid suggests the presence of a host defence mechanism in the amniotic fluid, and the recent widespread use of amniocentesis for diagnosing various foetal conditions has not shown any increase in its incidence. The organisms isolated from these cases of chorioamnionitis were staphylococcus aureus. staphylococcus epidermidis, *E. coli,* Proteus, Pseudomonas and sometimes streptococci. Most of these organisms are reflective of a vaginal or foecal flora and suggest that bacteria may gain access into the amniotic fluid from the vagina, even through intact foetal membranes.

Cattazeo in 1949 reported antibacterial activity of amniotic fluid. Since then several workers (Walsh Hildebrandt and Prystowsky, 1963; Sarkany and Gaylards, 1968; Galask and Snyder, 1968, 1970, 1973, 1974, Florman and Teubner, 1969, Bercovici and Sacks, 1972, Schlievert, 1974) have reported antimicrobial properties of amniotic fluid.

We present here our results about the effect of amniotic fluid on some microorganisms which are usually responsible for chorioamnionitis and perinatal infections.

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Material and Methods

Both *in vivo* and *in vitro* studies have been made. Only those cases were selected who had received no antibiotics in the past one week. The *in vivo* effect of amniotic fluid was studied by observing vaginal cultures before and after rupture of membranes in 25 cases. All these cases were in the third trimester of pregnancy. High vaginal swabs were taken with aseptic measures and then artificial rupture of membranes was done (20-25 ml. of amniotic fluid was collected in cases where it was possible for *in vitro* studies). About 1 hour later vaginal swabs were collected again. The swabs were transported to the laboratory and cultured for 24 hours. For the *in vitro* studies 65 samples of amniotic fluid were obtained at different periods of gestation and tested against staphylococcus aureus, strepto-coccus viridans and *E. coli* by spectrophotometry and plate diffusion technique. Amniotic fluid was collected trans abdomenally from the patients who came for mid-trimester abortions and from others by amniocentesis and by transvaginal amniotomy from women who

came in labour. Samples contaminated with blood or meconium were discarded. Amniotic fluid was stored at 20°C and prior to use was centrifuged at 2500 r.p.m. and sterilized by filtration through a 0.45 μ Millipore filter.

In the spectrophotometric studies, growth in amniotic fluid of the above mentioned organisms in 24 hours was compared with their growth in suitable growth media i.e. peptone water for staph. aureus and *E. coli* and glucose broth for Strept. viridans.

In the plate diffusion tests blood agar plates were used for Staph. aureus and Strept.viridans and McConkey plates for *E. Coli*. In the centre of these plates, plated with the above organisms a disc impregnated with amniotic fluid was kept and the zones of inhibition produced around these discs were noted. Controls were similar plates with discs impregnated in sterile water. Samples which produced more than 10 mm. zone of inhibition were regarded as bactericidal.

Results

Of the 25 cases of *in vivo* studies i.e. observation of vaginal cultures before and after rupture of membrane—17 cases (68%) showed decreased growth of bacteria in the swabs collected after rupture of membranes. 6 cases showed no change and only two cases showed increased growth (Photograph No. 1).

In vitro studies: Upto 15-16 weeks of gestation amniotic fluid permitted staph. aureus to grow relatively freely. Thereafter its inhibitory activity appeared and increased gradually, manifesting diminishing growth of bacteria. After 31-32 weeks growth was barely perceptible in 63% of samples by spectrophotometry. Slight decrease in this inhibitory activity of amniotic fluid was noted in samples after term i.e. 40 weeks. With Strept. viridans the inhibitory activity of amniotic fluid was less marked. It showed inhibition only after 27-28 weeks and by 33-34 weeks growth was inhibited in 62% of cases. After 40th week the same phenomenon was observed.

Similar results were obtained against *E. coli*, but the inhibitory effect at 32-34 weeks was seen, and was less marked as compared to Staph. aureus and strept. viridans.

Plate diffusion test: With Staph. aureus no zone of inhibition is produced upto 30 weeks of gestation. Thereafter increasing zones of inhibition appeared upto term. At 37-38 weeks more than 10 mm. zone of inhibition was seen in 50% of cases which increased to 77.7% at 39-40 weeks. Again at 41-42 weeks only 50% of the samples showed this property.

With Strept. viridans the pattern of inhibition was about the same uptil 38 weeks but at 39-40 weeks only 72% of the samples showed more than 10 mm. zone of inhibition (Photograph No. 2). No zone of inhibition was produced at any period of gestation with *E. coli*.

Discussion

The study of inhibitory effect of amniotic fluid is relatively new but in recent years considerable progress has been made on the subject. The presence of a host defence mechanism in amniotic fiuid is implied by the absence of clinical infection inspite of the occasional presence of micro-organisms in amniotic fluid. Indirect evidence of bacterial inhibiting properties of amniotic fluid is further suggested by the fact that after rupture of membranes an alteration in the vaginal flora is observed.

Diminution in the bacterial flora was seen after rupture of membranes in 17

out of 25 cases (68%) studied by us. Bercovici and Diamant (1973) also found diminished growth after rupture of the membranes in 61.5% of their cases.

In our study we had deliberately ruptured the membranes and allowed about 1 hour to elapse before collecting the second sample of swabs for our study. The other workers had not specified the time interval between rupture of membranes and collection of sample. As the time interval after rupture of membranes increases the incidence of chorio-amnionitis shows some rise. This may explain the difference in the figures obtained by Bercovici and Diamant (61.5%) as against ours (68%) where the separate culture was done after a shorter period. Sudden rupture of membranes may also be decreasing organisms of the vaginal flora by washing away a large population of bacteria in the vagina.

In 1949 Cattaneo using plate diffusion technique and Micrococcus as test organism and later Thadepalli (1977) and Applebaum (1978) using the same technique but working with *E. coli* and Staph. aureus demonstrated bacterial growth inhibition around wells filled amniotic fluid. On the other hand Walsh Hildebrandt and Prustowsky (1962) and Sarkany and Gaylards (1967) using the same technique failed to show any inhibition of bacterial growth by amniotic flud.

Galask and Snyder (1968) studied spectrophotometrically the growth curves of organisms grown in amniotic fluid and some standards nutrient media and demonstrated complete inhibition of Proteus mirabilis, streptococcus, bacillus subtilis and candida albicans and partial inhibition of pseudomonas, streptococcus foecalis, staph. aureus and staph. epidermidis.

A more elaborated method—the plate count technique—where bacterial colonies are counted was used by Florman and Teubner (1969), Bergman Bercovici and Sasks (1972) and Patrick Schlievert (1974). They have also found positive evidence of anti-microbial properties in amniotic fluid.

For the present study the spectrophotometric studies of Galask and Snyder and the Plate diffusion technique of Cattaneo and Thadepalli have been combined. We used Staph. aureus, Strept. viridans and E. coli as test organism as these are frequently encountered in chorio-amnionitis, perinatal and puerperal infection.

65 Samples ranging 9-42 weeks have been studied both by spectrophotometry and plate diffusion technique. Spectrophotometry revealed that from 9 to about 20 weeks amniotic fluid tended to support the growth of all three organisms tested. The inhibitory activity set in gradually from about 20 weeks and went on increasing towards term. After 32 weeks there was no increase in the turbidity of the amniotic fluid samples showing complete inhibition of bacterial growth.

By the plate diffusion method we observed that no zone of inhibition is produced by amniotic fluid with any of the test organisms upto 32 weeks of gestation. After this period zones of inhibition appeared in the case of staph. aureus and strepto. viridans with a maximum at 39-40 weeks and thereafter showed a slight fall. No zone of inhibition was produced with E. coli at any period of gestation.

On combining the results of the above experiments the overall picture which emerges is that in the early period of gestation upto about 20 weeks, the inhibitory capacity is very poorly developed—in fact upto 16-18 weeks of gestation the

amniotic fluid seems to support bacterial growth. After 20 weeks of gestation the inhibitory capacity becomes manifest and goes on increasing till it reaches a maximum at 39-40 weeks and thereafter shows a slight fall. These results are in agreement with those of Patrick Schlievert (1974) who showed the inhibitory activity of amniotic fluid was demonstrated as early as 20 weeks reaching a maximum at 36-40 weeks and then declining slightly. Thadepalli in 1978 found absence of any antimicrobial activity in amniotic fluid in the first trimester, but found positive results in the second and third trimester.

The results also show that the antimicrobial activity is more marked for Staph. aureus and strept. viridans and such less for *E. coli*. The results of plate diffusion tests shows the amniotic fluid has no bactericidal effect on *E. coli* and this might possibly explain the higher incidence of *E. coli* as the causative organism in perinatal and puerperal infections.

Several factors have been held responsible for the antimicrobial property of amniotic fluid. The ultimate effect appears to depend on the presence of various combinations of different antimicrobial factors. Though this work is not concerned with the identification of various factors, several factors have been isolated by other workers.

Some of the important factors are Lysozymes, a B-lysin, transferrin, immunoglobulins, peroxidase antimicrobial systems, fatty acids and steroids, metal mediated systems and some cationic peptides. The mechanism of action of these various factors forms a separate but very interesting study.

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See Figs. on Art Paper I