COMPARATIVE STUDY OF THE LEVEL OF CREATININE UREA AND THE FREE AMINO ACID OF AMNIOTIC FLUID OF HUMAN AND RAT AMNION

by

ANJAN BISWAS
A. SEN GUPA
and
ACHINTYA K. MUKHERJEE

SUMMARY

The changes in the level of creatinine, urea and amino acid of amniotic fluid have been assessed at different stages of pregnancy in women and compared with that of rat. The pattern of changes of these three constituents of amniotic fluid in rat is strikingly similar to women. The results of the present investigation suggest that assessment of toxic effects of compounds/drugs on fetus can be predicted by assessing of these parameters in pregnant rats.

Introduction

The study of the amniotic fluid composition and estimation of individual constituents has helped in the assessment of fetal status and thus impart a prognostic tool for clinical obstetrician. Pitkin and Zwirek (1967) and Pitkin *et al* (1968) noted the rise of creatinine level of amniotic fluid with the advancement of pregnancy. Parmley and Muller (1969) observed that the creatinine levels of amniotic fluid predicted with a good accuracy of the presence of fetus of atleast 250 gms or gestational age of 35 weeks. Roychowdhury *et al* (1969) suggested a positive correlation between creatinine content and the fetal distress. De *et al* (1969) observed the rise of the urea content of amniotic fluid with advancement of pregnancy is an indication of placental insufficiency.

The importance of the amniotic amino acid contents with regards to the fetal development and nutrition has been suggested by various workers. In the present study an attempt has been made to evaluate the mechanism of changes of the level of creatinine, urea and free amino acid in amniotic fluid at different stages of pregnancy.

Material and Methods

The amniotic fluid (human) was collected by amniocentesis from hospitalized normal pregnant subjects at various stages of pregnancy viz. 33-36 weeks (early stage of pregnancy), 37-40 weeks (normally terminated pregnancy), 40 weeks onwards (post mature pregnancy).

From: Department of Physiology, Presidency College, Calcutta-700 073 and Department of Obstetrics and Gynaecology, Calcutta National Medical College, Calcutta.

Accepted for publication on 28-1-85.

Before collection of amniotic fluid in rats, both male and female rats (Charls Foster) of proven fertility were put in breeding cages and vaginal smear was examined under microscope every day. The day of detection of sperm in the smear was designated as the first day of pregnancy. Amniotic fluid was collected from pregnant rats after laparotomy under pentobarbital anaesthesia (40 mg/kg b.wt.i.p.) at early (7 days), midterm (14 days) and late term (21 days) of gestation. Amniotic fluid was collected from atleast 20 pregnant women and rats in each group. The creatinine was estimated by the method of Owen *et al* (1954) urea and free amino acid was done by the method of Chaney and Marbach (1962) and by Folin (1934) respectively.

Results

Creatinine content of amniotic fluid rises along with the advancement of pregnancy. The level of creatinine in amniotic fluid at early stage of pregnancy (33-36 weeks), normally terminated pregnancy (37-40 weeks) and post mature pregnancy (40 weeks onwards) was found to be 0.92 ± 0.04 mg/100 ml, 1.75 ± 0.07 mg/100 ml and 2.65 ± 0.12 mg/100 ml respectively (Fig. 1a). Difference between the creatinine level of amniotic fluid of three stages of pregnancy were found to be highly significant ($P < 0.001$).

The creatinine content of amniotic fluid of rat at early stage (7 days) and midterm (14 days) and late term (21 days) were found to be 0.39 ± 0.06 mg/100 ml, 0.61 ± 0.01 mg/100 ml and 1.66 ± 0.04 mg/100 ml respectively (Fig. 1b). The increasing level of creatinine with the advancement of pregnancy is also highly significant in rats ($P < 0.001$).

The content of urea in amniotic fluid increased with the progress of pregnancy in human. The level of urea in amniotic fluid at early stage of pregnancy (33-36 weeks), normally terminated pregnancy (37-40 weeks) and post matured pregnancy (40 weeks onward) was found to be 22.27 ± 0.39, 24.60 ± 0.39 and 32.02 ± 0.36 mg/100 ml respectively (Fig. 2a). Difference between the urea content of amniotic fluid of three stages of pregnancy were found to be highly significant ($P < 0.001$).

The level of urea of rat amnion at 7 days, 14 days and 21 days of gestation corresponding to early stage mid term and late term are 7.62 ± 0.19, 13.07 ± 0.25 and 25.90 ± 0.36 mg/100 ml respectively (Fig. 2b).

Unlike creatinine and urea the free amino acid content of amniotic fluid decreased with the advancement of pregnancy. Free amino acid content of amniotic fluid at early stage of pregnancy (33-36 weeks), normally terminated pregnancy (37-40 weeks) and post mature pregnancy (40 weeks onwards) was found to be 37.49 ± 0.44, 27.57 ± 0.55 and 17.44 ± 0.62 mg/100 ml respectively (Fig. 2a). This changes in content of free amino acid level in amniotic fluid are highly significant ($P < 0.001$).

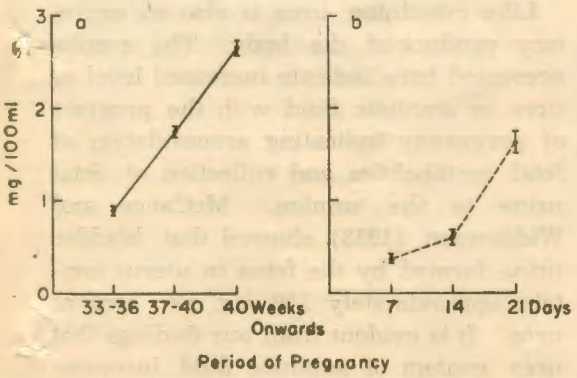

Fig. 1.

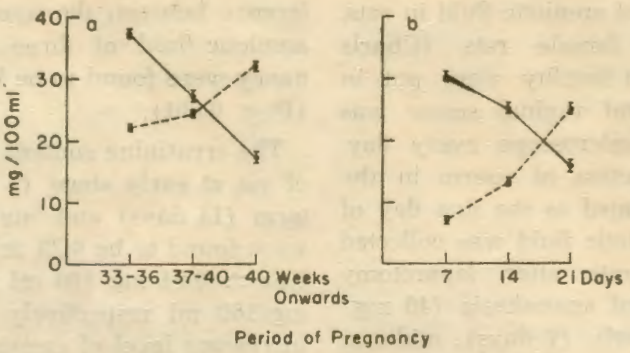

Fig. 2

Free aminoacid content of rats amniotic fluid collected at different days of gestation also show a significant ($P < 0.001$) fall in the amino acid values with the advancement of pregnancy at early stage (7 days), 30.54 ± 0.36, midterm (14 days) 25.75 ± 0.55 and in late term (21 days) 16.55 ± 0.71 mg/100 ml respectively (Fig. 2b).

Discussion

Creatinine a non-protein nitrogen is an excretory product of the body. Excess of creatinine indicates protein catabolism. The results presented here indicate increased level of creatinine in amniotic fluid with the progress of pregnancy, maximum at post mature pregnancy in case of human being. The high creatinine content in the amniotic fluid may be attributed to excretory products of the fetus which is concentrated in the amniotic fluid volume. Alternately, the incipient hypoxia increases the capillary permeability and thereby creatinine diffuses through the capillary membrane and accumulate in the amniotic fluid. Normally a maternal-fetal barrier exists which regulate the diffusion of creatinine in normal pregnancy. However, in post mature pregnancy this maternal-fetal barrier is deranged resulting in increased level of creatinine in amniotic fluid. However, in case of normally terminated pregnancy (37-40 weeks) increased level of creatinine in the amniotic fluid (Fig. 1a) has been observed. These results are in agreement with that of Pitkin and Zwirek (1967). The increase in level of creatinine in normally terminated pregnancy is possibly due to increased activity of the fetal kidney and increased transplacental exchange. It is interesting to note that there is parallelism between increased level of creatinine content of amniotic fluid both in the pregnant rat and woman with the advancement of pregnancy indicating the similarity in the regulatory mechanism of creatinine level in amniotic fluid.

Like creatinine, urea is also an excretory product of the body. The results presented here indicate increased level of urea in amniotic fluid with the progress of pregnancy indicating accumulation of fetal metabolities and collection of fetal urine in the amnion. McCance and Widdowson (1953) showed that bladder urine formed by the fetus in uterus contain approximately 100 mg. per cent of urea. It is evident from our findings that urea content of amniotic fluid increases with the advancement of pregnancy in

both human and rat. This increase is due to addition of fetal urine and concentration of amnion. The physiological mechanism of this elevation of urea in both rat and human indicate a similar metabolic pattern in the two different species of mammals. It may also be speculated that the fetal metabolic pattern runs a similar physiological pattern in mammals.

The free aminoacid in amniotic fluid plays a prominent role in the development of fetus. Transplacental absorption and utilization of aminoacid by the fetus for its growth and development results in lowering of the free aminoacid level in the amniotic fluid. Because of the above facts the free aminoacid level of the amniotic fluid declared with increasing demand of the fetus for its rapid development at the later phase of pregnancy. The results of the present investigation clearly indicates that the amniotic aminoacid metabolism follows the similar pattern in both pregnant rats and in the pregnant women.

The levels of creatinine, urea and free aminoacid in the amniotic fluid have been correlated to the fetus status, placental insufficiency and maturation of the fetus during the prenatal stage. The results presented here clearly indicate the pattern of changes of the three parameters of rat and is strikingly similar to human being. These observations are particularly important in predicting the adverse effect of experimental drugs or environmental pollutents by assessing their effects on levels of creatinine, urea and free aminoacid in amniotic fluid of pregnant rats.

Acknowledgement

Authors gratefully acknowledge to Dr. S. K. Chaudhuri, Organon Research Centre, Calcutta for his help and guidance in preparation of this manuscript.

References

1. Chaney, A. L. and Marbach, E. P.: Clin. Chem. 8: 130, 1962.
2. De, K. C., Roychowdhury, N. N. and Mukherjee, A. K. Cal. Med. Jour. 66: 73, 1969.
3. Folin, O. J. Biol. Chem. 106: 311, 1934.
4. McCance, R. A. and Widdowson, R.: Proc. Royl. Soc. Lond. Ser. B., 141: 488, 1953.
5. Owen, J. A., Iggo, B., Scandrett, F. J. and Stewart, C. P.: Biochem. J. 58: 426, 1954.
6. Parmley, I. Miller, E.: Am. J. Obstet. Gynec. 98: 1135, 1969.
7. Pitkin, R. M. and Zwirek, S. J.: Am. J. Obstet. Gynec. 98: 1135, 1967.
8. Pitkin, R. M., Reynolds, W. A. and Burchell, R. C.: Am. J. Obstet. Gynec. 100: 835, 1968.
9. Roychowdhury, N. N., De, K. C. and Mukherjee, A. K.: Cal. Med. Jour. 66: 98, 1969.