

# CHARACTERISATION OF TERM PREGNANCY AND LABOUR BY CYTOCHEMICAL STUDIES ON VAGINAL EXFOLIATED CELLS

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Considerable information has been obtained regarding the variations in morphology in exfoliated cells during menstrual and reproductive cycle (Papanicolaou, 1946; Liu, 1955; Stern *et al*, 1966; Rubio, 1966; Malek, *et al*, 1967; Nyklicek, 1968; Wahi, 1969). It has been observed from this laboratory that a series of biochemical changes take place in the exfoliated cells during the advancement of pregnancy and in disturbed pregnancy (Bose *et al*, 1971; Bhowse *et al*, 1971). Further impetus developed when it was noted that alkaline phosphatase undergoes changes in terms of its affinity towards different substrates during labour (Bhowse, *et al*, 1971). To explore further whether there are any changes of the in-situ biochemical constituents of the exfoliated cells in term pregnancy and labour, a scrutiny of different macromolecular constituents were made by cytochemical means.

## Materials and Method

Vaginal smears were taken from the posterior fornix from 108 cases of term pregnancy. The diagnosis of duration of pregnancy were done clinically and were followed for confirmation. Smears were

also collected at the onset of labour. Vaginal smears from normal premenopausal fertile women were taken at 16-18 days of the menstrual cycle when estrogen and progesterone are operating at a moderate level. Any symptoms or signs indicating slightest departure from normality were excluded from this series (Taylor, 1967).

## Localization of Glycogen

Best's carmine method was employed for localization of glycogen described by (Mc. Manus, and Mowry 1960a). The staining time was for thirty minutes. Control slides were treated with diastase and salivary amylase to determine specificity of reaction. The differential counts were made with respect to cells displaying positive or negative reaction for glycogen and the different intensities of positive reaction were graded with respect to different cell types (Figs. 1 & 2).

## Localization of Acid Mucopolysaccharide

Acid mucopolysaccharide was localized following Mowri's technique cited by McManus and Mowri (Mc. Manus, *et al*, 1960b). The incubation period was for thirty minutes. Control slides were kept by digesting the slides with hyaluronidase, and also by deleting colloidal iron solution from the staining procedure. The rating was done according to the intensity of reaction. Percentage counts of cells

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displaying positive and negative reaction were also determined.

#### Localization of Succinic Dehydrogenase

The activity of this enzyme was demonstrated according to the technique of Nachlas *et al* (Nachlas *et al*, 1957) using Nitro B. T. as electron acceptor. This site of enzyme activity is demonstrated by the deposition of formazan granules. The localization was seen to be strictly cytoplasmic. Subjective grading was done according to the intensity of reaction, and percentage of cells displaying positive reaction for the enzyme activity has been determined by counting approximately 500 cells per slide.

#### Results

The occurrence and distribution of different macromolecular species studied in present investigation reveal certain striking features. It appears from the glycogen distribution (Tables I & II) that

TABLE I

*Distribution of Glycogen in Vaginal Exfoliated Cells During Term Pregnancy and First Stage of Labour*

Cell types	Non-pregnant	Term pregnancy	Labour
Superficial	2+	4+	±
Intermediate	3±	4+	±
Parabasal	1+	2+	±

TABLE II

*Mean Percentage of Cells Showing Different Intensities of Glycogen During Pregnancy and Labour*

% of Cells displaying glycogen	Non-pregnant	Term pregnancy	Labour
Total	97.0	99.2	18.8
High	85.7	96.2	6.5
Low	11.3	3.0	12.3
Negative	3.0	0.8	81.2

it is sharply increased during term pregnancy than from non-pregnant condition. During labour the glycogenic mass sharply declines, leaving only 6.5% of high glycogen containing cells as against 96.2% of that in term pregnancy. However, a reverse phenomenon pertaining to the distribution of acidmucopolysaccharide has been noted in similar situations (Tables III & IV). This mucosubstance

TABLE III

*Distribution of Acid Mucopolysaccharide in Vaginal Exfoliated Cells During Term Pregnancy and First Stage of Labour*

Cell types	Non-pregnant	Term pregnancy	Labour
Superficial	1+	3+	4+
Intermediate	1+	3±	4±
Parabasal	±	2+	3+

TABLE IV

*Mean Percentage of Cells Showing Positive Reaction For Acid Mucopolysaccharide During Term Pregnancy and Labour*

	Non-pregnant	Term pregnancy	Labour
% of cells with positive reaction	45	61.3	86.8

has been found to be steadily increased from non-pregnant condition to the stage of labour, where 86.2% cells show intense reaction throughout the cytoplasm. On the other hand, a parallelism is seen to exist between distribution of glycogen and succinate dehydrogenase under the above three conditions.

The percentage of cells showing positive reaction for succinate dehydrogenase rises significantly with the advancement of pregnancy. At the third trimester of pregnancy, about 76.5% cells show positive reaction as against 33.8% in case of non-

pregnant condition. The number, however, sharply drops at labour wherein 25.3% cells show positive reaction for this enzyme activity. Among the cells with positive reaction, the distribution pattern in the different cells also display regular increase in all the cell types with advancement of pregnancy, being maximum during the third trimester. However, with the decrease of number of positive cells during labour the intensity of enzyme reaction sharply falls in all the cell types. (Tables V & VI)

TABLE V

*Succinate Dehydrogenase Activity in Vaginal Exfoliated Cells During Term Pregnancy and Labour*

Cell types	Non-pregnant	Term pregnancy	Labour
Superficial	±	3+	1+
Intermediate	1+	3+	1+
Parabasal	2+	3+	1+

TABLE VI

*Mean Percentage of Cells Showing Positive Reaction of Succinate dehydrogenase in Non-pregnant Condition and in Term Pregnancy and Labour*

	Non-pregnant	Term pregnancy	Labour
% of Cells with positive reaction	33.8	76.5	25.3

#### Discussion

It appears from the above results that the occurrence and distribution of certain macromolecules in vaginal exfoliated cells show a distinct relationship with the change of physiological status. Recently we have reported a change in respect of alkaline phosphatase activity as well as its differential hydrolytic behaviour pertaining to various substrates under similar

conditions (Bhose *et al*, 1971). There are several evidences which show that hormonal status plays a significant role in the occurrence and distribution of certain macromolecules (Tock and Shilkin, 1970; Vacek, 1965; Foraker *et al*, 1963; Frost & Dakin, 1970). In the present results the increase of glycogen, mucopolysaccharides and succinate dehydrogenase during pregnancy indicates hormonal influence over these macromolecules during this period. However, the decrease of glycogen and succinate dehydrogenase during labour assumes significance in the light of cessation of placental hormonal functions. There are several evidences which reveal the increase of mucopolysaccharides in vaginal epithelium under progesterone influence (Botella-Llusia *et al*, 1958). Strangely enough, alcian blue positive substances are more abundant in the exfoliated cells during labour. It needs mentioning that pregnancy and labour are opposite physiological manifestations with respect to hormonal status and hence the abundance of acid mucopolysaccharides in labour cannot be explained in terms of progestational influence. There are several evidences which reveal that acid mucopolysaccharide exist in different molecular forms (Shiller *et al*, 1962). Probably all these forms are not progesterone dependent. Since the metabolism of mucopolysaccharide is profoundly influenced by various hormonal factors rather than progesterone alone (Dorfman, 1963; Schiller and Dorfman, 1957; Schiller *et al*, 1962); the increase of this polymer in labour might be due to increase of certain forms of acid muco-substance dependent on hormonal factors other than progesterone operating during labour (Ohakwa, and Ohakwa 1970). Biochemical evidences regarding the electrophoretic mobility of acidmuco-

substances from connective tissue of cervix under conditions of pregnancy and labour indicate a distinct differential mobility of the individual components of mucopolysaccharides which could be correlated with specific cervical conditions.

#### Summary

Cytochemical studies of glycogen, acidmucopolysaccharides and succinate dehydrogenase have been made in the vaginal exfoliated cells in cases of term pregnancy and labour. The results show that while all the three macromolecules increase markedly during pregnancy, glycogen and succinate dehydrogenase show a sharp decrease with the onset of labour. Such change of the chemical environment of the exfoliated cells possibly reflects the complex phenomenon involving the shift of hormonal status leading to the onset of labour.

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See Figs. on Art Paper I