ESTIMATION OF NUCLEIC ACIDS AND PROTEIN AS AN INDEX OF PLACENTAL GROWTH IN PLACENTAE OF NORMAL AND LOW BIRTH WEIGHT INFANTS

by

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Introduction

Increasing interest in evaluation of the newborn infant by birth weight in relation to gestational age has led to a great deal of work on intrauterine growth retardation resulting in small for dates infants (Gruenwald, 1963; Scott and Usher, 1966).

The average birth weight of babies in India is substantially lower than that in developed countries. The vast majority of these babies who are premature according to the internationally accepted definition of prematurity, behave like full term infants and do not need special care (Raghiviah, *et al*, 1962; Ghosh and Beri, 1962).

An important question that has to be decided in the study of these infants is the relative value of genetic and nutritional influences on intrauterine growth and development. In experimental animals there is evidence that birth weight is determined primarily by maternal nutrition rather than genetic factors (Wiggleswor'h, 1964). Undernutrition of the mother during pregnancy was not considered a significant cause of the growth retardation in humans since the growth retardation encountered in underprivileged malnourished population group is not as severe as pathological growth retardation due to placental insufficiency. Clinical studies could not provide satisfactory answers to the question of relative importance of genetic and nutritional factors in these populations.

The work done by Winick (1970) showing that the deviants from normal growth due to intranatal and postnatal nutritional deprivation may be decided by estimating the nucleic acid and protein content of the placenta and foetal organs has given us a new approach to the problem of determining the relative importance of nutritional and genetic factors in low birth weight infants in our population. This study has been carried out with the following aims.

1. To study the pattern of cellular growth in the human placenta of normal birth weight babies.

2. To compare the above findings with those of placentae of infants showing

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intrauterine growth retardation, i.e. 2 standard deviation below mean weight for gestation according to two standards:

(a) Usher's (Canadian figures).

(b) Indian figures (calculated from Irwin Hospital data).

Material and Methods

Eighty-six human placentae from singleton birth were collected and studied at Maulana Azad Medical College and associated Irwin and G. B. Pant Hospital.

Placentae of subjects having diabetes, erythroblastosis foetalis, twins and of still born and malformed babies were excluded from the study.

Pregnancies complicated by toxaemia, hypertension, anaemia, antepartum haemorrhage compatible with foetal life and deliveries after 29 weeks of gestation were included.

Gestational age was calculated by Naegele's rule and expressed in terms of completed weeks. On careful inquiry from the mother it was possible to obtain gestational age in all cases. The accuracy of dates according to the mother was further checked by Usher's criteria of maturity.

Baby's birth weight was recorded in grams on an Avery beam type weighing scale.

Collection of Placentae

Placentae were collected after delivery and examined for any abnormality. In most of the cases it was possible to do the processing immediately after delivery but in a few cases delivered at night, placentae had to be kept in the deep freeze working at —20°C till morning.

Placental membranes were removed, blood was carefully drained by gentle squeezing and placentae blotted to remove as much blood as possible. Umbilical cord was cut flush with the placental surface. Placentae were then weighed. Small pieces were removed from central and peripheral portions of placentae and washed with ice-cold normal saline to remove blood. The tissue was next blotted with filter paper, 2 G was weighed out accurately and homogenised in the cold in a pestile type of homogenizer. 20% homogenate was prepared in distilled water and filtered through a piece of muslin.

The filtered homogenate was used for the estimation of protein content by Lowry *et al* (1951) method and for extraction of R N.A. and D.N.A. by Schmidt and Thanwhauser (1945) procedure. R.N.A. was estimated by Mejbaum (1939) procedure and D.N.A. by Indole method.

The values of protein, R.N.A. and D.N.A. content per gram wet weight placenta thus obtained were considered in relation to infants birth weight. The birth weights were plotted against the period of gestation on the growth rate charts of Usher (1969), and Irwin Hospital data (1972) as follows:

Group I: Thirty-nine normally grown infants with appropriate birth weight for the period of gestation. These were further divided into two groups:

(a) Pre-term infants between 32-36 weeks of gestation (10 infants).

(b) Term infants born after 37 completed weeks of gestation (29 infants).

Group II: Twenty-four infants having birth weight 2 S.D. below mean for their period of gestation according to Usher.

Group III: Twenty-three infants having birth weight 2 S.D. below mean for their period of gestation according to Irwin Hospital figures.

There were no pre-term babies in Groups II and III.

Observations

In 86 placentae R.N.A. and D.N.A. and protein content per unit weight placenta, placental weight feto-placental-weight ratio were studied to note the changes in placental growth pattern associated with deviants from the normal.

The mean birth weights of the term infan's was 2770 G and of pre-term infants was 1860 G in Group I and 2242 G and 1761 G for Groups II and III (Table I).

Feto-placental weight ratio: Though there was a linear correlation between foetal and placental weights, the mean feto-placental ratio remained the same in Groups I and II (7.9). In Group II it was as high as 9.4 showing a marked reduction in placental weight in comparison to foetal weights (Table I).

Protein (Table II)

The mean protein content expressed in mg/gm wet weight placenta was 28.7 in pre-term controls rising to 33.05 in control term infants of Group I. In Group II it was almost the same, i.e. 33.29 but in Group III there was statistically significant rise to 47.74 ($P < 0.001$). The interpretation and significance of these values is discussed.

R.N.A. (Table III)

The mean R.N.A. content expressed in mg/gm wet weight placenta was 0.63 in

TABLE I

Mean and S.D. of Birth Weight, Placental Weight and Fetoplacental Ratio

Groups	Weight of foetus (G) Mean ± S.D.	*Weight of placenta (G) Mean ± S.D.	@ Fetoplacental ratio Mean ± S.D.
I	a. 1866 ± 321.5	237 ± 43	7.9 ± 1.12
	b. 2770 ± 385	325 ± 53	8.5 ± 0.57
II	2243 ± 97	288 ± 48	7.9 ± 1.1
III	1761 ± 214	187 ± 19	9.4 ± 1.0
Comparison of Group means	I (b) and II: t = 1.84 for 51 d.f. t = 2.3 for 51 d.f. 0.05 < P < 0. 10 N.S. P> 0.05 N.S.		
	II and III: t = 8.64 for 50 d.f. t = 4.150 d.f. P<0.001 S P <0.001 S		
	S. Significant — N.S. Not significant.		

TABLE II

Protein Content

Groups	Protein mg/g. Mean	Wet Weight ± S.D.	Placenta
I	a. 28.7	± 4.4	
II	b. 33.03	± 3.67	
	33.29	± 5.22	
III	47.74	± 7.04	
Comparison of Group means	I (b) and II t = 0.18 for 50 d.f. P> 0.8 N.S.		
	II and III t = 8.64 for 49 d.f. P <001 S.		
	S. Significant — N.S. Not significant.		

pre-term, rising to 0.77 in control term infant of Group I. In Group II it was almost the same i.e. 0.735 but in Group III it was significantly higher to 1.150 ($p < 0.001$).

RNA/DNA Ratio (Table V)

The mean RNA/DNA ratio in Placentae of control term infants was practically the same for control pre-term

TABLE III

Ribonucleic Acid Content

Groups	RNA mg/g Mean	Wet weight placenta ± S. D.
I	a. 0.639	± 0.169
	b. 0.774	± 0.100
II	0.735	± 0.108
III	1.150	± 0.109

Comparison of Group means: I (b) and II: $t = 1.0$ for 51 d.f. $P > 0.3$ N.S.
II and III: $t = 11.9$ = for 50 d.f. $P < 0.001$ S.
S. Significant — N.S. Not significant.

D.N.A. (Table IV)

In the control group DNA increased linearly from 1.167-1 394 till placental weight reached 270 G, birth weight 2300 G or at 36 weeks of gestation, thereafter the curve levels of showing cessation of hyperplasia at the stage of gestation. In Group II the DNA values were almost the same as in controls but in Group III the DNA values were significantly lower ($P < 0.001$). This value was even lower than that of pre-term controls showing that cell hyperplasia stopped earlier than 34 weeks.

Group I and Group II. In contrast the RNA/DNA of Group III was significantly elevated.

Discussion

Foetal Weight, Placental weight and Feto-placental Weight ratios

A linear correlation is demonstrated between weight of the baby and weight of the placenta, though there is a wide variation in the weight of the placenta in relation to birth weight. Many authors have commented on the wide range of the placental weight compatible

TABLE IV

Desoxy Ribonucleic Acid (DNA) Content

Groups	DNA mfi/g Mean	Wet weight placenta ± S.D.
I	a. 1.167	± 0.19
	b. 1.394	± 0.11
II	1.353	± 0.045
III	0.955	± 0.12

Comparison: I (b) and II: $t = 1.12$ for 43 d.f. $P > 0.02$ N.S.
II and III: $t = 13.2$ for 43 d.f. $P < 0.001$ S.
S. Significant — N.S. Not significant.

TABLE V
R N A : D N A Ratio

Groups	* RNA/DNA ratio Mean ± S.D.	
I	a. 0.55	± 0.17
	b. 0.56	± 0.095
II	0.54	± 0.087
III	1.21	± 0.213

Comparison of Group means: I (b) vs II* $t = 0.924$ for 43 d.f. $P > 0.3$ not significant.
II vs III* $t = 13.2$ for 43 d.f. $P < 0.001$ significant.

with any given birth weight due to variation in timing of clamping the cord, method of trimming the membranes and drainage of blood from the placenta.

In this study a ratio of 7.9 in Group II which is lower than the ratio of 8.5 for term babies is statistically significant ($P < 0.05$). This is difficult to explain.

It is possible that the increase in size of placenta of infants in this group showing very mild intrauterine growth retardation might be due to a compensating mechanism to increase its functional capacity.

The mean placental weight of the severe growth retarded infants in Group III was significantly less than that in the term babies of Group I (b) ($P < 0.001$). Intrauterine growth retardation of foetus as a result of placental insufficiency is a well documented fact. (Gruenwald, 1963 and Mac Burney *et al* 1947). Usher *et al* (1966), Winick (1967) and Younoszai and Howarth (1969) have also shown that placentae of infants with intrauterine growth retardation were smaller than those of normal term infants but they did not find any striking deviation in the average ratio of the placental weight to birth weight, shows that in their series there was proportionate decrease in weight of the placenta and baby. In this study fetoplacental ratio is significantly more 9.4 as compared to 8.5 in Group I (b) ($P < 0.001$), showing that the decrease in weight of the placenta is much greater in proportion to the decrease in weight of the infants.

Biochemical Analysis

In this study biochemical analysis was carried out on bits of tissues which were taken from different parts of the placenta and pooled together, not on the whole placenta. The values are therefore expressed per unit weight placenta and not as total values. As the co-efficient of variation between individual values in each group was very low the values expressed per unit weight were taken as representative of the whole placenta.

Protein

There was a proportionate increase in placental protein content per gm. wet tissue with an increase in weight of the placenta and period of gestation in control group as also reported by Winicks (1967). Protein content was significantly higher ($P < 0.001$) in the placentae of group III, i.e. growth retardation by Indian standard, while there is no difference between the other two groups. Some of this high protein can be explained by the difficulty in draining of blood encountered in these cases.

Garrow and Fhawes (1971) has emphasized the importance of obtaining blood free tissue for analysis when estimating the cellular protein of the placenta. In this study, though every attempt was made to drain blood from the decidual surface and foetal vessels of the placentae in order to minimise variability resulting from inclusion of blood upon the chemical composition, in some placentae from postmature and dysmature babies it was found to be difficult. Garrow and Fhawes (1971) found that in placentae from term deliveries which were trimmed and drained, in standard manner the amount of trapped blood after draining ranged from 25 G to 270 G and in placentae of post-mature and dysmature infants trapping was larger than in normal term babies.

Winick (1967) by analysing the total placental protein content of intrauterine growth retarded infants showed a reduction in protein content proportionate to the reduction in placental weight indicating normal cell size. Since in this study the protein was estimated per gm. wet weight without reference to placental size, the same conclusion as Winicks (1967) cannot be drawn.

Younoszai and Howarth (1969) like Winick estimated the total placental protein content and yet found an increase in the protein content of (mainly non-Collagenous) the placentae of infants with intrauterine growth failure. They suggested that it may be due to a larger number of cells or cellular hypertrophy. As they did not estimate DNA content the former possibility cannot be proved.

As in this study only soluble protein has been determined and not insoluble collagen protein, it is not possible to make a direct comparison between results of this study and those previously repeated in the literature.

DNA

DNA is essentially limited to the nucleus. Since it is present in a constant amount within the deploid nucleus of each species the total quantity of DNA reflects the number of cells.

Winick (1967) has stated that growth of the placentae is a continuous process of cell replication. It seems though that a point is reached when its growth level of presumably sufficient size is reached when replication plateus, total DNA content will too, indicating that growth has ceased considerably before growth stops and final phase of growth is without cell division.

In this study it was found that DNA/gm wet weight placenta in group I controls increased linearly only until placental weight approximated about 270 gm., which is lower than Winicks (1967) placental weight, birth weight reached 2300 or upto 36 weeks of Pregnancy which corresponds with Winicks (1967) figures.

DNA content of Group II did not differ much from Group I term infants. ($P > 0.2$). When the two are combined together the DNA plateaus at a lower birth weight of 2200 G. In this study there was very little difference in DNA content of the placentae of pre-term and term infants of Group I. This may be due to the fact that most of the cases were between 34-36 weeks (only two were less than 34 weeks) and has already attained the adequate number of cells.

The DNA content of the placentae of infants in Group III is significantly less ($P < 0.001$) than in Groups I and II at term. This shows that cell number is

reduced in these placentae. DNA content in Group III is even less than that of preterm groups. As in the human placenta there is no further increase in cell number after approximately 36 weeks of gestation and since cell number is reduced in these placentae, it would appear that whatever stimulus retarded the growth, it must already be operating before this, even prior to the 34th week of gestation as most preterm infants were between 34-36 weeks and DNA levels in Group III infants are even lower than these.

RNA

RNA/gm. wet weight placenta increases linearly with placental weight showing that RNA contents increases with placental weight and continues until term. Winick (1967) has also observed the same linear pattern. There is no significant difference between Groups II and Group I term values ($P > 0.3$) RNA concentration in the placentae of infants with intrauterine growth failure of Group III, which is also reported by Winick (1967).

The RNA/DNA ratio is however definitely elevated in placentae of infants, intrauterine growth retardation increased RNA/DNA ratio has been noticed by various authors whenever there is tissue stress, e.g. in cardiac hypertrophy secondary to experimental aortic ligation (Gluck *et al* 1964) uterine hypertrophy due to hermonate factors (Moore and Hamilton 1964) and human bone marrow has shown a similar rise in association with various maligrant Neoplasm (Povlovsky 1966).

Increased RNA/DNA ratio is not dependent on a lack of foetal growth alone. Since it does not occur when growth is compared in conjunction with congenital malformation (Winick, 1967); it is possible that this indicates placental insufficiency in the placentae of infants with intrauterine growth failure and no other abnormalities.

Summary

The conclusion drawn from this study was that the values of RNA, DNA and protein per unit weight placenta show statistically significant difference in babies 25 D below mean weight for gestation according to Usher but within the normal range of Irwin Hospital as compared to control term infant of Group I. In Group III, i.e. babies 25 D below mean weight for gestation according to Irwin Hospital there is a highly significant difference with a change similar to that showing Winick to be associated with intrauterine malnutrition. Smallness at birth can therefore be attributed to both genetic and nutritional factors, the minor deviants being due to the genetic factors while the more marked deviants show intrauterine malnutrition.

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