Ascorbic acid concentration of human fetal tissues in relation to fetal size and gestational age

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1. Studies were carried out on the distribution of ascorbic acid in human fetal tissues with the progress of gestation.

2. Fetuses and stillborn babies varying in gestational age from 12 to 38 weeks were obtained from various Baroda hospitals. Ascorbic acid levels were determined in selected tissues: brain, adrenal, liver, kidney, lung, heart and placenta.

3. Ascorbic acid concentration in the brain was higher than that in the adrenal at all gestational ages, suggesting the importance of this vitamin in brain development. The concentrations of this vitamin in liver, kidney, lung and placenta were comparable, but that in the heart tended to be lower. In all the tissues, there was a fall in ascorbic acid during late gestation. However, the levels in tissues of stillborn babies were higher than those reported for adults.

It is well known that reproductive performance imposes physiological and nutritional stress on the maternal organism. When the maternal diet is already poor, it is likely to become more so with the advent of pregnancy and lactation. The demands for ascorbic acid are very high during reproduction as the fetus is born with a store of 700–1000 mg of this vitamin (Rajalakshmi *et al.* 1974).

It is also known that the placenta has a high concentration of ascorbic acid (Subbulakshmi, 1970) and that cord blood has a higher concentration of ascorbic acid than maternal blood (Vobecky *et al.* 1982). This was also found to be true of poorly nourished women with low intakes of ascorbic acid in previous studies in this laboratory (Rajalakshmi & Ramakrishnan, 1969). The latter study showed the satisfactory transfer of ascorbic acid to infants even by mothers on low intakes of the vitamin. A question arises as to the status of the growing fetus with regard to ascorbic acid. The present studies are concerned with the distribution of ascorbic acid in human fetal tissues in relation to gestational age and fetal growth.

MATERIALS AND METHODS

Studies were made on 121 fetuses obtained from three hospitals in Baroda. Fetuses less than 20–22 weeks were largely obtained by medical termination of pregnancy, whereas those above 20–22 weeks were largely derived from spontaneous abortions and stillbirths. They were immediately kept in ice after abortion, transported to the laboratory, dissected and the tissues taken for analysis.

Gestational age was taken as menstrual age (as reported by the mother after careful questioning) minus 2 weeks to allow for ovulation. Ascorbic acid levels were determined in selected fetal tissues: brain, adrenals, kidney, liver, lung, heart and placenta. The method used for the estimation of total ascorbic acid was the dinitrophenyl hydrazine procedure (Roe & Kuether, 1943). DNA was estimated by the procedure of Schneider (1957). Student's t test was used to determine the significance of differences between groups.

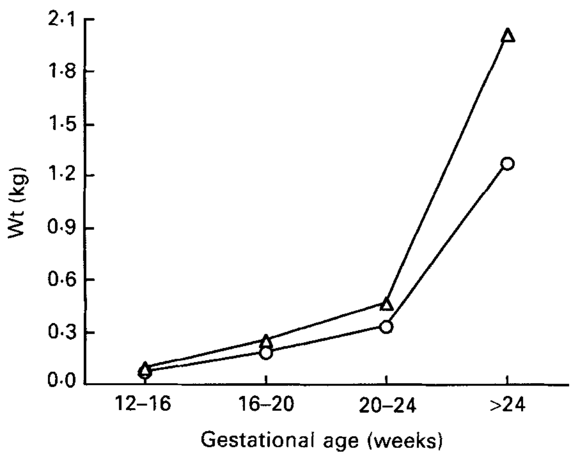

Fig. 1. Fetal weight with progress of gestation for growth-normal (△) and growth-retarded (○) human fetuses (for details of fetuses, see p. 601). Numbers of observations for the different age groups are given in parentheses. Growth-normal: 12–16 weeks (24), 16–20 weeks (48), 20–24 weeks (12), > 24 weeks (10). Growth-retarded: 12–16 weeks (4), 16–20 weeks (8), 20–24 weeks (11), > 24 weeks (2).

RESULTS

Fetal weights were found to increase significantly with the progress of gestation; they were less than those reported for corresponding gestational age in the West (Gruenwald, 1966; Thompson *et al.* 1968), but the values for weeks 12–16 and 16–20 compare with those reported by others in this country (Iyengar & Apte, 1972; Lakshminarayan *et al.* 1974).

When fetal weights were considered as a percentage of expected weight for gestational age using Widdowson's (1968) norms, they were classified as growth-normal and growth-retarded, using a cutoff point of 60%. The differences in fetal weights between growth-normal and growth-retarded fetuses are shown in Fig. 1. The growth-normal fetuses exhibited higher tissue weights than growth-retarded fetuses, which is consistent with the differences in their body-weights.

The ascorbic acid concentration in fetal tissues with the progress of gestation is presented in Table 1. The pattern for adrenal and brain tissues was unexpected, the latter being greater than the former. This was true for all gestational ages. The brain values were very high and in the range 0·104–0·785 mg/g during 12–20 weeks of gestational age.

Ascorbic acid concentration showed a decrease during late gestation in all tissues, the decrease being significant in the case of brain and adrenals. The decrease in concentration of this vitamin in the lung was at an earlier age.

The growth-retarded fetuses showed a significantly lower concentration of ascorbic acid than the growth-normal fetuses during weeks 12–16 of gestation in the brain, liver and kidney, but a similar pattern was not observed thereafter (Table 2).

DISCUSSION

Brain ascorbic acid levels were greater than adrenal ascorbic acid levels at all gestational ages. The mean moisture content of the brain at weeks 16–20 of gestation was found to be 909 mg/g and protein concentration was 44 mg/g, so that non-protein solids accounted for 47 mg/g. Ascorbic acid appeared to form 1% of non-protein solids.

The ascorbic acid levels in the brain declined significantly after 32 weeks of gestation. Even in stillborn babies, ascorbic acid in the brain remained higher than those reported for

Table 1. *Ascorbic acid concentration in human fetal† tissues with the progress of gestation (mg/g)*

(Mean values with their standard errors; no. of observations in parentheses)

Gestational age (weeks)...	< 16		16–20		20–24		28–32		> 32	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Brain	0·349 (27)	0·024	0·408 (43)	0·030	0·423 (23)	0·037	0·525*** (5)	0·050	0·254 (7)	0·057
Adrenals	0·224 (27)	0·020	0·206 (43)	0·013	0·232 (23)	0·015	0·295* (5)	0·060	0·142 (7)	0·020
Liver	0·197 (27)	0·018	0·166*** (46)	0·012	0·229 (23)	0·018	0·159 (5)	0.039	0·114 (7)	0·016
Kidney	0·197 (27)	0·015	0·186 (32)	0·014	0·221 (12)	0·028	0·162 (5)	0·024	0·110 (7)	0·035
Lung	0·210 (11)	0·020	0·184 (32)	0·011	0·220*** (9)	0·027	0·111 (5)	0·020	0·096 (7)	0·019
Heart	0·110 (11)	0·009	0·111 (25)	0·008	0·122 (9)	0·008	0·121 (5)	0·019	0·079 (7)	0·017
Placenta	0·157 (6)	0·027	0·166 (19)	0·011	0·161 (9)	0·016	0·197 (5)	0·029	0·150 (2)	0·050

Mean values were significantly different from those for the subsequent gestational age-group: * $P < 0.05$, *** $P < 0.001$.

† For details of fetuses, see p. 601.

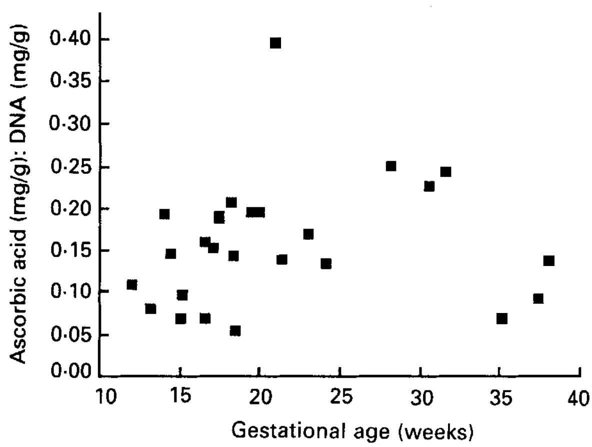

Fig. 2. Human fetal brain ascorbic acid: DNA with progress of gestation (for details of fetuses, see p. 601).

adults (Hornig, 1975). Similar observations have been made by Adlard *et al.* (1974), who reported that forebrain ascorbic acid levels in full-term babies (37–42 weeks gestational age) were at least three times higher than the adult levels. In this connection, it is known that the rate of decrease in ascorbic acid is greatest during early life, but continues to some extent into adult life (Schaus, 1957).

Autoradiographical studies by Hammarstrom (1966) on pregnant mice have confirmed that the fetal brain at full term concentrates ascorbic acid up to 100-fold from maternal

Table 2. *Ascorbic acid concentration in tissues of growth-retarded (GR) and growth-normal (GN) human fetuses (mg/g)*†

(Mean values with their standard errors; no. of observations in parentheses)

Gestational age (weeks)...		12–16		16–20		20–24		28–38	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Brain	GR	0·226** (4)	0·044	0·399 (5)	0·093	0·437 (11)	0·058	0·453 (3)	0·152
	GN	0·369 (23)	0·025	0·410 (38)	0·031	0·410 (12)	0·047	0·338 (9)	0·057
Adrenals	GR	0·172 (4)	0·042	0·187 (7)	0·015	0·228 (11)	0·022	0·180 (3)	0·022
	GN	0·233 (23)	0·023	0·210 (36)	0·014	0·237 (12)	0·022	0·214 (9)	0·046
Liver	GR	0·106*** (4)	0·005	0·180 (7)	0·028	0·249 (11)	0·021	0·155 (3)	0·038
	GN	0·210 (23)	0·019	0·163 (39)	0·013	0·211 (12)	0·029	0·131 (9)	0·020
Kidney	GR	0·149* (4)	0·013	0·180 (5)	0·048	0·193 (6)	0·032	0·142 (3)	0·030
	GN	0·206 (23)	0·017	0·187 (27)	0·015	0·250 (6)	0·046	0·129 (9)	0·017
Lung	GR	0·284 (2)	0·104	0·168 (7)	0·024	0·245 (5)	0·029	0·108 (3)	0·036
	GN	0·194 (9)	0·019	0·189 (25)	0·011	0·188 (4)	0·048	0·100 (9)	0·015
Heart	GR	0·116 (2)	0·016	0·104 (6)	0·020	0·127 (5)	0·014	0·129 (3)	0·037
	GN	0·108 (9)	0·012	0·113 (19)	0·009	0·117 (4)	0·008	0·090 (9)	0·017
Placenta	GR	—		0·151 (4)	0·011	0·151 (2)	0·010	0·155 (2)	0·045
	GN	0·157 (6)	0·027	0·170 (15)	0·014	0·166 (7)	0·019	0·195 (5)	0·030

Mean values were significantly different from GN values: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† For details of fetuses, see p. 601.

plasma. The following roles have been designated for ascorbic acid in the brain: (*a*) it is essential for catecholamine metabolism and its regulation, particularly, as a cofactor of dopamine β-mono-oxygenase (dopamine β-hydroxylase, *EC* 1.14.17.1) (Friedman & Kaufman, 1965). Cerebral regions in rat brain which have high concentrations of catecholamines often have correspondingly high ascorbate levels although no direct correlation has been shown (Milby *et al.* 1982); (*b*) there is speculation that ascorbic acid and its oxidized forms are regulators of cell division (Edgar, 1970).

The high ascorbate content of the immature brain may have an anatomical basis. With the progress of myelination, it is possible that the ascorbic acid compartment, i.e. neurons, is diluted because of glial growth and myelination. Shimizu *et al.* (1960) have observed histochemically that brain ascorbic acid was predominantly localized in neuronal cell bodies, which supports this possibility. The ascorbic acid (mg/g):DNA (mg/g) ratio in the fetal brain with progress of gestation is shown in Fig. 2; the fall in the fetuses of weeks 35–38 from the ratio in fetuses of weeks 28–31 was significant ($P < 0.01$).

The adrenal ascorbic acid levels declined significantly after 32 weeks of gestation. The bulk of the fetal gland is composed of the fetal zone, overlying which is a thin rim of cells which come to constitute the definitive or adult cortex of the gland after birth. The fetal zone begins to degenerate shortly before birth (Lanman, 1962). The fetal zone, the placenta and the fetal liver function as an integrated unit to produce and metabolize the major steroid hormones necessary for proper fetal development and maintenance of pregnancy (Yeasting, 1986). In the adrenals, both cortex and medulla require ascorbic acid for the synthesis of corticosteroids and catecholamines respectively.

In the case of liver, lung, kidney and heart, ascorbic acid levels tend to decrease after 24 weeks of gestation and to show a further decrease at term but the differences were not significant, perhaps because of wide intragroup variation and sample size. It is known that ascorbic acid exists in greater concentrations in embryonic tissues where synthetic processes occur with intensity. The ascorbic acid concentrations in these tissues are comparable to those reported by Yavorsky *et al.* (1937) and Bessey & King (1933). Placental ascorbic acid levels were comparable to those reported by Toverud *et al.* (1950).

Placental ascorbic acid concentrations remained constant and were comparable to those of liver and kidney during early pregnancy. Studies in the rat have suggested that at all times during gestation, the placenta has greater ability to concentrate labelled ascorbic acid than the fetus (Rosso & Norkus, 1976). It is thought that the placenta may also actively transport ascorbic acid by a mechanism similar to that for amino acids (Dancis *et al.* 1968; Longo *et al.* 1973).

Growth retardation was associated with a significant decrease in ascorbic acid concentration in brain, liver and kidney during weeks 12–16 of gestation.

In conclusion, the present studies indicate that human fetal brain has a higher concentration of ascorbic acid than the adrenals. The ascorbic acid concentrations in the liver, kidney, lung and placenta were comparable while those in the heart tended to be less. In the case of all the tissues, a decline was found after 32 weeks of gestation but it was significant only in the case of the brain and adrenals. These studies point to the importance of ascorbic acid for the functional maturity of the fetal brain. The marked differences in the concentrations in the brain and adrenals of the human fetus emphasize the need for comparative studies on species differences with regard to this aspect.

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