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Glycosaminoglycans in human fetal liver in relation to water and electrolytes

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Abstract. The acidic mucopolysaccharides secreted into the extracellular space are thought to play many important functions amongst which are binding of water and electrolytes on the polyanionic glycosaminoglycans. Characteristically these components undergo continuous changes during growth and development of the fetuses. Relationships of the concentrations of glycosaminoglycans to the water and principal electrolytes at different periods of gestation were studied in human fetuses. It was found that during growth of the human fetuses there was a progressive decrease in water, thiocyanate space, total sodium content and glycosaminoglycans. However the decrease of glycosaminoglycans was greater than the rate of decrease of the other constituents. Hence mucopolysaccharides were thought to play more important roles than just binding of water and cations.

Keywords. Glycosaminoglycans; electrolytes; human fetuses; thiocyanate space.

Introduction

A growing fetal organ like the liver and brain is characterized by growth and prolixferation of endodermal or ectodermal cells in a matrix provided by mesenchymal tissue which supports and nourishes the characteristic cellular proliferation. The matrix consists of collagen fibres and proteoglycans secreted by the fibroblasts. It is envisaged that during growth of a fetal organ like the liver prior provision of the matrix ensures 3 dimentional proliferation of hepatic parenchymal cells. The matrix forms and fills the extracellular space, which is permeated by the collagen fibres and contains the nutrients, water and minerals in transit from the capillary to the tissue cells and the excretory products in the reverse direction. The nonfibrous part of the matrix consists mostly of proteoglycans which are proteins to which oligosaccharide chains consisting of repeating disaccharide units containing a hexosamine and an uronic acid (UA) are covalently attached. The structure and biosynthesis of proteoglycans have been extensively reviewed (Muir and Hardingham, 1983; Hascall and Hascall, 1981; Rodin, 1980). The oligosaccharide chains are known as glycosaminoglycans (GAGs). They are essential for maintenance of the structural integrity of many connective tissues and because of their characteristic polyanionic physicochemical properties they have been implicated in a number of functions like binding of water, microions, distribution of various molecules by steric exclusion and cell-cell and cell-substrate interactions (Hook *et al.,* 1984; Comper and Laurent, 1978; Muir and Hardingham, 1975). During growth and differentiation of fetuses, there is a change in the water electrolytes and GAG contents of an organ like the brain (Tower, 1969; Margolis, 1969). The sodium content of the fetus was found to be

Abbreviations used: UA, Uronic acid; GAG, glycosaminoglycan; HY, hyaluronic acid; MPS, mucopolysaccharides; TCA, trichloroacetic acid; CPB, cetyl pyridinium bromide; CS, chondroitin sulphate; NaSCN, sodium thiocyanate; AMPs, acidic mucopolysaccharides.

relatively higher than that of the adult (Behrman and Vaughan, 1983). Whereas in the adult exchangeable sodium was 40 mEq/kg in the fetus the corresponding value was 85 mEq/kg. This was attributed to a higher content of extracellular fluid in the fetus than in the adult. However, the relationship between water, electrolytes and GAGs has not yet been studied satisfactorily. The present paper was an attempt to study this relationship in human fetal liver at different gestation periods.

Materials and methods

The fetuses were obtained from the Medical Termination of Pregnancy clinics of the Institute of Post Graduate Medical Education and Research, Calcutta. The program was cleared by the Ethical Subcommittee of the Institute, mothers gave written consent to the use of the fetuses for biological research. The authors had no say in the selection of cases for hysterotomy which was performed by the professor of obstetrics. Mothers who did not want to continue their pregnancies and wanted ligation at the same time formed the subject of the investigations. It was difficult to determine the exact period of gestation in some cases, because some mothers were vague about the last date ofmenstrual periods. Therefore, a combination oflast date of menstrual period abdominal palpation and anthropometry of the fetuses was used to determine the period of gestation. All the fetuses were so called 'normal' fetuses and were delivered by hysterotomy. Sterile sodium thiocyanate solution (0·3 ml of 5% solution/kg) was injected to the mother 4 h before the conceptuses were taken out. Fetuses were put on ice in Dewar flask in the operation theater and brought to the laboratory where they were weighed and measured anthropometrically. They were dissected within 30 min after removal from the uterus. The entire liver was cut and used for the following estimations. Total water was measured by weighing and drying in an oven at 110°C for 18 h. Ashing of the dried specimen was carried out by incinerating in the mufle furnace at 550–650°C for 4 h. The weighed ashes were dissolved in 0·5 N HNO_3 and sodium and potassium concentrations were carried out by flamephotometry. Fetal blood was obtained by right atrial puncture into a heparinized syringe and immediately centrifuged to obtain the plasma. Aliquots of tissues were homogenised in the cold with water (1 ml/g) for determination of the thiocyanate space according to Eder (1951). Extraction of GAGs and their fractionation were carried out by the method of Singh and Bachhwat (1968). UA was determined in the dialyzate by the method of Dische (1947) as modified by Bitter and Muir (1962). UA was multiplied by 2·4 to get an approximate value of GAG content. GAGs were fractionated according to the method of Schiller *et al.* (1961). Hexosamines in the fractions of GAGs were hydrolyzed in 6 N HCl in sealed ampoules and the hydrolysate were dried over NaOH pellets in the vacuum desiccator. The dried samples were dissolved in a small volume of water in which the hexosamines were determined by the method of Ludowicz and Benmaman (1968). Total sulphate content was measured by the method of Dodgson and Price (1962). Glucuronolactone, acetylacetone, gelatine, fructose-6-phosphate, glutamine, glucosamine, galactosamine, hyaluronic acid (HY), chondroitin-4-sulphate, chondroitin-6-sulphate, sodium salt of heparin were purchased from Sigma Chemical Company, St. Louis, Missouri, USA. All other chemicals were of analytical grade and bought from British Drug House or E. Merck. Solvents were redistilled.

Results

For the sake of convenience the fetuses have been divided into 6 groups—A, B, C, D, E and F (table 1) according to a difference in the gestation period of 4 weeks. The gestational age was ascertained from the date of the last menstrual period, the body weight of the fetuses, the crown rump and crown heel lengths. Fetuses of group A had gestational ages of 9–12 weeks; their body weights were from 1·1–14·5 g. Group B represented fetuses of 13–16 weeks with body weights of 14·6–110 g. Group C included fetuses of 17–20 weeks gestation with body weights of 111–315 g. Group D included fetuses of 21–24 weeks with body weights from 360–680 g. Group E contained fetuses of 25–28 weeks with body weights from 681–1050 g. Another group represented specimens of body weight 900–3000 g including premature and full term babies from 1–8 days of age. Of the 115 fetuses investigated in the present series 15 belonged to group A, 26 to B, 36 to C, 21 to D, 14 to E and 3 to F.

Table 1. Grouping of fetuses and weight length (crown-rump) relationship.

Group	No. of fetuses	Week	Weight (g)	Length (mm)	Length change / Weight change
A	15	9–12	1·5–14·5	25–58	4·6
B	26	13–16	15·6–108	62–109	1·1
C	36	17–20	115–295	110–157	0·90
D	21	21–24	330–660	163–211	0·37
E	14	25–28	715–1025	203–252	0·20
F	3	29–32	1055–1650	230–277	0·17

Weight of the liver

Table 2 and figure 1 shows the weights of the fetal livers at different periods of gestation, expressed as the total weight of the organ and in figure 2 as g per kg of body weight. With progress of gestation, there is an increase in the weight of the

Table 2. Weight of liver.

Weight of fetus (g)	No. of samples	(g/kg body wt.) Mean ± S.D.	Mean ± SEM
0–100	37	47 ± 8·8	47 ± 1·5
100–200	19	40 ± 3·6	40 ± 0·83
200–300	14	39 ± 2·5	39 ± 0·74
300–400	9	38 ± 3·1	38 ± 1·0
400–500	4	40 ± 2·9	40 ± 1·5
500–600	4	35 ± 2·2	35 ± 1·1
600–700	3	42 ± 7·2	42 ± 4·2
700–800	4	41 ± 5·3	41 ± 2·7
800–900	2	39	39
900–1000	3	33 ± 3	33 ± 1·1
1000–2000	6	39 ± 4·2	39 ± 1·1

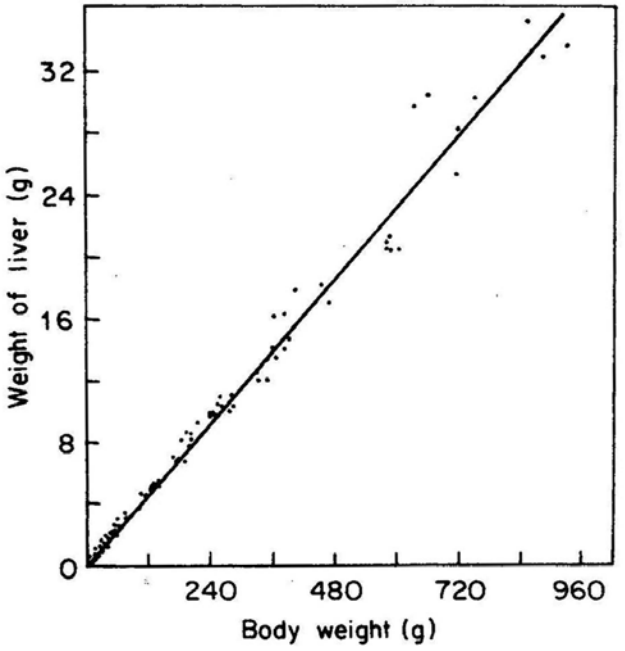

Figure 1. Total weight of human fetal liver.

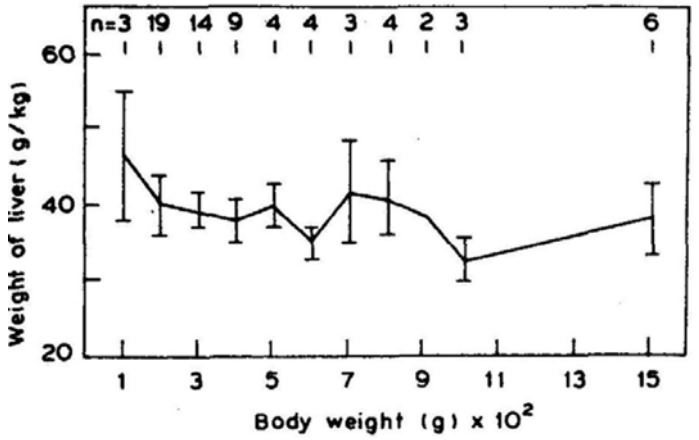

Figure 2. Weight of human fetal liver (g/kg of body weight).

liver. But the rate of increase is not the same at the different ages. When the weight of the liver is expressed as g per kg of body weight, it is seen that except at the earliest period investigated, *i.e.* at 9–12 weeks of gestation the weight of liver was almost exactly proportional to the weight of the fetus at all other ages, even upto 8 days after birth. It forms about 4% of the total body weight. At 9–12 weeks of gestation the liver was comparatively a bigger organ. In the fetuses of very early gestation period, the liver constituted 5·5% of body weight. Subsequently from about 12 weeks of age it came to about 4% of the total body weight.

Mucopolysaccharides (MPS) of human fetal liver

The dehydrated defatted tissue was digested with papain followed by alkali and the proteins were precipitated with trichloroacetic acid (TCA). UA was determined in the dialyzed supernatants. Results are shown in table 3.

Table 3. Total MPS of human fetal livers of different gestation period.

Group	Age (weeks)	No. of fetuses	Total UA mg/g dry defatted tissue
B	13–16	3	0·74 ± 0·16
C	17–20	10	0·65 ± 0·07
D	21–24	10	0·56 ± 0·07
E	25–28	5	0·55 ± 0·09
Postmortem	0–8 days	6	0·35 ± 0·02

The amount of liver obtainable in fetuses of 9–12 weeks gestation was not enough to process extracts for total MPS and subsequent fractionation. The total UA content per g of dried defatted liver in the fetuses of gestation period from 13–32 weeks was not different and the difference between the groups was not statistically significant. Although the MPS content of the fetal liver did not change much during the progress of gestation, its amount became reduced within the first few days of life. Whereas the hexuronic acid content of fetal liver varied from 0·5–0·7 mg/g, in post-natal life the amount was around 0·3 mg/g.

Fractionation of MPS in developing human liver

The MPS in 0·04 M NaCl was precipitatedby cetyl pyrinidium bromide (CPB) (3 mg per mg of MPS) and centrifuged after the addition of celite (20 mg/mg of MPS). The precipitation was judged complete when hexuronic acid was found to be absent in the supernatant. The precipitate was washed with small volumes of 0·04 M NaCl containing 0·1% CPB. The washings were checked for any hexuronic acid contents, which were found to be absent. Thus the precipitation of MPS in the remaining extract was treated as being quantitative.

The CPB polysaccharide Celite complex was extracted repeatedly with small volumes of 0·4 M NaCl containing 0·1% CPB until the supernatant after centrifugation was free of hexuronic acid. This fraction (fraction 1) usually contains hyaluro nate. However, in this eluate, variable amounts of chondroitin sulphates and possibly heparan sulphate too may be eluted. The eluates were, therefore, analysed for glucosamine, galactosamine and sulphate. In hyaluronate the ratio of glucosamine to UA should be 1, there should be no galactosamine or sulphate, both of which are constituents of chondroitin sulphate (CS). Results are shown in table 4.

In group A, the livers were too small to be analyzed. In group B, we could determine the total amount of UA in fraction 1, *i.e.* the MPS extractable in 0·4 N NaCl, but the determination of the different hexosamines and sulphates could not be undertaken in the small amount of material available. In group B and C the total amount of fraction 1 was found to be same; the hexosamine present was mostly

Table 4. Composition of MPS of human fetal liver (Fraction-1).

Group	Age (weeks)	No. of specimens	UA mg/g dry defatted tissue	Hexosamine*	Glucosamine*	Galac-tosamine*	Sulphate*
B	13–16	3	0·40				
C	17–20	12	0·40 ± 0·02	1·30	1·27	0·03	0·9
D	21–24	10	0·30 ± 0·05	1·25	1·02	0·23	1·0
E	25–28	5	0·28 ± 0·08	0·90	0·75	0·15	0·8
F	29–32	—	ND	ND	ND	ND	ND
Postmortem	0–8 days	7	0·15 ± 0·02	0·75	0·60	0·15	1·37

*Expressed as molar ratio of UA.
ND, Not done.

glucosamine but the content of sulphate was almost equal to that of the UA. Thus this fraction contained the nonsulphated HY, some amount of heparitin sulphate and small amount of CS. In groups B and E, total MPS slightly decreased and this fraction also contained predominantly HY but considerable amounts of chondroitin and heparan sulphate was found to be eluted in this fraction. In post mortem specimens of babies of 0–8 days ages, the total amount of fraction 1 was found to be much less than in the fetal specimens but the nature of the material eluted in this fraction was observed to be a mixture of HY and CS and heparitin sulphate.

The CPB polysaccharide complex which remained after fraction 1 was eluted out of the complex was next extracted with small volumes of 1·2 M NaCl containing 0·1% CPB, until no more hexuronic acid was eluted. This fraction should contain CS. In order to check whether this fraction contained other MPS too, this fraction was checked for glucosamine, galactosamine, and sulphate. In CS, the aminosugar is Glam and the ratio of UA to sulphates is less than one depending on the sulphation of the hexosamines. Results are shown in table 5. In group B, the fraction was insufficient in amount for determination of various constituents. In group C, the amount of fraction II as determined by the total hexuronic acid content was higher than in the livers of babies dying after 0–8 days of life. When the fraction was, however analyzed for the glucosamine and galactosamine contents, most of the aminosugar was found to be galactosamine rather than glucosamine, which should be the preponderant aminosugar in this fraction.

The CPB-polysaccharide complex was next eluted with 2·1 M NaCl containing 0·1% CPB in order to elute the heparin. Results are shown in table 6. The content of heparin was low as compared to fraction 1 and 2 but by and large the heparin

Table 5. Composition of MPS of fetal liver (Fraction-2).

Group	Age (weeks)	No. of cases	UA mg/g dry defatted tissue	Hexosamine*	Glucosamine*	Galac-tosamine*	Sulphate*
B	13–16	3	0·26				
C	17–20	12	0·21 ± 0·02	0·97	0·13	0·84	0·87
D	21–24	10	0·24 ± 0·04	1·20	0·13	1·07	1·5
E	25–28	5	0·19 ± 0·07	0·86	0·38	0·48	0·98
Postmortem	0–8 days	7	0·16 ± 0·02	0·70	0·20	0·50	0·98

*Expressed as molar ratio of UA.

Table 6. MPS in human fetal liver (Fraction 3).

Group	Age gestation	No. of fetuses	UA mg/g dry defatted tissue	Hexosamine*	Glucosamine*	Galac-tosamine*	Sulphate*
B	12–16	3	0·08 ± 0·04	ND	ND	ND	ND
C	16–20	9	0·04 ± 0·02	0·88	0·44	0·44	1·0
D	20–24	8	0·02 ± 0·02	0·90	0·77	0·15	3·0
E	24–28	7	0·07 ± 0·02	0·71	0·68	0·03	3·1
G (Postmortem)	0–8 days	6	0·040 ± 0·02	1·2	1·1	0·1	1·0

*Expressed as molar ratio of UA.
ND, Not done.

content in fetal life was slightly greater in Group B and E than in postmortem liver in postnatal life. This fraction also cannot be considered to consist of heparin only as a considerable amount of galactosamine was present. The sulphate content was characteristic of heparin in groups D and E but low in group C and postmortem.

Water and electrolyte content of human fetal liver

Water content of human fetal livers at different periods of gestation is shown in table 10. In varied from 80·5% of the organ weight at 9–12 weeks to 77·6% at 25–28 weeks. The decrease was progressive except in the last two groups where the water content was almost unchanged. We could obtain livers of 4 children who died from prematurity and other causes; the water content in them was also like that at 21–28 weeks, namely around 78%. The water content of adult livers obtained by surgical biopsy was around 69% of organ weight. Measurement of extracellular water has been attempted with many compounds like inulin, sucrose, sodium thiocyanate (NaSCN), radioactive Na^+, Cl^-, $So_4^=$ etc and each of these substances have their own uses and defects (Deane *et al.,* 1951; Gaudino and Levitt, 1949). Originally, we tried to use insulin to determine extracellular space (Deane, 1951) but we ran into troubles. The determination of inulin concentration in the blank became a serious problem and we had to abandon the method in favour of a substance like NaSCN which in fetal tissues had a zero blank value. Briefly, the method consisted of injecting sterile solutions of sodium thiocyanate into mother and carrying out hysterotomy 4 h later. Table 7 shows thiocyanate concentrations of different fetal fluids after maternal injection of 0·3 ml NaSCN (5% in saline)/kg of body weight. The concentration of thiocyanate in maternal serum was higher than that of fetal serum. However, fetal serum concentration was more uniform than the maternal. The amniotic fluid, fetal bladder fluid

Table 7. Thiocyanate concentrations in bodyfluids.

		mg%
Maternal serum	(9)	4·7 ± 0·91
Fetal serum	(9)	2·7 ± 0·21
Amniotic fluid	(3)	0·48, 0·22, 0·94
Fetal bladder fluid	(2)	0·81, 0·48
Fetal C.S.F.	(1)	0·44

and fetal cerebrospinal fluid had lower concentrations than fetal serum. Since thiocyanate was found to be permeable across the placenta and since we assumed that fetal circulation was greater and fetal urinary excretion was slower than the adult, we presumed that thiocyanate had reached equilibrium concentration in 4 h. For determination of thiocyanate in the liver, the organ was homogenized in water and proteins were precipiated with same volume of 10% TCA. Thereafter the method was followed as described for the plasma by Eder (1951). The extracellular space per g of tissue was calculated according to the formula extracellular fluid = $x/y \times 100 \times 1{\cdot}1$ where x and y were thiocyanate concentrations in mg per cent in the organ and plasma respectively and 1·1 was a constant for Donnan Equilibrium. Total water was calculated by drying the tissue to constant weight at 110°C. The intracellular water taken as the difference of total organ water and extracellular space. Results are shown in table 8.

Table 8. Water content and distribution of human fetal liver.

Body weight	Approx. gest. period	Total water (g%)	Extracellular (g%)	Intracellular (g%)
95	16	80	53	27
101	16	79	51	28
107	16	79	51	28
120	17	80	53	27
250	20	81	45	36
330	23	79	46	33
375	24	80	46	34
402	25	79	46	33
877	27	78	43	35

The thiocyanate space varied from 53 ml per 100 g of liver at 16 weeks of gestation to 43 ml at 27 weeks. As the total water did not change very much during the different gestation period studied the corresponding intracellular water (*i.e.* total water-thiocyanate space) increased from 27 ml per 100 g at 16 weeks to 35 ml at 27 weeks. Whether the difference observed was due to any peculiarity of thiocyanate movement in fetal liver or to real movement of water it is difficulty to say. Such an increase in intracellular water at later periods of gestation cannot be ascribed to a greater number of cells per unit area at this time, because the DNA content per 100 g of liver tissue was higher at earlier period of gestation than at later periods. The increase in intracellular water might be due to an increase of volume and mass of the individual cells, since the total protein concentration per g of tissue was found to be higher at later periods of gestation than at earlier periods.

Thiocyanate space in human adult liver

Adult women undergoing operation for cholelithiasis were the subjects of the experiments. Informed consent was taken from these patients about the nature of experiments. Sterile sodium thiocyanate (15 ml of 5% solution) was slowly injected intravenously 4 h before the operation. At the time gall bladder was being taken out a sample of venous blood was collected and a liver biopsy was performed. Thiocyanate concen-

trations in the serum and the specimen of liver were measured and the total amount of water was also determined in an aliquot of the liver sample. Results are shown in table 9. In the determination of thiocyanate concentration in the TCA extract of liver, a blank determination was found to be essential which contained the equivalent amount of TCA filtrate and 1·5 ml of 2·5% HNO_3. A further blank was also necessary to correct for nonspecific substances giving colour with ferric nitrate (5–8% of the SCN space). The thiocyanate space varied from 30–35% and intracellular water from 34–42% of the wet weight of the liver.

Table 9. Thiocyanate space in female human adult liver.

Speciman No.	Age (years)	Total liver water	Thiocyanate concentration in serum (mg%)	Thiocyanate space (ml/100 g)	Intracellular water (ml/100 g)
1	46	72·5	4·6	30·0	42·5
2	39	69·3	5·1	35·0	34·3
3	48	70·0	5·2	35·0	35·0

In the 3 specimens, the thiocyanate space varied from 30–35% and the intracellular water (total water-thiocyanate space) 34·3–42·5%. None of the livers showed any histological abnormality.

Sodium and potassium contents of the human fetal liver

The metabolism of water, electrolytes and MPS is closely interrelated. The matrix containing the polyanionic acidic mucopolysaccharides (AMPs) is supposed to attract the hydronium ions and the common cations like sodium and potassium ions. The more charged it will be, the more will it attract the cations which again, in their turn, will bring the anions like the chloride and bicarbonate around them. We, therefore, determined the sodium and potassium concentrations in the ashed material of the liver. Results are shown in table 10. Potassium concentration in mEq/kg of body weight varied from 41–51; virtually there was no change at difference periods of gestation. Sodium content, of the liver, however, in earlier periods of gestation *e.g.* in group A and B was higher than in later periods.

Table 10. Total water, ash, sodium and potassium contents of human fetal liver.

Group	Gest. period (weeks)	Water (g%)	Ash (g%)	K^+ (mEq/kg)	Na^+ (mEq/kg)
A	9–12 (7)	80·7 ± 1·2	1·62 ± 0·93	41 ± 5	94 ± 10
B	13–16 (10)	79·4 ± 0·5	1·90 ± 0·13	43 ± 4	71 ± 7
C	17–20 (20)	78·5 ± 0·3	1·63 ± 0·02	51 ± 2	67 ± 8
D	21–24 (11)	78·3 ± 0·6	1·68 ± 0·15	48 ± 5	60 ± 10
E	25–28 (8)	77·6 ± 0·4	1·31 ± 0·18	44 ± 4	66 ± 10
Postmortem	0–8 days (4)	78·2 ± 1·3	1·57 ± 0·19	44 ± 3	62 ± 3
	8 (Adult)	69·0 ± 1·6	1·52 ± 0·07	57·3	63 ± 12

Values are expressed in wet tissue.

Extracellular sodium and potassium concentration was derived by multiplying the plasma concentrations 1·1 times the thiocyanate space. Intracellular sodium and potassium concentrations were determined by subtracting the extracellu lar ions from the total ions. Results are shown in table 11. The thiocyanate space decreased from 520 ml/kg of the organ weight at 17 weeks to 460 ml/kg at 28 weeks. The intracellular water kept more or less constant at 32%. The total sodium of the fetal liver was all in the thiocyanate space. Actually the calculated extracellular sodium was found to be higher than the total sodium content, so that it may be assumed that there was little sodium in the intracellular space. In contrast, in the adult liver about 13% of sodium content could be calculated to be within the extracellular compartment. Potassium was chiefly intracellular. In most instance the osmolar equivalent of intracellular and extracellular fluid calculated from the sodium and potassium concentrations matched exactly.

Discussion

Growth of liver at different gestational periods

In a growing organ like the human fetal liver, the term growth envisages both increase in cell number and increase in cell mass. The two processes are probably not synchronous. In the early stages of gestation the organ may be preoccupied with increase in cell number and in the later stages with both numerical as well as dimensional increase.

Both these two types of growth involves utilization of nutrients. In postnatal life the nutrients come from outside and are subject to external control. In utero, however, the different nutrients are derived from the mothers' circulation and in normal pregnancies in healthy mothers they are presumably not in deficient supply. Consequently the growth of the liver in utero can be envisaged as an intrinsic process, dictated by the genotype. In the early stages *e.g.* between 9–12 weeks of gestation the liver forms a greater percentage of body weight than at later stages. This may be due to two reasons. At this time, the other organs of the body, at least the bulkier ones like the muscles, bones and brain are comparatively smaller so that relatively a greater percentage of body weight is constituted by the liver. Or the rate of division of individual liver cells may be very high in this stage so that the organ is relatively a big one. It appears that both of these statements are true. At very early stages of development, *e.g.* at 7 weeks, the abdominal wall is open; when it begins to close, the space is not enough for retaining all the abdominal organs inside; thus the elongating intestinal loop herniates into the umbilical cord and returns only when the abdominal cavity has increased in size both absolutely and relatively. The relative increase of the abdominal cavity is due to a decrease in the rate of cell division of the liver. But, later on, from 12 weeks onwards the weight of the liver bears an almost constant proportion to the body weight. It appears that the liver subserves vital metabolic functions for the controlled growth of the whole fetus. Hence as the fetus grows, the liver also grows in proportion to the whole fetus. But this is only a teleological explanation; we are totally ignorant of the forces which control the growth of any organ, let alone the liver.

Table 11. Sodium and potassium concentrations in human fetal liver (in different compartments) values are expressed in wet tissue.

Gr.	Gest. period (weeks)	Water (%)	CNS space (ml/kg)	Intra-cellular water (ml/kg)	Plasma Na^+ (mEq/L)	Plasma K^+ (mEq/L)	Extra-cellular liver Na^+ (mEq/kg)	Extra-cellular liver K^+ (mEq/kg)	Intra-cellular liver Na^+ (mEq/kg)	Intra-cellular liver K^+ (mEq/kg)
C	17–20 (4)	79·5	520	275	140	11·6	79·1	6·6	0·0	44·4
D	21–24 (4)	78·5	465	320	139	10·1	70·6	5·2	0·4	42·8
E	25–28 (4)	78·5	460	325	135	12·5	68·4	6·3	0·6	37·7
Adults	(8)	69·0	320	370	142	4·8	49·9	2·0	7·1	55·0

Intracellular water = Total water − CNS space.
Extracellular Na = Plasma Na × 1·1 (Donnan Eq) × CNS space.
Extracellular K = Plasma K × 1·1 (Donnan Eq) × CNS space.
Intracellular Na = Total sodium − extracellular sodium.
Intracellular K = Total potassium − extracellular potassium.

MPS of human fetal organ

In almost all fetal organs, the epithelial elements in their developmental process becomes surrounded by mesenchymal tissue. The mesenchyme is an extraordinarily versatile tissue with many potentialities which find expression under the varied conditions offered during the course of development. Thus they may give rise to connective tissue, cartilage, bone, blood, smooth muscle and endothelium. Of these various derivatives, the connective tissue performs a predominantly mechanical function of support. This supporting function is exhibited by a composite of 3 things, a ground substance, some protein fibres and fibroblasts. The former two are products of the fibroplasts. The ground substance is considered to be structureless (by light microscopy) and nonliving and to be the actual component, responsible for the characteristic mechanical function. The maintenance of the ground substance is a function of the associated specialized cells in active condition.

In any developing organ, including the liver, the mesenchymal cells at first form a compact layer around the epithelial out growth. However, some mesenchymal cells are found to be loosely arranged around the mesenchymo-epithelial tubes forming a sort of labyrinthine space. They secrete the ground substance consisting of MPS's and collagenous fibres. The MPS are thought to be structureless; but the structurelessness refers to light microscopy only; molecule-wise the MPS's form a group of highly oriented organized molecules.

Just as the mesenchymal cells can take up various lines of differentiation, like the connective tissue, cartilage, bone, blood or endothelium, the characteristic cell of the connective tissue, *i. e.* the fibroblast can secrete may different kinds of molecules into the interstitial space. One such class of molecules is the MPS, the ones that we are dealing with here are called AMPs; they contain hexuronic acid. The amount of hexuronic acid in the extracted, partially purified MPS material is, therefore, a measure of the total MPS of an organ. In embryos the mesenchyme is principally cellular but in fetuses the matrix is quantitatively greater than the actual cellular element. The higher UA in fetal organs of early gestation period may be due to a relatively greater content of mesenchymal cells in a given volume of tissue. It must, however, be cautioned that these cells are relatively undifferentiated and less ready to extrude the proteoglycans in the interstitial space.

The procedure adopted for fractionation of the MPS of human livers was not ideal because none of these fractions were pure classes of compounds. Fraction 1, comprising HY should not contain Galm and sulphate. Although Galm content was proportionally low, there was quite a large amount of sulphate. It appeared, therefore, that some amount of CS and heparan sulphate was present in this fraction. There was a diminution in the amount of fraction 1 per g of dry defatted tissues, at later periods of gestation and in immediate postnatal life. Fraction 2 was also not a pure class of compounds inasmuchas in this fraction a large quantity of glucosamine was found. The sulphate content more or less corresponded with an equimolar quantity of hexosamine. The contamination might therefore, be either due to heparitin sulphate or even to heparin. The quantity was almost half of that found in fraction 1. Fraction 3 constituted less that 10% of the total UA content and the fraction also contained a mixture of a variety of compounds. If fraction 3 constituted entirely of heparin, it should have a hexuronic acid ratio to sulphate more than 2. However in the earlier period of gestation end in adult livers this ratio was less than 2. It is only in the later

periods of second trimester (21-28 weeks) that the ratio corresponded to that of heparin. The low sulphate may be due to a defect in the isolation procedure whereby mucopolysaccharises of low sulphate content like that of CS might have crept in. During the progress of gestation there was a decrease in the total UA content of the liver; the decrease was mostly in the HY fraction. We do not know the contribution of the individual proteoglycans to the overall function of the MPS. The decrease in HY fraction with presumably a greater stability of the matrix specially in extra-uterine life would go against the indispensability of HY to form a stable structure with link protein for the other polysaccharide.

Water and electrolytes of the human fetal liver

The main differences in the sodium and potassium concentrations of the serum in fetal and adult sera consisted in the higher serum potassium content in fetal life. Whereas adult serum potassium concentrations were 4·8 mEq/L±0·5, the corresponding concentrations in fetal sera were 10·8 mEq/L± 1·3. Since there was no differences in the sodium contents of fetal and adult sera, the total cation concentration and the total osmolarity of fetal sera were higher than those of adult sera. This higher osmolarity of fetal water probably has something to do with the floatation characteristic of the fetus in the amniotic fluid. The density of a few smaller fetuses was found to be around 1·103 by water displacement method, whereas that of the amniotic fluid was around 1·117. The maintenance of this density was a function of the osmotic tension of the body fluids of the fetus. A teleological explanation of the higher serum potassium in fetal life way also be given. A growing organ actively synthesizing protein and depositing the protein in the tissues needs potassium. The high serum potassium level in fetal life ensures a liberal supply. Furthermore, potassium concentration in the intracellular fluid of fetal liver was lower than that of the adult liver. Perhaps, the sodium pump in the tissue is not as effective in fetal liver as in adult liver so that potassium leaks out into the extracellular fluid.

The thiocyanate space in fetal liver was greater than in adult liver. The extracellular fluid was therefore present in greater quantity in fetal life than in adult life. In fact, the higher total water content of fetal liver may be ascribed entirely to the greater quantity of extracellular fluid. The intracellular fluid was not increased in fetuses. This huge amount of extracellular fluid is not present as free water but must be in a soluble form in combination with a macromolecule. The macromolecule is not presumably a protein but a polysaccharide, a MPS to be exact. Total MPS contents (table 3) was higher in earlier gestation period than in later. The decrease was still more manifest in neonatal livers on postmortem examination. The total water content of fetal liver (table 8) was lower than that of the adult liver. However, the decrease in total MPS from fetal to neonatal liver was more than the decrease in total water from fetal to adult life. It might therefore, be assumed that among the various functions of the MPS, only one function is related to its binding property with water. There are other functions of these classes of compounds, which are perhaps, more important in fetal life. The decrease of MPS during progress of gestation was almost entirely ascribable to a decrease in hyaluronates. Since the binding of hyaluronate to water is a well known phenomenon, the relation between the decrease of hyaluronate content and water is more than coincidental.

The presence of a high amount of water in the extracellular space necessitates retention of salts to maintain osmotic equilibrium. The total cation content of the extracellular fluid per kg of organ weight in fetal liver amounted to 75 mEq whereas in the adult liver it was only 52 mEq. The increase of extracellular water in fetal liver as compared to adult liver was between 50 and 70%. Therefore, the total cation content in extracellular fluid of fetal liver was increased relatively as Dell as absolutely. The total MPS contents of fetal liver in earlier gestation periods was about double that of neonatal livers. Hence the MPS must play important role in the greater binding of not only water but of cations as well.

Moreover, the other important functions of GAGs like interchain binding (Fransson, 1976), binding to collagen (Toole, 1976), platelet factor 4 (Moore *et al.,* 1975) and in cell-cell and cell-substrate interactions (Hook *et al*., 1984) may determine the behaviour of the matrix polysaccharides.

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