OSMOTIC ACTIVITY OF TISSUES DURING FETAL AND POST-NATAL GROWTH

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Preceding studies have shown that the tissue of certain secreting organs, namely, liver, kidney, pancreas, and submaxillary gland when immersed in solutions of sodium chloride exhibit osmotic pressure considerably above that of physiological salt solution or of blood serum (1, 2). Evidence indicating that this osmotic pressure is referable to physical conditions present during life has been described. As the extracellular fluid of the tissues, which constitutes from 15 to 20 per cent of their volume, is isotonic with blood plasma it is evident that this high level of pressure is maintained by the cells of the part. When the tissues are immersed in solutions of sodium chloride of graded concentration from 0.1 to 0.5 molar water movement quickly reaches an equilibrium which is maintained during a period of approximately 15 minutes; later, doubtless as the result of injury, there is increased permeability of the tissue. Water movement during 10 minutes of immersion has been found to be constant in tissues tested from 5 to 90 minutes following their removal from the living body (2).

The osmotic pressure measured by immersion of tissue slices is presumably referable to the molecular concentration maintained within the cytoplasm by the metabolism of the cell. This osmotic homeostasis may be impaired by severe injury to cells, as that caused by chloroform or by carbon tetrachloride in the liver or by potassium chromate in the kidney cortex. Isotonicity in solutions of sodium chloride then falls to the level of blood serum but with recovery from the injury the higher level is regained. Osmotic homeostasis is maintained for a time at least little changed when food is withdrawn, when lipidosis is produced by low protein diet or when common bile duct or ureter is obstructed.

In the course of these studies it was noted that isotonicity of the liver tissue of very young animals (white rats) when immersed in solutions of sodium chloride was at a level lower than that of the adult liver. The experiments which will be described show that tissues of the embryo at an early stage of development may exhibit osmotic pressure much lower than that of maternal blood serum (Figs. 1 and 2). They show that the level of isotonicity of liver or of kidney tissue immersed in salt solutions increases rapidly shortly before birth, more slowly after birth, and obtains the maximal level of adult life only after several weeks of growth.

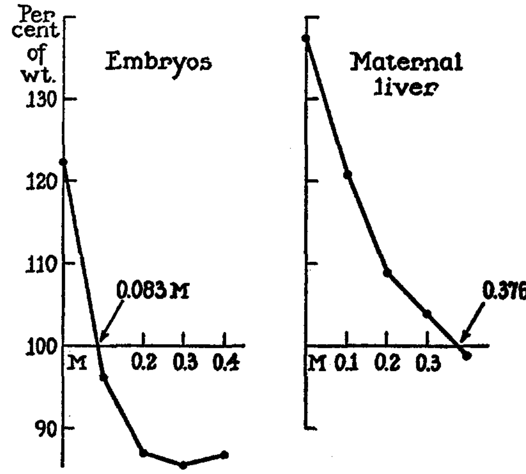

FIG. 1. Showing changes in per cent of weight of whole embryos weighing about 24 mg. and slices of maternal liver immersed during 10 minutes in solutions of sodium chloride from 0.1 to 0.4 molar and in distilled water.

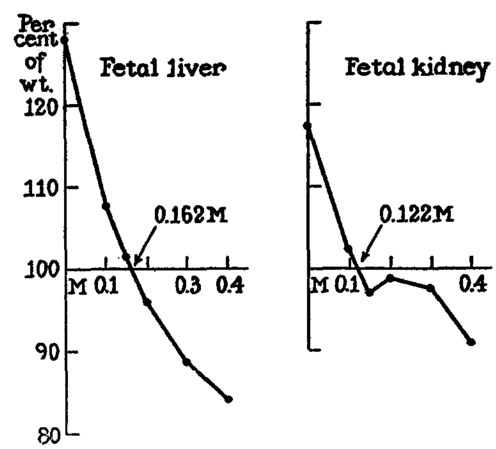

FIG. 2. Showing changes in per cent of weight in fetal liver and fetal kidney immersed during 10 minutes in water and in solutions of sodium chloride.

Method

The procedure for determination of the isotonicity of liver or kidney tissue immersed in distilled water and in solutions of sodium chloride from 0.1 to 0.4 molar is illustrated by Fig. 1. The per cent intake of water by liver tissue when plotted pursues a linear course, but with kidney deviation from a linear course may occur in the stronger solutions. In Fig. 2 intake of water by whole embryos from the same litter, one in water and in each of several concentrations of sodium chloride is compared with that of the maternal liver similarly treated.

White rats have been used and fetuses of graded size have been obtained. The weight of pregnant rats begins to increase at a minimum period of 14 days after mating and gives approximate information concerning the size of fetuses. The liver has been readily removable from a fetus weighing 0.22 gm. but is so small that a whole liver from individuals of the same litter must be immersed in each of the solutions that have been used, these being usually water, sodium chloride 0.1, 0.15, 0.2, 0.3, and 0.4 molar. With larger fetuses livers have been divided into two or more parts corresponding with the separation of lobes.

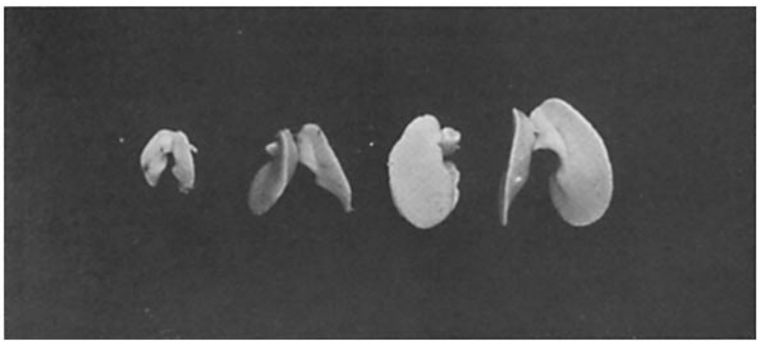

FIG. 3. Caudate lappets showing actual size of those from white rats weighing (from left to right) 26, 51, 88, and 143 gm., respectively.

Isotonicity of liver tissue of fetuses and of very young animals has been determined by immersing whole lobes in solutions, whereas in the case of larger animals slices of liver cut with a razor blade have been similarly immersed. It has seemed desirable to compare the water exchange in immersed slices with that in liver lobes, intact save for their separation from the main mass of liver tissue. In young animals the caudate lappets of the liver are well adapted to this comparison because they are flat bodies (Fig. 3) covered everywhere by the delicate liver capsule save at the hilum where each is attached to the other lobes by a narrow isthmus 2 or 3 mm. across. The isthmus when cut across represents a very small part of the surface in contact with the immersion fluid.

Table I shows the isotonicity of liver slices and of caudate lappets from animals with increasing weight from 22 to 100 gm. divided arbitrarily into two groups of which the weight is respectively greater and less than 60 gm. In the one group the caudate lappets have been approximately oval about 7 by 11 mm., with a maximum thickness less than 3 mm. and weighing less than 100 mg. These dimensions approximate those of liver slices save for thickness, which is greatest in a small area in the center of the lappet. In the second group the caudate lappets are larger with diameters approximately 9 by 13 mm., maximum thickness greater than 3 mm. and weighing from 90 to 180 gm. When liver tissue was obtained from animals weighing less than 60 gm. the average level of isotonicity for slices and for caudate lappets was approximately the same, namely, 0.222 and 0.228 molar sodium chloride respectively, but from larger animals thin slices had a somewhat higher level of isotonicity (0.273) than the much larger caudate lappets (0.243). These observations show that water movement can be measured by immersion of small livers or liver lobes in sodium chloride solutions and indicate that the mechanical injury of slicing has had little influence upon water movement under the conditions of the procedure.

TABLE I

The Isotonicity of Slices of Liver and of Caudate Lappets When Immersed in Solutions of Sodium Chloride

Reference No.*	Average body weight	Molar concentration of sodium chloride solution isotonic with liver tissue		Average weight of caudate lappets
		Slices	Caudate lappets	
	gm.			*gm.*
19	22	0.194	0.166	26.4
21	39	0.232	0.257	50.8
22	42	0.241	0.260	50.8
23	43	0.194	0.214	70.4
24	51	0.260	0.230	80.8
26	54	0.252	0.240	70.4
29	69	0.242	0.248	105.6
30	70	0.241	0.194	114.3
33	83	0.306	0.251	117.5
34	84	0.290	0.248	91.9
36	100	0.287	0.277	181.1

* Experiments in this table have been given the numbers listed in Table II.

Osmotic Activity of Liver and Kidney Cortex

In earlier studies (1, 2) more information has been obtained concerning the osmotic activity of liver and kidney cortex than of other tissues and in the present study attention has been chiefly focused upon these organs though several other tissues have been examined. The weight of the fetus has served as measure of the progress of growth (Table II, Fig. 4). During the period of growth in which the fetuses (Nos. 1 to 4) weighed from 0.22 to 1.43 gm. the liver tissue immersed in solutions of sodium chloride was isotonic with solutions approximating 0.1 molar, the average being 0.97 molar. In fetuses (Nos. 5 to 10) weighing from 2.1 to 3.4 gm., that is, after from 19 to 21 days of gestation (Donaldson (4)) isotonicity was at a somewhat higher level, namely from 0.106

TABLE II

The Isotonicity of Tissues Immersed in Solutions of Sodium Chloride

Reference No.	Stage of growth	Body weight	Molar concentration of sodium chloride solution isotonic with tissue		
			Liver	Kidney	Maternal liver
		gm.			
1	F(6)	0.22	0.091		0.294
2	F(3)	0.46	0.087		0.288
3	F(4)	1.39	0.121		0.320
4	F(6)	1.43	0.089		0.244
5	F(4)	2.11	0.158		0.249
6	F(4)	2.37	0.122		0.298
7	F(4)	2.68	0.110		0.331
8	F(4)	3.27	0.106		0.267
9	F(5)	3.3	0.121		0.282
10	F(6)	3.4	0.137		0.253
11	F(4)	5.0	0.113		0.310
12	F(6)	5.5	0.162	0.122	
13	NB(2)	5.7	0.181	0.063	
14	F(2)	6.2	0.087	0.067	0.286
15	NB(2)	6.2	0.196	0.118	
16	Y(2)	8.9	0.187	0.133	
17	Y	11.9	0.199	0.154	
18	Y(2)	15.3	0.191	0.150	
19	Y(2)	22.0	0.194	0.206	
20	Y(2)	23.5	0.210	0.181	
21	Y	39	0.232	0.241	
22	Y(3)	42	0.241		
23	Y	43	0.194	0.187	
24	Y	51	0.260	0.207	
25	Y	53	0.208	0.194	
26	Y	54	0.252	0.232	
27	Y	60	0.249	0.280	
28	Y(2)	60	0.226	0.255	
29	Y	69	0.242	0.192	
30	Y(2)	70	0.241		
31	Y	79	0.302	0.189	
32	Y	81	0.283	0.196	
33	Y	83	0.306	0.222	
34	Y	84	0.290	0.247	
35	Y	88	0.255	0.194	
36		100	0.287		
37		107	0.271	0.200	
38		110	0.342	0.255	
39		114	0.275	0.175	
40		119	0.367		
41		120	0.361		
42		123	0.328		
43		124	0.255	0.269	
44		126	0.294	0.215	
45		135	0.289		

TABLE II—*Concluded*

Reference No.	Stage of growth	Body weight	Molar concentration of sodium chloride solution isotonic with tissue		
			Liver	Kidney	Maternal liver
		gm.			
46		143	0.298	0.181	
47		145		0.232	
48		146	0.293		
49		151	0.279		
50		160	0.345	0.263	
51		164	0.288	0.252	
52		174	0.313	0.261	
53		178	0.281		
54		183	0.339	0.240	
55		185	0.280	0.220	
56		187	0.350		
57		190	0.342	0.247	
58		190	0.256	0.197	
59		195	0.345	0.247	
60		195	0.297	0.256	
61		196	0.326		
62		197		0.206	
63		200	0.351		

The stage of growth is indicated as fetal (F), newly born (N.B.), that is, within 24 hours, young (Y). Those with no similar designation, weighing more than 100 gm. are presumably mature. The accompanying figures in parenthesis indicate the number of litter mates that were in each instance used to determine movement of water.

to 0.158 molar sodium chloride, the average of livers from litters of six mothers being 0.126 molar. At the end of gestation fetuses (Nos. 11 to 15) weighed from 5 to 6.2 gm. and the level of isotonicity shortly before or within 24 hours after birth was from 0.087 to 0.196 molar, the average in the progeny of 5 mothers being 0.148 molar. Liver tissue was approximately isotonic with maternal blood. The isotonicity of the maternal liver during pregnancy varied from 0.244 to 0.331 molar sodium chloride, the average being 0.285 molar. This figure is less than that of the normal adult liver (1, 2).

The livers of fetuses weighing approximately 5 or 6 gm. examined before birth (Nos. 11, 12, and 14) had lower levels of isotonicity, the average being 0.121 molar sodium chloride, than those examined shortly after birth (Nos. 13 and 15) with an average of 0.189 molar. Livers from one litter (No. 14) had the lowest level of isotonicity and were from fetuses which, though they weighed 6.2 gm. had remained unborn presumably because the mother carried only two.

Shortly after birth in young animals (Nos. 16 to 23) weighing from 8.9 up to 50 gm. (Table II, Fig. 4) and by comparison with the approximate figures given by Donaldson (4) from 5 to 35 days old, isotonicity of liver tissue varied from 0.187 to 0.232, the average in 8 instances being 0.206 molar. In the period represented by young animals (Nos. 24 to 35) weighing from 50 up to 100 gm. and about 35 to 60 days old, isotonicity varied from 0.208 to 0.306 molar, the average of 12 animals being 0.260. In the period (Nos. 36 to 48) represented by body weight from 100 to 150 gm. up to the age of about 90 days isotonicity varied from 0.255 to 0.367 molar, the average in 12 instances being 0.304 molar.

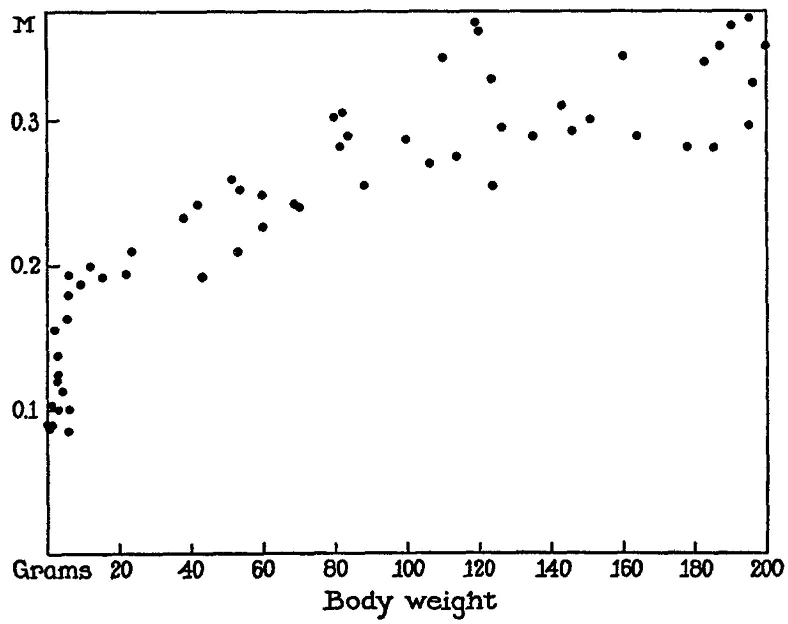

FIG. 4. Showing isotonicity of liver tissue before and after birth (see Table II) determined by immersion of the tissue in solutions of sodium chloride. Each point indicates the molar concentration of the solution with which the liver was in water equilibrium and these are arranged from left to right in the order of the increasing body weight of the animals from which the liver was obtained.

It may be assumed that isotonicity reached a maximum level within this period and with larger animals (Nos. 49 to 63) weighing from 150 to 200 gm. there was little change in isotonicity, the average level in 14 instances being 0.314 molar.

Isotonicity of kidney tissue (Table II and Fig. 5) measured by its water equilibrium with solutions of sodium chloride followed a course similar to that of liver but at a lower level. The size of the organs admitted approximately accurate measurements of weight and water exchange only when the body weight of fetuses had reached 5 or 6 gm., that is, at or just before birth. Isotonicity of kidney tissue from these animals (Nos. 12 to 15) as determined by immersion of whole organs from one or several animals of the same litter was from 0.063 to 0.122, the average being 0.093 molar. Following birth increase of this level proceeded more rapidly than with liver. In 7 young animals (Nos. 16 to 23) weighing from 8.9 to 50 gm. the average was 0.179; in 11 animals (Nos. 24 to 35) weighing between 50 and 100 it was 0.219; in 7 animals (Nos. 37 to 47) between 50 and 100 gm. it was 0.218 and in 10 animals (Nos. 50 to 62)

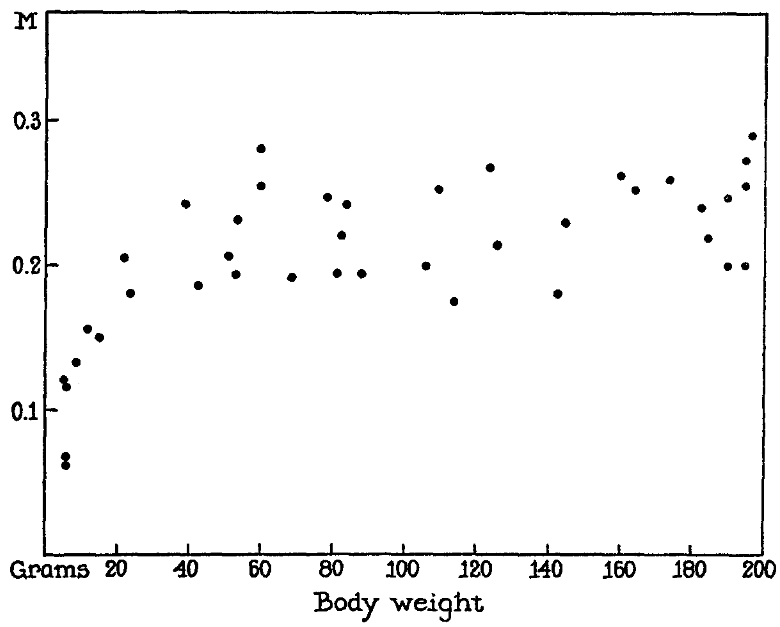

FIG. 5. Showing isotonicity of kidney tissue (see Table II) with measurements arranged as in Fig. 4.

between 150 and 200 gm. it was 0.217. It attained a maximum level in animals weighing between 50 and 100 gm. and about 35 to 60 days old.

Isotonicity of Whole Embryos and Fetuses

In one instance (Fig. 1) it has been possible to immerse whole embryos in solutions of sodium chloride and determine the concentration with which they were in water equilibrium. Embryos from one mother weighed approximately 24 mg. and were isotonic with 0.083 molar sodium chloride. In another instance fetuses weighing 0.41 gm., all from the same litter, when immersed in sodium chloride exhibited isotonicity at the level of 0.073 molar sodium chloride. These figures are approximately the same as those representing isotonicity of liver tissue in the earliest fetuses that were examined (Table II).

Water Movement of Fetal Tissues Immersed in Maternal Blood Serum

In several instances it has been possible to immerse fetal liver tissue or in one instance a whole fetus in maternal blood serum and to compare the change in weight with that of maternal liver similarly treated (Table III). The quantity of serum obtainable has been small not exceeding 2 cc. In an earlier study water exchange of adult liver in blood serum has been measured in 5 instances (Fig. 7 of reference 1) and found to cause an average weight increase of 6.8 per cent. The maternal livers in 4 instances (Table III) have caused somewhat

TABLE III

Movement of Water in Fetal Liver and Whole Fetus Immersed in Maternal Blood Serum

Reference No.*	Weight of fetus	Tissue immersed in maternal serum	Molar concentration of sodium chloride isotonic with tissue	Change of weight of fetal tissue in maternal serum	Change of weight of maternal liver in maternal serum
	gm.			*per cent*	*per cent*
1	0.22	Fetal liver	0.091	−17.5	+5.2
9	0.33	" "	0.121	−0.2	+4.5
10	0.34	" "	0.137	+0.2	+4.5
a	0.41	Whole fetus	0.073	−4.0	

* Numbers are the same as in Table II; experiment indicated by letter *a* is not included in Table II.

TABLE IV

Water Content of Fetus and of Liver

Reference No.*	Body weight of fetus	Water content of fetus	Water content of liver
	gm.	*per cent*	*per cent*
4	1.43	88.00	81.59
		88.35	81.07
13	5.7		81.78
			82.21
b	9.4		74.53
			74.98
c	50.0		70.23
			70.33
d	66.4		69.61
			69.75
33	82.5		66.58

* Numbers and letters as in Table III.

less weight increase, namely 4.8 per cent. The difference is not significant but may be referable to the lower level of isotonicity of the pregnant animals as compared with other adult rats.

A whole fetus weighing 0.41 gm. and presumably representing the 16th or 17th day of gestation (Donaldson) has been isotonic with 0.073 molar sodium chloride solution and has diminished in weight 4 per cent when immersed in the maternal blood serum.

Water Content of Tissues

The water content of whole fetuses and of the liver of fetuses and of young animals were determined (Table IV) in order that it might be compared with the isotonicity of these tissues in animals of the same body weight. Tissues were dried in an oven at 100 to 103°C. and weighed after 7 days, at a time when minimum weight was attained. The table shows, as Lowrey (5) and others have shown, that the water content of tissues is high in fetal life and diminishes during a period of growth after birth.

DISCUSSION AND RECAPITULATION

The osmotic pressure in eggs and embryos of aquatic and of amphibious animals has been repeatedly studied in the attempt to explain the relation of the developing organism to the water about it and in the case of birds to determine the relation of the embryo to the nutritive components of the egg yolk and white. The earlier literature of the subject has been reviewed by Needham (6) in his book published in 1931. Depression of the freezing point of frog's eggs at different stages of development was used by Bachmann and Runnström to measure osmotic pressure and to compare it with that of the water about the eggs. They found that ripe eggs of the ovary exhibited a freezing point depression which differed very little from that of the blood serum of the adult frog but when the eggs were expelled from the oviduct depression of the freezing point dropped to a level which was almost identical with that of the pool water about them. They evidently acquired some independence later and depression of the freezing point increased gradually so that when tadpoles were formed after about 20 days the freezing point of their substance approximated that of the adult. Krogh, Schmidt-Nielsen, and Zeuthen (8) measured osmotic activity in frog's eggs by comparing their vapor pressure with that of solutions of sodium chloride. At the time of first cleavage there was a definite initial fall in the level of isotonicity, then during 2 days a gradual decline followed by some increase, but all of the changes were much smaller than those observed by Bachmann and Runnström.

When Runnström (9) studied eggs of salmon which develop in fresh water but not in sea water they found no diminished depression of the freezing point of the egg content with fertilization and very little change throughout the course of development. Gray (10) obtained similar results when he studied the development of eggs of the trout and these were confirmed by Krogh and Ussing (11).

In the avian egg water passes from the white and yolk to the embryo and though the proportion of water in the embryo chick diminishes continuously from a very early period until the time of hatching, the actual water content increases along with the increase of formed material. The osmotic pressure of the embryo was found to increase continuously with development (Bialascewicz 12) and the passage of water from the white to the yolk was explained by the higher osmotic pressure of the latter, but later studies of Howard (13) based on freezing point determinations, dialysis, and vapor pressure measurements indicated that white and yolk had the same osmotic pressure.

Little attention has been given to the osmotic pressure maintained by the tissues of the mammalian embryo. At the beginning of the present century Sabbatani (14) measured depression of the freezing point of tissues of the dog and determined that of five fetuses found in one animal. The depression recorded by him was greater than that of the mother's blood.

Many studies demonstrate that the per cent of water in the mammalian fetus is greater than that of the adult and tends to diminish with the progress of gestation. This diminution continues after birth and in the albino rat stops, Hatai (15) states, after about 21 days, at a time when the young animal still receives its mother's milk.

The review of Needham of the extensive literature concerning the osmotic pressure of maternal and fetal blood at term, as measured by depression of freezing point, indicates that the two fluids have the same pressure and no difference between the sodium and potassium content of the two has been demonstrable. Studies of the urea nitrogen and of the non-protein nitrogen (Slemmons and Morriss, 16), of uric acid (Slemmons and Bogert, 17) and of amino acid nitrogen (Morse, 18) in fetal and maternal blood have disclosed no essential difference between the two and indicate that the substances concerned at least in the late stage of fetal growth pass the placental barrier freely.

The present study has compared the osmotic pressure maintained by cells of liver or of kidney of full grown animals with that of the corresponding tissues during embryonic development and with the fetus as a whole when removed from its membranes. The level of isotonicity of fetal tissues of the white rat measured by their water equilibrium when immersed in solutions of sodium chloride has been found considerably lower than that of physiological salt solution or of blood serum but reaches this level at about the time of birth. The corresponding isotonicity of kidney is somewhat lower than that of liver but it too reaches the level of blood serum shortly after birth. Following birth isotonicity of the two organs continues to rise. That of liver reaches the level of the adult when young animals weigh from 100 to 150 gm. and are about 60 to 90 days old whereas the kidney reaches the adult level in animals weighing from 50 to 100 gm. and about 35 to 60 days old.

The water content of the tissues of the fetus is higher before than after birth and in early embryos may approximate 90 per cent of the weight. No satisfactory explanation of the high water content of the fetus which is in intimate contact with maternal tissues and circulation has been offered. The mucopolysaccharides of the fetal tissues may undergo hydration but there is no information concerning the ability of material in the abundant interstitial tissue of the embryo to hold water. The evidence that is available indicates that at the end of human gestation electrolytes, urea, uric acid, incoagulable nitrogenous compounds, and substances represented by nitrogen attributed to amino acids pass readily from the maternal to the fetal blood. Nitrogenous substances available for the building of proteins may be utilized with such rapidity that the molecular concentration of the cytoplasm is kept at a low level. As the velocity of growth diminishes small molecules may accumulate, so that finally with the subsidence of growth molecular concentration of the cytoplasm may be sufficient to maintain the osmotic homeostasis characteristic of liver, kidney, and some other tissues of the fully grown animal.

SUMMARY AND CONCLUSIONS

The osmotic pressure maintained by liver tissue of the white rat preceding birth is less than that of the maternal blood serum and shortly after birth approximates this level.

Following birth osmotic pressure of liver tissue, continuing to increase, reaches after about 60 to 90 days the level found in the liver of mature animals and is then isotonic with solutions of sodium chloride with concentration slightly more than twice that isotonic with blood serum.

Osmotic pressure maintained by kidney tissue pursues with growth a similar course but at a lower level and about 35 to 60 days after birth reaches that found in the mature animal being represented by isotonicity with a concentration of sodium chloride slightly less than twice that isotonic with blood serum.

The tissues of the whole fetus are isotonic with sodium chloride solutions less concentrated than that isotonic with the maternal blood serum.

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