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Biochem. J. (1960) **77**, 30

The Effect of Growth and Function on the Chemical Composition of Soft Tissues

BY ELSIE M. WIDDOWSON AND J. W. T. DICKERSON
Medical Research Council, Department of Experimental Medicine, University of Cambridge

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During the second half of the nineteenth century a considerable amount of work was done in European countries on the chemical composition of the mammalian body and of its component tissues. The subject then lapsed, and papers on it appeared only occasionally until 10 or 15 years ago, when there was a revival of interest, particularly in the United States. Much of the work has been done on animals, but human tissues have also been studied, generally to see how various diseased states affected their composition.

In spite of all this activity there have, up till now, been very few systematic studies of the changes which take place in tissues with normal development. It has been known for at least 100 years that foetuses and new-born animals contain a higher proportion of water than do adults of the same species (von Bezold, 1857). This is to some extent accounted for by an increasing proportion of fat in the body, but even on a fat-free basis the percentage of water in the whole body falls from embryonic to some time in post-natal life. This percentage then becomes approximately constant at an age which varies from species to species. This 'steady state' was termed by Moulton (1923) 'chemical maturity'. More recently it has been appreciated that the fall in the percentage of water is due largely to a decrease in the proportion of the body occupied by extracellular fluids. Coincident with this there is a rise in the proportion of the body occupied by cells, the cells first increasing primarily in number and later in size.

The body is made up of many different organs and tissues, each with a different chemical composition, and each contributing a different proportion to the body as a whole. The proportion which the various organs contribute to the body, moreover, is not the same at all ages; for example, the brain always accounts for a larger proportion of the body in early life, and skeletal muscle accounts for more of the weight of the adult than of the foetus or new-born baby. The present investigation was designed to study, in man and the pig, the rate at which different parts of the body become chemically mature, and if possible to relate this to the function of these parts at different ages. The effect of development on the chemical composition of the whole bodies of these two species was described some years ago (Widdowson & Spray, 1951; Widdowson, McCance & Spray, 1951; Spray & Widdowson, 1951). More recently the findings on the serum and erythrocytes have been published (Widdowson & McCance, 1956; McCance & Widdowson, 1956*a*) and also those on the skeletal muscle (Dickerson & Widdowson, 1960).

MATERIAL AND METHODS

The sources of the material for this investigation were the same as those for the skeletal muscle described by Dickerson & Widdowson (1960). Skin, heart, liver, kidney and brain have been studied at four or five stages of development in man and five or six stages of development in the pig. The number of samples analysed in each case is given in the tables. Each sample from the foetal and new-born

pigs consisted of pooled material taken from several animals in the same litter; 44 foetal pigs at 46 days of gestation, 22 at 90 days and 12 new-born pigs have been used in the whole investigation.

Skin. This was taken from the abdomen or thigh in new-born and post-natal material and from both these sites in the foetuses. All visible fat was scraped off and the skin was finely cut up with scissors. It included epidermis and corium.

Heart. In some instances whole hearts have been analysed and in others the organ has first been dissected into right and left ventricles and auricles before sampling. All visible fat was removed before dissection and sampling, and the tissue was cut up finely with scissors.

Liver. The organ was blotted free from superficial blood and weighed. The whole of the foetal and new-born livers were taken for analysis and portions of the adult organs were taken from the centre of more than one lobe. The material was homogenized in a Waring Blendor without added water.

Kidney. The capsule was stripped off, and with it the perirenal fat. Both kidneys were taken from the foetuses and new-born babies and piglets, but only one from the adults. The organs were homogenized without added water.

Brain. In every instance the whole brain was taken and again homogenized without added water.

The methods of sampling and all chemical techniques were the same as those described by Dickerson & Widdowson (1960) for skeletal muscle. Fat was determined as the difference in weight before and after extracting the dry solids with light petroleum. Pieces of skin and cardiac muscle taken from the left ventricle were fixed in formalin–0·9% sodium chloride solution (1:9, v/v) for histological examination.

RESULTS

Skin

Tables 1 and 2 show the composition, on a fat-free basis, of human and pig skin at various ages. Development in both species was associated with a fall in the concentration of water, sodium and chloride, and an increase in that of total nitrogen and collagen nitrogen. Collagen nitrogen increased faster than the total nitrogen, and there was therefore an increase in the proportion of the total nitrogen present as collagen. This process had proceeded further in the skin of human babies at birth than in that of new-born piglets. The skin of the infants at 3–6 months was mature in so far as percentages of water and total nitrogen were concerned, but not in the proportion of the total nitrogen contributed by collagen. In the pig, this aspect of development was complete at 6 weeks, although the proportions of total nitrogen, collagen nitrogen and water did not reach a mature level until after this age. The amounts of the cellular constituents, potassium, phosphorus and magnesium, increased during pre-natal growth to a maximal value during early post-natal life, and fell to a lower level in the adult organ. The concentration of calcium in human skin increased during pre-natal growth to a maximum at the end of gestation and decreased during subsequent growth. The results of the analyses of adult human skin are similar to those of Eisele & Eichelberger (1945), and agree with all previous work on the skin of a variety of animals in that they show a higher concentration of chloride than is present in any other tissue except tendon.

Heart

Tables 3 and 4 show the weights and composition of the whole hearts of man and the pig at various stages of development before and after birth.

In man the heart contributed about the same proportion of the body weight at each age, but in the pig the proportion fell. The decrease after birth in the latter species is partly accounted for by the deposition of body fat during post-natal growth.

As in other tissues the proportion of water fell with development and the concentration of nitrogenous constituents rose. High values for calcium were found in the heart muscle of the foetal pig at 46 days of gestation, as they were in foetal skeletal muscle (Dickerson & Widdowson, 1960). There was insufficient material to make the determination in the foetal hearts of man.

The intracellular ion potassium and the extracellular ion sodium behaved in a rather unexpected way. Although the results were rather variable from one heart to another there seemed no doubt that the foetal heart of man generally contained a much higher concentration of potassium and a lower concentration of sodium than did the heart of the baby at term; at 4–7 months the figures were similar to those found at birth, and thereafter the concentrations of the two elements moved in the usual directions. The heart of the pig foetus at 46 days of gestation also contained more potassium and less sodium than did the heart of the new-born animal. The differences were smaller than in human heart, but were none the less statistically significant (potassium, $P < 0{\cdot}01$; sodium, $P < 0{\cdot}01$). In both human and pig heart the concentration of chloride hardly changed throughout development and, if chloride space may be taken as a measure of extracellular fluid, it must be concluded that the heart of the foetus is very near its adult composition in the proportion of it occupied by extracellular fluid; in this respect heart muscle is to be contrasted with skeletal muscle, described by Dickerson & Widdowson (1960).

The heart is a composite structure consisting of two auricles and two ventricles, separated by a strong muscular septum, and valvular, bundle and nodal tissues, and these form different proportions of the whole heart at different ages. It has been shown by other workers that the walls of the different chambers of the heart of an adult man do

Table 1. *Composition of human skin*

Results are expressed per kg. of fresh fat-free skin. Average and range are given.

	Foetus		Baby		
	13–14 weeks	20–22 weeks	New-born	3–6 months	Adult
No. of samples analysed	2	4	4	6	5
Water (g.)	917 (910, 924)	901 (893–910)	828 (800–850)	675 (620–770)	694 (680–710)
Total N (g.)	11·6 (10·1, 13·0)	11·9 (10·9–13·0)	26·5 (22·8–29·2)	54·5 (37·8–69·6)	53·0 (51·0–55·0)
Collagen N (g.)	—	2·4 (1·5–3·7)	16·8 (9·8–21·5)	39·2 (23·2–47·1)	45·7* (45·2, 46·1)
Collagen N (% of total N)	—	20·2 (13·8–28·4)	63·4 (43·0–73·6)	70·5 (61·2–78·4)	89·8* (87·5, 92·0)
Na (m-equiv.)	—	120 (107–130)	87·1 (73·0–104)	69·4 (61·7–76·8)	79·3 (70·5–83·0)
K (m-equiv.)	23·8 (22·0, 25·6)	36·0 (30·0–43·5)	45·0 (40·3–52·0)	43·7 (39·8–49·5)	23·7 (19·2–28·8)
Cl (m-equiv.)	90·6 (86·9, 94·3)	96·0 (91·0–102)	66·9 (63·2–72·0)	72·3 (61·3–82·5)	71·4 (62·0–77·0)
P (m-moles)	41·8 (40·0, 43·6)	28·2 (23·7–31·3)	31·7 (29·4–33·5)	34·9 (31·0–45·3)	14·0 (11·6–17·1)
Mg (m-equiv.)	—	3·8 (1·5–6·0)	4·7 (2·3–7·1)	7·4 (5·3–9·5)	3·1 (1·2–4·3)
Ca (m-equiv.)	4·4 (4·3, 4·4)	6·1 (6·0–6·2)	10·0 (6·5–12·1)	11·4 (10·0–13·4)	9·5 (7·8–12·2)

* Two values only.

Table 2. *Composition of pig skin*

Results are expressed per kg. of fresh fat-free skin. Average and range are given.

	Foetus		Piglet			
	46 days	90 days	New-born	3 weeks	4–6 weeks	Adult
No. of samples analysed	5	2	4	3	3	6
Water (g.)	937 (935–940)	902 (900, 903)	836 (818–852)	782 (755–800)	778 (760–800)	685 (650–710)
Total N (g.)	6·5 (5·7–6·9)	10·6 (10·5, 10·6)	20·4 (18·1–21·7)	36·5 (34·2–41·2)	36·5 (33·3–39·0)	53·2 (47·6–61·0)
Collagen N (g.)	0·4 (all 0·4)	3·6 (3·6, 3·6)	9·6 (8·7–10·1)	18·8 (15·3–23·4)	30·9 (28·4–33·4)	48·5 (44·0–52·5)
Collagen N (% of total N)	6·2 (5·8–7·0)	34·1 (34·0, 34·2)	47·0 (46·6–48·0)	51·5 (44·8–56·8)	84·6 (82·3–89·7)	91·1 (86·0–92·4)
Na (m-equiv.)	127 (115–152)	96·0 (94·0, 98·0)	110 (100–127)	90·2 (79·0–100)	86·8 (86·0–88·0)	81·6 (68·2–105)
K (m-equiv.)	28·3 (24·3–32·9)	39·5 (39·2, 39·8)	39·5 (34·0–44·5)	44·5 (39·7–47·2)	41·9 (39·8–43·0)	26·2 (17·8–36·5)
Cl (m-equiv.)	93·7 (86·5–97·5)	81·0 (79·2, 81·7)	80·0 (78·0–84·5)	74·7 (67·5–79·0)	75·5 (70·0–81·0)	74·4 (66·0–83·5)
P (m-moles)	15·8 (12·6–17·4)	28·6 (28·2, 28·9)	31·2 (26·2–39·5)	38·8 (33·8–47·5)	34·4 (31·8–35·8)	17·2 (12·3–22·7)
Mg (m-equiv.)	4·3 (3·0–5·5)	7·2 (6·7, 7·7)	6·6 (5·7–8·1)	7·8 (6·3–9·0)	7·8 (7·3–8·1)	4·4 (3·0–5·8)
Ca (m-equiv.)	10·2 (9·2–11·4)	11·3*	9·1 (6·1–11·2)	10·8 (8·3–12·7)	8·4 (7·3–10·0)	9·2 (6·5–12·4)

* One result only.

Table 3. *Composition of human heart*

Results are expressed per kg. of fresh fat-free heart. Average and range are given.

	Foetus	Baby		
	20–22 weeks	New-born	5–7 months	Adult
No. of hearts analysed	4	4	2	2
Wt. of heart (g.)	1·6 (1·2–2·2)	17 (10–22)	40 (37, 43)	230 (216, 245)
Wt. of heart as % of body wt.	0·63 (0·57–0·71)	0·63 (0·40–0·75)	0·50 (0·47, 0·53)	0·42 (0·38, 0·46)
Water (g.)	860 (837–879)	841 (820–857)	830 (825, 835)	827 (825, 828)
Total N (g.)	14·0 (12·3–15·2)	19·6 (18·0–22·6)	21·0 (20·2, 21·8)	22·9 (22·7, 23·1)
Non-protein N (g.)	1·5 (1·3–1·6)	1·7 (1·5–1·9)	1·7 (1·6, 1·8)	2·2 (1·8, 2·6)
Collagen N (g.)	0·8 (0·7–0·9)	2·0 (1·5–2·4)	3·5 (3·4, 3·6)	2·4 (1·8, 3·0)
Na (m-equiv.)	46·1 (37·7–58·5)	64·2 (60·2–66·7)	59·8 (57·5, 62·0)	57·8 (57·8, 57·8)
K (m-equiv.)	81·1 (56·7–101·5)	54·3 (37·6–62·5)	49·3 (46·9, 51·6)	66·5 (65·5, 67·5)
Cl (m-equiv.)	41·0 (36·1–46·0)	45·2 (41·2–49·0)	49·3 (49·2, 49·4)	45·6 (40·0, 51·2)
P (m-moles)	49·7 (30·0–63·2)	47·0 (42·2–54·2)	49·5 (48·5, 50·5)	49·0 (47·2–50·8)
Mg (m-equiv.)	—	10·9 (8·9–12·5)	11·0 (10·4, 11·6)	13·2 (11·7, 14·6)
Ca (m-equiv.)	—	7·4 (5·3–8·3)	8·2 (7·1, 9·3)	— (5·5, 12·6)

Table 4. *Composition of pig heart*

Results are expressed per kg. of fresh fat-free heart. Average and range are given.

	Foetus		Piglet			
	46 days	90 days	New-born	3 weeks	4 weeks	Adult
No. of hearts analysed	5 litters	2 litters	6	2	2	2
Wt. of heart (g.)	0·31 (0·18–0·47)	3·5*	8·4 (6·7–10·3)	20 (15, 25)	40 (31, 49)	420 (410, 430)
Wt. of heart as % of body wt.	1·24 (1·03–1·46)	0·55*	0·71 (0·66–0·76)	0·57 (0·40, 0·73)	0·56 (0·45, 0·67)	0·21 (0·21, 0·21)
Water (g.)	876 (857–885)	868 (865, 870)	830 (817–858)	825 (815, 835)	820 (818, 822)	825 (815, 835)
Total N (g.)	15·1 (14·0–17·0)	16·3 (16·2, 16·3)	20·8 (18·4–22·0)	22·9 (22·6, 23·2)	23·8 (22·8, 24·8)	26·2 (24·4, 28·0)
Non-protein N (g.)	1·3 (1·2–1·5)	2·0 (1·9, 2·1)	1·9 (1·7–2·0)	2·1 (1·9, 2·2)	2·3 (2·2, 2·4)	2·5 (2·3, 2·7)
Collagen N (g.)	0·35 (0·32–0·38)	0·61 (0·60, 0·62)	0·81 (0·65–0·98)	0·60 (0·41, 0·80)	0·99 (0·95, 1·03)	1·7 (1·7, 1·7)
Na (m-equiv.)	46·0 (33·1–55·0)	52·8 (52·7, 52·9)	56·8 (45·7–69·5)	45·7 (44·4, 47·0)	43·5 (41·5, 45·5)	45·8 (45·0, 46·6)
K (m-equiv.)	93·9 (89·0–96·8)	75·4 (71·8, 79·0)	82·2 (74·3–94·0)	89·5 (82·5, 96·5)	100 (97·0, 103)	87·5 (86·3, 88·7)
Cl (m-equiv.)	36·3 (34·4–38·1)	38·4 (36·9, 39·8)	36·6 (29·2–44·8)	34·3 (33·5, 35·1)	36·0 (32·0, 40·0)	32·0 (29·7, 34·2)
P (m-moles)	46·8 (33·0–57·0)	53·1 (52·3, 53·8)	69·0 (62·5–75·0)	75·8 (67·6, 84·0)	76·0 (74·0, 78·0)	68·2 (66·4, 70·0)
Mg (m-equiv.)	14·2 (13·6–14·7)	10·5 (10·1, 10·8)	16·0 (14·5–18·4)	16·2 (14·0, 18·3)	18·7 (17·6, 19·8)	19·2 (17·2, 21·2)
Ca (m-equiv.)	13·5 (12·0–14·7)	5·9 (5·4, 6·3)	6·3 (3·9–8·2)	5·8 (5·4, 6·2)	2·9 (2·8, 3·0)	3·3 (3·1, 3·5)

* Hearts of only one litter weighed.

Table 5. *Composition of ventricles and auricles of human heart*

Results are expressed per kg. fat-free tissue solids. Average and range are given.

	Right ventricle				Left ventricle				Auricles			
	Foetus 20–22 weeks	Baby New-born	Baby 5–7 months	Adult	Foetus 20–22 weeks	Baby New-born	Baby 5–7 months	Adult	Foetus 20–22 weeks	Baby New-born	Baby 5–7 months	Adult
No. of hearts analysed	3*	7	2	2	3*	5	2	7	3*	7	2	2
Total N (g.)	116	128 (118–143)	121 (112, 129)	127 (121, 132)	111	115 (98–134)	123 (111, 135)	127 (121–129)	130	134 (113–143)	127 (125, 129)	135 (129, 140)
Na (m-equiv.)	314	408 (362–454)	364 (344, 384)	—	392	373 (328–454)	375 (356, 394)	253 (228–299)	422	371 (342–400)	343 (328, 358)	—
K (m-equiv.)	485	301 (264–365)	290 (270, 309)	341 (317, 365)	505	292 (244–325)	309 (281, 336)	404 (362–448)	410	243 (222–278)	248 (232, 264)	298 (276, 320)
Cl (m-equiv.)	323	310 (278–391)	308 (304, 312)	339 (292, 386)	305	265 (194–314)	282 (268, 296)	201 (152–236)	—	271 (229–349)	278 (271, 285)	334 (320, 348)
P (m-moles)	365	309 (278–357)	290 (280, 300)	265 (264, 266)	352	307 (280–354)	323 (323, 323)	295 (264–319)	318	242 (186–278)	216 (202, 229)	192 (189, 195)

* One pooled sample was analysed.

not have exactly the same chemical composition (Cullen, Wilkins & Harrison, 1933; Wilkins & Cullen, 1933; Mangun, Reichle & Myers. 1941; Clarke & Mosher, 1952). For example, the auricles contain most water, sodium and chloride, and least potassium and phosphorus; the left ventricle contains least water, sodium and chloride, and most potassium and phosphorus; the right ventricle contains intermediate amounts of all constituents. In ox heart the nodal tissues and tricuspid valve have been shown to contain considerably more sodium and less potassium than the auricles (Davies, Davies, Francis & Whittam, 1952). Changes in composition of the heart, as a whole, are therefore the result of changes in the composition of the different parts, and of the contribution of these different parts to the whole.

In order to obtain more information about the unexpected decrease in potassium and increase in sodium which had been found to take place in the whole hearts of man between 20 weeks of gestation and birth, a further series of hearts was collected, and these were dissected into right and left ventricles and auricles and analysed. The different parts of the hearts, particularly those of the individual foetuses, had to be stored for some time at −20° before analysis and it was evident from the values obtained for the various constituents that a variable amount of drying had taken place during storage. For this reason, the composition of the different parts of the heart has been expressed per kg. of dry fat-free solids. Table 5 shows the results. Again, the decrease in concentration of potassium during the latter part of gestation is seen, and it is evident that all the chambers participate. Two possible explanations for this are suggested below.

The results in Table 5 for the different chambers of adult heart agree with the previous results of the workers cited above. For most constituents there was a smaller difference between the composition of right and left ventricles in the immature hearts than there was in the adult organs.

Liver

Tables 6 and 7 show the gross composition of the liver at various ages in man and the pig. There are certain features of these results common to both species, and other features in which the two species differ. The general increase in weight of the liver with development is part of the process of growth, and in other species it is known to be accompanied by an increase in the number and size of the cells (Kennedy & Pearce, 1958). The liver formed a more uniform percentage of the body weight in man than in the pig over the age range studied. Had younger human foetuses been available larger livers in proportion to the body weight would no doubt have been found (Hamilton, Boyd & Mossman, 1945). The

Table 6. *Composition of human liver*

Results are expressed per kg. of fresh liver. Average and range are given.

	Foetus		Baby		
	13–14 weeks	20–22 weeks	New-born	4–7 months	Adult
No. of livers analysed	2	4	4	3	4
Wt. of liver (g.)	1·18 (1·10, 1·26)	10·7 (9·2–12·3)	125 (120–130)	310 (290–325)	1570 (1335–2035)
Wt. of liver as % of body wt.	4·0 (3·9, 4·1)	4·2 (3·7–4·9)	4·6 (3·7–5·2)	4·0 (3·6–4·4)	2·4 (2·2–2·7)
Water (g.)	849 (826, 871)	812 (792–825)	786 (780–798)	764 (761–767)	711 (636–739)
Total N (g.)	20·2 (17·4, 23·1)	22·1 (20·3–22·7)	22·6 (20·1–26·7)	24·4 (25·1–26·3)	28·2 (27·8–28·9)
Na (m-equiv.)	—	54·8 (49·5–59·7)	59·8 (58·6–61·9)	51·0 (48·0–56·2)	42·5 (39·8–50·0)
K (m-equiv.)	81·8 (78·5, 85·0)	92·9 (85·0–100)	58·7 (50·7–68·2)	66·2 (64·5–67·0)	75·0 (71·5–82·5)
Cl (m-equiv.)	62·2 (61·3, 63·0)	57·1 (50·5–61·8)	55·8 (53·0–59·0)	42·8 (40·5–44·3)	38·3 (30·0–47·6)
P (m-moles)	82·5 (73·0, 92·0)	88·0 (79·6–102)	56·5 (47·5–71·0)	82·5 (81·0–84·2)	86·0 (74·3–95·0)
Mg (m-equiv.)	—	14·7 (13·6–16·8)	10·4 (9·2–12·4)	11·8 (11·7–12·2)	15·2 (13·5–18·0)
Ca (m-equiv.)	—	2·3 (1·9–2·9)	3·0 (2·3–4·2)	4·4 (3·8–5·0)	2·8 (2·6–2·9)

Table 7. *Composition of pig liver*

Results are expressed per kg. of fresh liver. Average and range are given.

	Foetus		Piglet			
	46 days	90 days	New-born	3 weeks	4 weeks	Adult
No. of samples analysed	5	1	6	1	2	5
Average wt. of liver (g.)	2·2 (1·3–3·5)	15·5	37 (20–54)	92	198 (190, 206)	2570 (2246–3102)
Wt. of liver as % of body wt.	9·4 (7·8–10·8)	2·5	3·0 (2·2–3·4)	2·7	2·8 (2·6, 3·0)	1·3 (1·1–1·6)
Water (g.)	822 (813–833)	816	784 (759–806)	759	746 (744, 747)	716 (708–724)
Total N (g.)	22·9 (19·7–24·8)	21·8	20·2 (19·4–24·8)	27·4	26·8 (26·7, 26·8)	34·5 (33·2–36·7)
Protein N (g.)	20·2	18·5	17·4	23·8	23·3	30·6
Na (m-equiv.)	59·3 (45·0–74·2)	63·6	50·7 (38·4–62·3)	51·0	34·4 (31·4, 37·4)	35·8 (32·6–40·9)
K (m-equiv.)	74·6 (61·8–86·0)	64·0	85·1 (67·0–95·0)	71·6	87·1 (84·0, 90·2)	82·2 (78·3–84·5)
Cl (m-equiv.)	51·4 (45·2–61·0)	56·2	39·7 (29·8–49·0)	32·4	29·0 (27·8, 30·2)	29·2 (27·1–31·6)
P (m-moles)	100 (91–108)	78	82 (72–94)	103	116 (115, 117)	119 (117–123)
Mg (m-equiv.)	19·0 (14·8–21·1)	16·2	16·8 (12·9–20·6)	18·7	18·1 (18·0, 18·2)	19·4 (18·9–20·9)
Ca (m-equiv.)	3·4 (2·3–4·3)	4·2	2·2 (1·9–2·5)	2·6	2·0 (1·8, 2·1)	2·6 (2·2–2·9)

small percentage of the body occupied by the liver in adult pigs is due, in part, to the large amount of body fat in these animals.

There was a steady fall in the proportion of water and a rise in the proportion of nitrogen in both species, broken for a short period at birth in piglets, probably owing to the large amount of glycogen accumulating in the organ at that time (McCance & Widdowson, 1959). The livers of the human foetuses, like the hearts, contained quite remarkably large amounts of potassium and there is no obvious explanation unless it was associated

Table 8 *Composition of human kidneys*

Results are expressed per kg. of fresh whole kidney. Average and range are given.

	Foetus		New-born baby	Adult
	13–14 weeks	20–22 weeks		
No. of samples analysed	2	4	4	4
Average wt. of two kidneys (g.)	0·20 (0·18, 0·22)	1·73 (1·41–1·89)	28 (24–32)	340 (213–452)
Wt. of kidneys as % of body wt.	0·64 (0·60, 0·68)	0·68 (0·60–0·74)	1·02 (0·91–1·16)	0·52 (0·35–0·60)
Water (g.)	915 (914, 915)	884 (876–891)	841 (825–853)	810 (795–821)
Total N (g.)	12·5 (11·9, 13·0)	14·2 (11·4–15·7)	19·2 (17·6–21·1)	24·5 (21·3–27·6)
Na (m-equiv.)	—	68·0 (57·0–73·5)	75·5 (70·7–80·3)	82·0 (79·0–84·4)
K (m-equiv.)	—	66·5 (59·5–78·5)	56·0 (50·5–61·0)	57·0 (52·5–59·7)
Cl (m-equiv.)	66·6 (65·4, 67·8)	59·6 (55·5–66·0)	56·4 (49·5–60·0)	67·8 (59·8–73·0)
P (m-moles)	72·0 (71·0, 73·0)	65·5 (60·0–69·0)	61·0 (57·5–66·0)	57·5 (49·0–66·0)
Mg (m-equiv.)	5·3 (5·3, 5·3)	8·6 (6·5–12·4)	8·7 (6·6–11·5)	8·6 (7·1–9·7)
Ca (m-equiv.)	18·4 (18·0, 18·8)	17·4 (14·7–19·5)	7·7 (5·1–9·9)	7·0 (5·6–8·0)

Table 9. *Composition of pig kidneys*

Results are expressed per kg. of fresh whole kidney. Average and range are given.

	Foetus		Piglet			
	46 days	90 days	New-born	3 weeks	4 weeks	Adult
No. of samples analysed	5	2	4	2	2	5
Average wt. of two kidneys (g.)	0·45 (0·21–0·89)	4·6*	9·8 (7·7–11·9)	25 (22, 28)	38 (32, 43)	436 (396–474)
Wt. of kidneys as % of body wt.	1·8 (1·5–2·0)	0·70*	0·82 (0·70–0·93)	0·70 (0·63, 0·76)	0·54 (0·47, 0·60)	0·23 (0·22–0·23)
Water (g.)	889 (878–927)	874 (873, 875)	825 (790–860)	811 (811, 811)	808 (802, 814)	812 (801–824)
Total N (g.)	13·6 (13·4–14·6)	14·3 (14·3, 14·4)	20·3 (18·0–21·5)	21·5 (20·8, 22·1)	22·0 (21·7, 22·3)	23·7 (22·7–24·4)
Na (m-equiv.)	96·2 (78·5–115)	78·3 (76·5, 80·1)	76·0 (57·5–105)	71·4 (70·0, 72·8)	57·8 (57·6, 58·0)	62·9 (48·7–73·3)
K (m-equiv.)	48·6 (45·0–52·8)	54·5 (52·6, 56·4)	77·4 (60·0–97·5)	67·9 (62·6, 73·2)	76·5 (65·0, 88·0)	60·1 (50·5–68·5)
Cl (m-equiv.)	61·9 (59·5–65·8)	59·1 (57·3, 60·8)	54·9 (49·2–59·0)	59·6 (57·3, 61·8)	48·0 (44·8, 51·2)	57·0 (52·2–60·5)
P (m-moles)	63·0 (60·0–69·0)	54·8 (54·0, 55·5)	84·0 (64·6–108)	81·6 (71·6, 91·6)	89·0 (84·0, 94·0)	69·0 (62·5–75·0)
Mg (m-equiv.)	8·8 (8·5–9·1)	11·2 (11·0, 11·3)	13·2 (10·9–15·6)	13·8 (12·1, 15·5)	13·7 (12·2, 15·2)	8·9 (7·2–10·3)
Ca (m-equiv.)	7·9 (6·8–9·0)	5·0 (4·4, 5·5)	4·6 (4·5–4·7)	9·3 (8·3, 10·3)	4·4 (3·5, 5·4)	4·4 (3·2–5·5)

* Kidneys from only one litter were weighed.

with the haemopoietic activity of the organ before birth (Hamilton *et al.* 1945). The concentration of sodium was not correspondingly low, and the sum of the sodium and potassium was higher than at any other age. The values for sodium, potassium and chloride in the livers of the foetal and new-born pigs showed rather wide variations from one sample to another. Active formation of glycogen is probably one reason for the high values for potassium in the liver of the new-born pig (McCance & Widdowson, 1959). Over the whole age range the protein nitrogen/potassium ratio increased in both species. This has been attributed to a decrease in the amount of water in the cells (McCance & Widdowson, 1956*b*); there is less than 1% of collagen in human liver (Lowry, Gilligan & Katersky, 1941), so that most of the nitrogen must be in the cells, but if haemopoietic tissue has a lower nitrogen/potassium ratio than true liver, this would account for the change. Human liver contained appreciably less phosphorus and magnesium than pig liver at comparable stages of development. High values for calcium, similar to those found in foetal skeletal muscle and heart, were not found in foetal liver.

The immature livers of both species contained more sodium and chloride than the adult organs, suggesting that they contained more extracellular fluid, but the possibility that the cells contained sodium and chloride cannot be ruled out (Yannet & Darrow, 1940). It has not been deemed wise to make calculations of intracellular water on the basis of chloride space.

Kidney

Tables 8 and 9 show the weight and composition of the kidneys. In man the kidneys at birth formed a higher proportion of the body weight than they did at any other age, but in the pig the kidneys contributed most to the total weight of the body at 46 days of gestation. At this stage of the pig's existence the mesonephros, which has been a large functional organ (Stanier, 1960), is shrinking rapidly and the metanephros is growing very fast; 2 days earlier (at 44 days of gestation) the metanephros formed about the same proportion of the body weight as it did at 46 days, and the mesonephros comprised another 1%.

As in other soft tissues, chemical development of the kidneys involved a decrease in the proportion of water and an increase in the proportion of nitrogen. The figures for inorganic constituents were rather erratic, owing no doubt to the presence of fluids filtered off or secreted by the organ, even in the earliest stages. The high values for calcium in the foetal kidneys should perhaps be noted, though at present they remain unexplained. High concentrations of calcium have been reported in foetal membranes (Economou-Mavrou & McCance, 1958), as well as in foetal skeletal muscle (Dickerson & Widdowson, 1960) and heart (see above).

Brain

The weights and gross chemical composition of the brains of the two species are shown in Tables 10 and 11. At all ages the brain of man formed a much larger proportion of the body than did the brain of the pig, and both in the new-born and in the adult the human brain weighed more than ten times as much as the brain of the pig.

The changes in composition in the two species followed the same course, the proportion of water falling from over 90% in the foetus to 76–77% in the adult. The proportion of nitrogen increased, but was always higher at the selected stages of development in the pig than in man, and the proportion of water was correspondingly lower. The concentration of phosphorus was higher in the pig at all ages. In both species the concentration of sodium and chloride decreased with development and the concentration of potassium increased; the big change towards the adult level had already occurred by the time of birth in the pig, but did not occur until after birth in man. Figures for calcium in the pig brains have not been included because some of the adult skulls were sawn in half before the brain was removed, and the values for calcium suggested that the brain samples were contaminated with bone dust.

DISCUSSION

The form and structure of the adult organism is reached by a process of differential growth. The chemical development of the organism is also a differential process, for from the results described above, and from those of Dickerson & Widdowson (1960) for skeletal muscle, there is no doubt that some organs and tissues mature faster than others. Foetal heart muscle and foetal liver and kidneys are much nearer the chemical composition of the adult organs than are skeletal muscle or skin. It is tempting to relate these differences in the rate of chemical development with variations in the speed of functional maturation. The heart sounds can usually be heard at 18–20 weeks of gestation in man, and in the pig the heart has begun to beat before the twenty-first day of gestation, when the animal still weighs only 0·2 g., and Stubbs & Widdas (1959) have shown that contraction of the heart muscle is associated with extrusion of interstitial fluid. The liver functions actively as a haemopoietic organ during gestation and the kidneys certainly produce a urine which is usually, if not always, hypo-osmotic with respect to sodium and chloride. In contrast to these organs, the voluntary

muscles and skin of the foetus have little or no work to do. Observations made on perfused non-viable human foetuses have suggested that the foetus may be quite active *in utero* (Westin, Nyberg & Enhörning, 1958), but even so the work done by the skeletal muscles must be extremely small compared with that done by the beating heart. In the skeletal muscle of the foetus the cells are small and are widely separated by extracellular material (Dickerson & Widdowson, 1960). In foetal heart muscle the cells are also small, but they are packed closely together and there is no space for the large amounts of extracellular material found in skeletal muscle.

Table 10. *Composition of human brain*

Results are expressed per kg. of fresh whole brain. Average and range are given.

	Foetus		New-born baby	Adult
	13–14 weeks	20–22 weeks		
No. of brains analysed	2	4	4	4
Wt. of brain (g.)	4·65 (4·42, 4·88)	34 (25–41)	365 (330–430)	1438 (1250–1640)
Wt. of brain as % of body wt.	15·0 (15·0, 15·0)	13·4 (12·5–14·5)	13·4 (12·2–15·1)	2·3 (2·1–2·8)
Water (g.)	914 (911, 916)	922 (911–929)	897 (891–903)	774 (763–785)
Total N (g.)	9·6 (9·4, 9·7)	8·4 (7·2–9·5)	9·3 (9·0–9·9)	17·1 (16·2–17·7)
Na (m-equiv.)	97·5 (91·0, 102)	91·7 (83·8–98·1)	80·9 (78·5–85·7)	55·2 (54·0–57·1)
K (m-equiv.)	49·6 (44·2, 55·0)	52·0 (47·8–60·0)	58·2 (55·0–60·0)	84·6 (74·0–90·9)
Cl (m-equiv.)	72·1 (68·0, 76·2)	72·6 (69·0–76·2)	66·1 (64·5–67·7)	40·5 (38·1–42·8)
P (m-moles)	57·0 (56·5, 57·5)	52·2 (45·5–58·5)	54·0 (51·2–57·5)	109 (101–115)
Mg (m-equiv.)	—	8·4 (7·8–8·9)	7·9 (7·3–8·3)	11·4 (10·9–11·7)
Ca (m-equiv.)	—	4·9 (3·9–6·0)	4·8 (4·4–5·0)	4·0 (3·0–5·5)

Table 11. *Composition of pig brain*

Results are expressed per kg. of fresh whole brain. Average and range are given.

	Foetus	Piglet			
	46 days	New-born	3 weeks	4 weeks	Adult
No. of brains analysed	5	4	2	2	5
Wt. of brain (g.)	0·87 (0·44–1·28)	33 (32–34)	44 (42, 45)	45 (41, 50)	100 (83–117)
Wt. of brain as % of body wt.	3·5 (2·8–4·2)	2·8 (2·4–3·4)	1·2 (1·1, 1·3)	0·6 (0·6, 0·7)	0·05 (0·04–0·06)
Water (g.)	908 (901–915)	847 (842–859)	820 (815, 825)	812 (805, 819)	758 (744–775)
Total N (g.)	10·0 (8·8–10·4)	14·2 (13·2–15·6)	14·3 (14·0, 14·6)	15·3 (15·0, 15·5)	19·1 (16·9–23·0)
Na (m-equiv.)	82·8 (74·4–85·2)	59·9 (52·6–75·2)	64·1 (61·0, 67·1)	61·4 (55·5, 67·2)	60·7 (50·5–67·9)
K (m-equiv.)	56·7 (54·3–58·0)	85·8 (80·5–89·0)	64·6 (57·0, 72·1)	75·9 (63·8, 88·0)	76·0 (66·6–83·0)
Cl (m-equiv.)	62·3 (59·5–65·6)	47·8 (47·0–49·0)	48·9 (44·5, 53·2)	45·2 (45·0, 45·3)	41·1 (36·4–45·3)
P (m-moles)	57·0 (50·0–64·0)	82·5 (80·5–84·0)	82·0 (80·5, 83·5)	92·5 (92·0, 93·0)	125 (122–130)
Mg (m-equiv.)	9·5 (9·2–9·8)	10·6 (9·9–11·8)	9·2*	8·0 (7·7, 8·2)	12·2 (10·2–13·9)

* One result only.

The changes in the electrolyte composition of the heart and of its several compartments between 20 weeks of gestation and the time of birth in man were rather puzzling, for the high concentration of sodium and low concentration of potassium at term as compared with the foetal heart at 20 weeks of gestation suggested that the whole process of development had been reversed, but, on the other hand, the proportions of water and nitrogen indicated that chemical development had proceeded in the expected direction.

There seem to be two possible ways in which this could have been brought about. Foetal heart muscle may contain some organic anion, for example a protein, peculiar to this time of life, which binds potassium. It will be recalled that the liver of the human foetus also contained more potassium than did the liver of the full-term baby. The high concentration of potassium in the foetal heart could hardly have been due to the formation of glycogen, for the hearts of two normal 20-week foetuses, obtained immediately after delivery, contained only 1·16 and 0·35 g. of glycogen/100 g. There may, however, be some other substance which has not as yet been identified.

Alternatively, it may be that the heart-muscle cells of the full-term babies studied in this investigation had become permeable to sodium, and a corresponding amount of potassium had left them. This is what happens when strips of rat ventricle are suspended in a physiological solution, and the supply of oxygen is cut off (Hercus, McDowall & Mendel, 1955; McDowall, Munro & Zayat, 1955); it has also been shown to take place in man in recently infarcted myocardium (Alexander, Boyle, Iseri, McCaughey & Myers, 1950). The still-born babies, whose hearts were analysed, probably died from a lack of oxygen, and the same thing is true of the older babies who were suffocated in their cots. Their hearts had undoubtedly gone on beating until the time of death, their failure indeed had probably been the cause of death, and it is possible that the anoxia had prevented the cells extruding sodium in the normal way, so that an exchange of sodium and potassium had taken place through the cell walls. Calculations of intracellular sodium, based on the chloride space as a measure of extracellular fluid, showed that the cells of the heart at birth contained more sodium than at any other age examined. Babies suffering from respiratory distress have been shown to have high concentrations of potassium in the serum (Usher, 1959), and this potassium may have come in part from the heart-muscle cells. These babies, moreover, had electrocardiographic abnormalities. It is at present impossible to decide between these two alternatives, and it may even be that both are involved. The matter is under further investigation.

The values obtained for the various constituents in the brain of the adult of the two species are in agreement with those collected by Rossiter (1955), from the literature, for the composition of mammalian brain. It seems certain that, at all stages of development, human brain contains a higher proportion of water than pig brain. The explanation for this may be that grey matter forms a greater percentage of the brain substance in man than in the pig, and grey matter is known to contain a higher proportion of water than white matter (Stewart-Wallace, 1939).

Kerpel-Fronius (1937) showed that the concentration of chloride in the brain of man, the dog and the rabbit fell during post-natal development, and Flexner & Flexner (1950) found that the proportion of extracellular fluid, measured as the chloride space, in the cerebral cortex of the guinea pig decreased steadily from the forty-second day of gestation to term (sixty-sixth day); in the newborn it had the same value as it had in the adult. The chloride space in human brain at the younger ages was found to be greater than it was in the pig at corresponding stages of development. This may mean that human brain contains a greater proportion of extracellular fluid and the same conclusion might be drawn from measurements of the sodium spaces. It has, however, been stated that the minute chemical structure of the brain is so complex that it would be 'unreasonable to interpret' the chemical results in terms of a simple mixture of extracellular and intracellular phases (Manery & Hastings, 1939). There is also evidence from perfusion experiments that much of the chloride of the brain, of some species at any rate, is intracellular (Amberson, Nash, Mulder & Binns, 1938; Oster & Amberson, 1939). Measurements *in vivo* of the volume of distribution in brain of inulin, iodide, *p*-aminohippuric acid and sucrose have given results of less than 5% of the wet weight (Davson & Spaziani, 1959; Morrison, 1959).

Whatever the real distribution of the chloride ion may be, it seems certain that at all stages of development, in both man and the pig, the volume of distribution of the sodium ion is greater, so that some of the sodium is certainly in the cells. Flexner & Flexner (1949) found that the influx of sodium into the cells of the guinea-pig's cerebral cortex, as calculated from the sodium and chloride spaces, coincided with the onset of electrical activity.

The extracellular phase of any tissue may be considered to consist of extracellular water and the connective-tissue proteins (Manery, Danielson & Hastings, 1938). Some of the extracellular water is associated with the fibrous connective-tissue proteins, collagen, elastin and reticulin, to constitute the dense fibrous connective tissue, and this part

of the extracellular water may be referred to as 'fibre' water. The rest of the water outside the cells is associated with the non-fibrous material making up the connective tissue, e.g. mucopolysaccharides and plasma proteins. This concept of the extracellular phase is of importance in relation to the composition of the skin because of the large amount of dense, fibrous, connective tissue which is present in the adult organ.

The partition of the extracellular water between the fibrous and non-fibrous connective-tissue proteins in the skin can be calculated from the amount of collagen in the tissue if it is assumed that the composition of the dense fibrous connective tissue is the same as tendon. A few samples of pig tendon were therefore analysed for water, total nitrogen, collagen and chloride. The values obtained for these constituents were similar to those given by Eichelberger, Eisele & Wertzler (1943) for dog tendon. On the assumption that all the water in tendon is associated with fibrous connective-tissue protein, the results of the analyses of pig tendon have been used to calculate the amount of water associated with the collagen fibres ('fibre' water) in the skin of the pig and man at the different stages of development. The amount of chloride present in this 'fibre' water, at the same concentration as in tendon water, has then been subtracted from the total chloride in the tissue to obtain the amount of this ion present in the 'non-fibre' water, the volume of which has been calculated in the usual way from the concentration of chloride in serum water, corrected for the Donnan equilibrium. The sum of the 'fibre' and 'non-fibre' water is the total extracellular water and this has been subtracted from the total amount of water in the tissue to give the amount of intracellular water. The basis of the calculation of the volume of 'non-fibre' water is the assumption that all the chloride in the tissue is outside the cells. Although this assumption may not be valid in some species at certain ages (Eichelberger *et al.* 1943), it nevertheless yields a picture of the changes in the distribution of skin water during development which can be correlated with the changes in histological structure that take place during this process.

Table 12 shows the amount of 'fibre', 'non-fibre' and intracellular water in the skin of man and the pig at the different ages expressed as a percentage of the amount of total water. At all ages, in both species, over 66 % of the total water in the organ was accounted for by extracellular fluid. The distribution of this extracellular fluid changed with the progress of development, for the proportion of it associated with the collagen fibres increased along with the increase in collagen, whereas the 'non-fibre' extracellular water decreased. In human skin the percentage of the total water in the cells increased to a maximum at the time of birth, and decreased to a lower level in the adult. This change roughly paralleled the change in the concentration of cellular substances in this species (Table 1). In the pig the proportion of cell water was very small in the youngest foetus and in the adult, and here again the concentrations of the cellular constituents were lower at these ages than at the intermediate ones (Table 2).

The fall in the proportion of non-collagen nitrogen during growth is due to a fall in the contribution of the proteins of the cells and ground substance to the total nitrogen in the tissue. The number of cells visible in a section of human foetal skin makes it seem likely that cell proteins contribute a fairly large proportion of the non-

Table 12. *Distribution of skin water*

The 'fibre' and 'non-fibre' water values were calculated from the average values for collagen and Cl per kg. of skin given in Table 1, and the results are expressed as a percentage of the total water. 'Fibre' water + 'non-fibre' water = total extracellular water. Total water − total extracellular water = cell water.

	Extracellular water			
	'Fibre' water	'Non-fibre' water	Total	Cell water
Human skin				
Foetus 20–22 weeks	2·5	90·6	93·1	6·9
Baby (New-born	19·4	47·3	66·7	33·3
Baby (3–6 months	55·7	34·0	89·7	10·3
Adult	63·7	23·1	86·8	13·2
Pig skin				
Foetus (46 days	0·4	98·0	98·4	1·6
Foetus (90 days	3·9	84·0	87·9	12·1
Piglet (New-born	11·0	78·0	89·0	11·0
Piglet (3 weeks	23·2	56·5	79·7	20·3
Piglet (4–6 weeks	38·0	43·0	81·0	19·0
Adult	68·0	23·4	91·4	8·6

collagen nitrogen before birth. The space between the cells must be filled with extracellular material of the same general composition as that in the other organs and tissues of the body. The amount of collagen in the skin at this age is small when compared with that in the adult tissue and, in fact, sections stained for collagen by the Van Gieson method did not show the presence of any mature collagen fibres. Sections stained with a silver-impregnation technique showed that the collagen estimated in human foetal skin was in the form of reticulin fibres, which formed a fine interlacing network in which the cells of the corium were embedded. Since the amount of collagen in foetal skin was small, there was a correspondingly small amount of extracellular water associated with it; most of the extracellular water was therefore associated with the mucopolysaccharides and non-fibrous proteins of the extracellular phase. This gave the skin an 'oedematous' appearance when it was removed from the body, and the sections show that the skin was thicker in the human foetus than in the full-term baby.

The skin of the new-born baby contains fewer cells, loosely scattered in the corium, but glands of mature appearance are present and the epidermis is also more mature than in the foetus. The glands and epidermis together probably contribute to the increase in the amount of potassium during the latter part of gestation. At birth the corium is loosely packed with collagen fibres.

The thickness of the skin increases during post-natal growth, and the combination of this with a decrease in cell density in the corium is largely responsible for the fall in the level of potassium in the skin after a post-natal age of 7 months.

These descriptions have been mostly concerned with human skin but the histological changes in pig skin during growth are, on the whole, very similar. There is much less collagen in the skin of the pig than in that of the baby, at birth.

Much of the interest in the composition of normal skin has been in its function as a depot for mobile water and salt (see Rothman, 1954). The whole of the extracellular water in the skin has been described as 'available water' (Flemister, 1941–42), and it has been shown that the amount of water in the skin changes more than that of skeletal muscle, for example, during dehydration (Rothman, 1954). It does not appear to have been shown whether this water represents a decrease in both fibre and non-fibre extracellular water, or if it is, in fact, drawn only from the latter. It would seem to be unlikely that the fibre water would be 'available' since analyses of tendon have shown that collagen is always associated with a constant amount of water and electrolytes (Manery *et al.* 1938).

After the skeletal muscles the skin is the largest soft tissue of the body, and the skin, the skeleton and the skeletal muscles in the adult human body together contribute about 70% of the body weight (Forbes & Lewis, 1956). Table 13 shows the proportion of the total nitrogen in the body contributed by each of these tissues and also the proportion of the total nitrogen in the body contributed by the collagen in them. The values for the weights of fat-free skin, skeleton and skeletal muscle and of the whole fat-free body have been taken from the data of Forbes, Cooper & Mitchell (1953), Forbes & Lewis (1956) and Forbes, Mitchell & Cooper (1956). The values for total nitrogen have been calculated from the data published by these workers for the same subjects. The amounts of collagen nitrogen in the whole skin and skeletal muscles have been calculated from the percentage of the total nitrogen contributed by collagen in these tissues as found in the present study and in that of Dickerson & Widdowson (1960). The value for the skeleton was obtained from the percentage of the total nitrogen accounted for by collagen in the adult human femur (J. W. T. Dickerson, unpublished data), on the assumption that this bone is representative of the whole skeleton. The results show that the three major tissues of the body contribute over 70% of the total nitrogen in the body and that the collagen of the skin and skeleton account for 25% of the total nitrogen. Since considerable amounts of collagen are also present in the gastrointestinal tract and in the lungs, it seems reasonable to suppose that at least a quarter of the protein in the adult human body is outside the cells (Neuberger, 1955). An increase in the amount of extracellular protein in the skin may well have accounted for some of the stored nitrogen in subjects who were recovering from a protein deficiency (Holmes, Jones & Stanier, 1954).

Table 13. *Contribution of the skin, skeleton and skeletal muscle to the total nitrogen in the adult human body*

	Wt. (fat-free) (kg.)	Total N (g.)	Collagen N (g.)	N in organ (% of total N in body)	Collagen N in organ (% of total N in body)
Total body	48·1	1840	—	—	—
Skin	3·3	176	158	9·5	8·5
Skeleton	8·0	331	271	18·1	14·8
Skeletal muscle	23·1	856	28	46·3	1·5

The skin probably accounts for a greater proportion of the body weight in the human foetus and new-born baby than it does in the adult (Wilmer, 1940), and the changes in its composition, particularly during early post-natal life, emphasize the importance of the contribution of this organ to the changes in the composition, of the whole body during development. McCance & Widdowson (1957) have stressed the importance of growth in the homoeostasis of the new-born. The skin may be as important as skeletal muscle in this regard for, in man, over 30 g. of nitrogen was found to be deposited in 1 kg. of fat-free skin during the first 3–5 months after birth, and in the pig 12 g. of nitrogen was deposited in each kg. of fat-free skin during the first 3 weeks of post-natal growth. This deposition of nitrogen not only relieves the kidney of dealing with it, but it also equips the skin better to carry out its own responsibilities of protecting the underlying tissues from desiccation, heat loss and the entry of noxious organisms.

SUMMARY

1. Skin, cardiac muscle, liver, kidney and brain of man and the pig have been analysed in the foetus, in the new-born, during the suckling period and in the adult.

2. Development was associated with a fall in the proportion of water and an increase in the proportion of nitrogen in all tissues; foetal heart muscle, and foetal liver and kidneys reached their adult composition before skin and skeletal muscle, and it is suggested that this is related to their early functional development.

3. Next to skeletal muscle the skin is the largest soft tissue of the body, and changes in its composition make a significant difference to the composition of the body as a whole. The proportion of collagen per kilogram of skin increased during development, both in absolute terms and as a percentage of the total nitrogen. The cellular constituents were at a maximum at about the time of birth.

4. The hearts of the still-born babies contained considerably less potassium than did the foetal hearts. Two possible explanations are put forward for this anomalous finding.

5. Human brain contained more water and less nitrogen and phosphorus than pig brain at corresponding stages of development. This may be due to a larger amount of grey matter in human-brain substance.

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Biochem. J. (1960) **77**, 43

Insulin and an Inhibitor of Glucose Uptake, in Protein Fractions of Normal Human Plasma

BY L. VARGAS,* K. W. TAYLOR† AND P. J. RANDLE
Department of Biochemistry, University of Cambridge

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Experiments in which the proteins of normal human serum or ox serum were fractionated by zone electrophoresis on columns of treated cellulose have suggested that insulin in serum migrates with fractions containing α_1-, β- and γ-globulins (Randle & Taylor, 1958; Taylor & Randle, 1959). Further subfractionation of the β- and γ-globulins suggested that insulin was associated predominantly with β- rather than γ-globulins and it seemed possible that insulin in γ-globulins is derived from contamination with β-globulins as a result of zone spreading during electrophoresis. In addition, evidence was obtained for the presence of an inhibitor of glucose uptake by isolated rat diaphragm *in vitro*, migrating in the α_2-globulin fraction. Detection of this inhibitor was made difficult by contamination of the α_2-globulin fraction with insulin derived possibly from adjacent α_1- and β-globulins (Randle & Taylor, 1958).

We have now sought to confirm these conclusions by an alternative method of protein fractionation, with ethanol at low temperatures, which effects a much better separation of β- and γ-globulin fractions of normal human serum than zone electrophoresis. In order to demonstrate the presence of the inhibitor with certainty it was necessary to subfractionate one of the fractions prepared with ethanol, by an improved method of zone electrophoresis. Details of this method of zone electrophoresis are given in the Appendix.

* Rockefeller Foundation Fellow. Present address: Facultad de Medicina, Universidad Catolica de Chile, Santiago, Chile.

† Elmore Research Student, University of Cambridge. Present address: Baker Clinic Research Laboratory, Boston, Mass., U.S.A.

EXPERIMENTAL

Blood samples. Human blood was drawn from an antecubital vein of non-fasting subjects and diluted immediately with 0·173 vol. of acid–citrate–dextrose (Lever *et al.* 1951). The diluted plasma was then separated by centrifuging.

Fractionation of plasma proteins. The proteins of diluted plasma were first separated into three fractions with ethanol at $-5°$ by the method of Lever *et al.* (1951). The fractions obtained have been designated according to Lever *et al.* as fraction I + III (mainly α_2- and β-globulins and fibrinogen), fraction II (γ-globulins) and fraction IV, V and VI (mainly albumin and α-globulins with a little β-globulin). The fractionation was confirmed in most instances by paper electrophoresis of the fractions, employing the vertical-tank method of Flynn & de Mayo (1951) and staining the protein with bromophenol blue by the method of Henry, Golub & Sobel (1957). The protein fractions were dialysed for 30 hr. against distilled water, in Visking tubing, at room temperature and freeze-dried.

Fraction IV, V and VI was subfractionated by zone electrophoresis at pH 8·4, on columns of treated cellulose, 50 cm. long and 2·0 cm. in diameter, (Porath, 1956) with the phosphate–borate buffer of Campbell & Stone (1956). The faster-moving proteins (albumin and α_1-globulin) were allowed to move off the column during