Calcium, Magnesium and Phosphorus in Foetal Tissues

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Surprisingly little is known about the amount, location, chemical combination and function of calcium, magnesium and phosphorus in foetal tissues. The present work was inspired by this comparative ignorance coupled with the availability of some fresh, well-dated foetal material from the pig and the rabbit. Some human foetuses have also been analysed, and the relevant literature will be mentioned with the presentation of the results. Some information about the physico-chemical structure of embryonic cells and fluids has already been published (Dickerson & McCance, 1957; McCance & Dickerson, 1957; Widdowson & McCance, 1956; McCance & Widdowson, 1956).

MATERIAL AND METHODS

The pigs and rabbits were from the Department's animal colony and were all healthy at the time of death. The pigs were the result of an original cross between Large White and Essex breeds with subsequent matings with a Large White breed of boar. The rabbits were Laboratory stock. The pigs were killed at the slaughter house. Samples of maternal blood were obtained there and the uteri removed and brought at once to the Laboratory. The mature human skeletal muscles were taken from the thigh during autopsies on young adults killed suddenly by street accidents and the foetal muscles, also from the thigh, were obtained after hysterotomy. The rest of the human material was collected direct from the operating theatre by someone who attended for that purpose. All the samples of foetal blood were obtained from the cord. Most of the figures given are the averages of determinations made on two or more individuals. In studying the pig and the rabbit, material from several foetuses in a single gestation was generally pooled for analysis. All the determinations were made in duplicate.

The chemical methods employed were as follows:

The tissues, including foetal membranes, were cut up finely with scissors and solids were determined by drying weighed portions at 100° to constant weight. These samples were then ashed at 450° and the ash was extracted with HCl as described by McCance & Shipp (1933). The Ca in the serum, foetal fluids and tissue-ash extracts was determined by the Clark and Collip modification of the Kramer and Tisdall method (Hawk, Oser & Summerson, 1954, p. 589). The ash extracts were brought to pH 6.4 before precipitation. The oxalate in the precipitate was determined by adding an excess of ceric sulphate and measuring the excess, after the addition of potassium iodide, by titration with thio-sul-

phate. After precipitation of Ca, the Mg was determined in the supernatant fluid by a modification of the Denis method (Hawk *et al.* 1954, p. 593) in which the precipitate was washed twice with aq. 5% (v/v) NH_3 soln. [i.e. a 1:20 dilution of conc. (sp.gr. 0.880) NH_3 soln. in water].

The inorganic P in the adult serum was determined by the method of Fiske & Subbarow (1925). In the foetal serum and fluids a turbidity developed when the molybdate reagent was added. The phosphomolybdic acid was therefore extracted from trichloroacetic acid filtrates with *iso*-butanol and the alcoholic extract washed with $\text{N-H}_2\text{SO}_4$ according to the method of Berenblum & Chain (1938). The colour was then developed by shaking the alcoholic phase with the aminonaphtholsulphonic reagent used in the Fiske & Subbarow method. The blue aqueous phase was collected, made up to a suitable volume and the optical density determined at 700 $\text{m}\mu$ in comparison with a blank and standard which had been similarly treated. The total P in the tissue and extracts was determined by the Fiske & Subbarow method after hydrolysis with a few drops of conc. H_2SO_4 to ensure that all P was converted into orthophosphoric acid.

The citric acid in the allantoic fluid of the pig was estimated by the Pucher, Sherman & Vickery (1936) method as modified by Hargreaves, Abrahams & Vickery (1951).

RESULTS

Sera

Calcium. Table 1 shows that concentration of calcium was higher in sera from the mixed arterio-venous blood of the two human foetuses than in the corresponding maternal sera, but the latter were rather low compared with normal values for adults. A depression of maternal serum calcium, especially during the latter half of pregnancy, has been repeatedly described (Bodansky & Duff, 1936; Harding, 1925; de Wesselow, 1922; Ramsay, Thierens & Magee, 1938, and Mull, 1936*a, b*). According to Mull (1936*a*) the calcium concentration in the foetal serum, although always higher than that in the maternal serum, is determined by the latter. This relationship between mother and foetus has been reported also in later pregnancy and at full term (Aburel & Ornstein, 1930; Bogert & Plass, 1923; Duckworth, 1942; Miletto & Gerli, 1947; Smith, 1951). Hallman & Salmi (1953) added the information that the concentration of calcium in serum taken from the umbilical vein was greater than that in serum from the artery. This

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had previously been reported for cattle by Matsuo (1933). The serum calcium of the pregnant rabbit was normal for this animal, in which it may be high even in the non-pregnant individual (Schmidt & Greenberg, 1935) and the figures for the foetus agree with those of Matsuo (1933). The current findings indicate that at the twenty-first day of pregnancy the concentrations of calcium were not quite so high in mixed cord blood as they were in maternal serum, but Matsuo (1933) found them higher. The concentration of calcium in the mixed cord serum of the foetal pig at 46 days from conception was certainly high by comparison with values accepted as normal for humans and adult pigs, for the average was nearly 18 as against about 11 mg./100 ml. in the corresponding maternal serum.

Magnesium, phosphorus and solids. The concentrations of magnesium in human sera were within the range usually found. Bogert & Plass (1923) and Zaharesco-Karaman, Alexiu & Ursu (1936) had previously found the concentration of magnesium in foetal serum in late pregnancy to be similar to, or higher than, that in maternal serum. The present findings are too few and too variable to allow any firm statement to be made about early pregnancy. The concentrations of magnesium in foetal and maternal sera of the rabbit were notably high, and both in the pig and rabbit the serum magnesium was higher in the foetus than in the mother. The figures for phosphorus are few in number, but the high figures in all three foetal sera are in accordance with the findings of previous investigators (Needham, 1931). The information about solids

reflects what was already known about proteins (Widdowson & McCance, 1956), and unless the serum proteins on the foetal side of the placenta differ in binding capacity for calcium and magnesium from those on the maternal side there are some problems to be solved about ionization and solubility in the foetal extracellular fluids.

Skeletal muscle

Table 1 summarizes the results; those for the adults are of the same order as those reported by many previous investigators. The figures for the foetal tissues all differ in similar respects from those for adults of the same species. Thus the calcium concentrations in fetuses were up to five times those in adults. Magnesium and phosphorus were only about half as great and the solids were much lower. Few previous investigators have interested themselves in these tissues, but Sheldon & Ramage (1931) commented on the weak spectral lines given by the magnesium in foetal muscle.

Amniotic and allantoic fluids

Table 2 contains the results which have been obtained for the solids, calcium, magnesium and inorganic phosphorus in the amniotic fluids of the human foetus at three stages of pregnancy, for the pig at 46 days after copulation and for the rabbit at 21 days. The fluids have been analysed by numerous investigators at various stages of pregnancy. The present figures are in general agreement with those of Abdine, Ghalioungui & El Ridi (1954); Cantarow, Stuckert & Davis (1933);

Table 1. *Concentrations of solids, calcium, magnesium and phosphorus in the serum and skeletal muscle of man, pig and rabbit*

The number of observations and their range follow each (average) figure.

Species and age (days)	Serum				Muscle			
	Solids (g. %)	Ca (mg./100 ml.)	Mg (mg./100 ml.)	Inorganic P (mg./100 ml.)	Solids (g. %)	Ca (mg./100 g. fresh tissue)	Mg (mg./100 g. fresh tissue)	Total P (mg./100 g. fresh tissue)
Human								
Adult	8.1 [2] (8.0-8.2)	8.08 [2] (8.05-8.1)	2.21 [2] (2.12-2.3)	3.8 [1]	23.8 [4] (22.6-25.6)	5.64 [4] (4.75-6.63)	22.7 [4] (19.3-27.3)	202 [4] (185-210)
Foetus (84-182)	4.0 [2] (3.9-4.1)	8.99 [4] (7.9-10.5)	2.77 [4] (1.89-4.3)	14.9 [1]	12.3 [9] (8.7-20.1)	13.3 [8] (6.7-23.7)	11.7 [9] (4.99-15.7)	113 [9] (66.6-147)
Pig								
Sow	8.68 [4] (7.9-9.8)	11.2 [8] (9.8-12.1)	2.60 [8] (2.2-4.42)	7.12 [6] (5.92-9.8)	25.2 [8] (23.7-26.7)	4.6 [8] (3.54-6.55)	26.0 [8] (22.4-28.3)	218 [8] (200-231)
Foetus (46)	3.8 [2] (3.0-4.6)	17.6 [4] (16.8-18.4)	3.79 [5] (3.54-4.2)	13.6 [1]	8.4 [7] (8.1-8.7)	14.7 [5] (11.9-17.1)	10.4 [7] (8.5-14.9)	84.9 [7] (73.5-92.7)
Rabbit								
Doe	8.13 [3] (8.0-8.3)	15.7 [4] (12.0-17.5)	5.47 [4] (4.1-6.6)	9.39 [2] (9.07-9.7)	24.6 [4] (23.4-25.4)	4.76 [4] (4.28-5.05)	29.2 [4] (28.2-30.5)	237 [4] (209-296)
Foetus (21)	2.6 [2] (2.5-2.7)	13.0 [3] (10.5-14.3)	6.93 [3] (6.3-8.0)	14.4 [1]	9.95 [4] (9.6-10.2)	25.5 [4] (18.7-32.6)	13.8 [4] (12.3-14.7)	152 [4] (137-162)

Davies & Routh (1957); Matsuo (1933); Makepeace, Fremont-Smith, Dailey & Carroll (1931); Paton, Watson & Kerr (1908-9); Shrewsbury (1933). Uraga-Imaz & Gascon (1950) and Uyeno (1919) found much less magnesium, but their technical methods may have been at fault. The amniotic fluid of dogs (Childs & Eichelberger, 1942), cows (Abdine *et al.* 1954; Döderlein, 1890; Matsuo, 1933; Paton *et al.* 1908-9; Sato, 1938), goats (Abdine *et al.* 1954) and sheep (McDougall, 1949; Malan, Malan & Curson, 1937; Paton *et al.* 1908-9) has also been examined. By neglecting isolated findings it seems reasonable to say that there do not seem to be large variations from one species to another in the composition of amniotic fluid, or from one stage of pregnancy to the next.

Table 2 also gives the solids, calcium, magnesium and inorganic phosphorus in the allantoic fluid of the pig at 19-21 days and 46 days after mating, and of the rabbit at 21 days. The allantoic fluids of the pig were analysed incompletely at unstated times during gestation by Paton *et al.* (1908-9) and reported to contain 17 mg. of calcium and 2 mg. of magnesium/100 ml. The stage of gestation is clearly important in the pig, not only for calcium, but also for sodium, chloride and potassium (McCance & Dickerson, 1957). Childs & Eichelberger (1942) studied the dog, but only at a late stage of pregnancy, and found 7.0 and 3.5 mg./100 ml. for calcium and magnesium respectively. The stage of pregnancy may make a big difference to the composition of the allantoic fluid in the sheep, for McDougall (1949) found over 20 mg. of

calcium and less than 0.1 mg. of phosphorus in two fluids at 84 days as against about 11 mg. of calcium and 12 mg. of inorganic phosphorus at 28 days. Malan *et al.* (1937), however, did not find such a difference. The present figures for the rabbit agree with those of Davies & Routh (1957) and of McCance & Dickerson (1957).

The presence of so much calcium and inorganic phosphorus in the allantoic fluid of the rabbit at 21 days and in that of the pig at 46 days raised the question of how the calcium was maintained in solution. Determinations of citrate were therefore made in pig fluid and the concentrations compared with those of calcium. The results are shown in Fig. 1, and indicate that the high concentrations of calcium were accompanied by concentrations of citrate quite large enough to play a major part in maintaining them in solution. The correlation coefficient between the calcium and the citric acid in allantoic fluids of the pig was 0.79 ($P < 0.01$). The only previous work to be found on the citric acid in embryos concerns the amounts in whole bodies. Dickens (1941) found these to range between 12.4 and 16.2 mg./100 g. in mice and between 9.4 and 31 mg. in chicks.

There was a wide spread in the concentrations of both magnesium and phosphorus in the allantoic fluids of the pig, and Fig. 2 demonstrates the relationship which has been found between them. The correlation coefficient was 0.83 ($P < 0.01$). The slope of the best line drawn through the points calculated by the method of least squares and the points calculated by the method of least squares

Table 2. *Concentration of solids, calcium, magnesium and inorganic phosphorus in the foetal fluids of man, pig and rabbit*

The number of observations and their range follow each (average) figure. Standard deviations have been given where the numbers justified them.

Species and foetal age (days)	Amniotic fluid				Allantoic fluid			
	Solids (g. %)	Ca (mg./100 ml.)	Mg (mg./100 ml.)	Inorganic P (mg./100 ml.)	Solids (g. %)	Ca (mg./100 ml.)	Mg (mg./100 ml.)	Inorganic P (mg./100 ml.)
Human								
105	1.3 [1]	7.7 [1]	1.7 [1]	3.8 [1]	—	—	—	—
119-126	1.3 [2] (1.2-1.4)	7.35 [2] (7.2-7.5)	1.64 [2] (1.42-1.87)	—	—	—	—	—
At term	1.26 [8] (0.92-1.7)	8.0 [8] (5.4-12.6)	0.95 [8] (0.54-1.4)	2.02 [5] (1.28-2.46)	—	—	—	—
Pig								
19-21	—	—	—	—	1.1 [11] (0.16-1.73) ±0.42	5.83 [18] (3.3-8.9) ±1.42	1.75 [15] (1.1-2.14) ±1.3	8.56 [28] (4.1-13.2) ±1.98
46	1.38 [21] (1.25-2.17) ±0.19	9.69 [25] (8.05-12.0) ±0.87	1.8 [24] (1.23-2.25) ±0.22	4.04 [30] (1.58-5.98) ±0.89	1.69 [22] (1.0-3.2) ±0.64	29.7 [27] (18.7-46.5) ±7.49	1.05 [27] (0.22-1.56) ±0.57	5.92 [35] (3.09-11.7) ±1.9
Rabbit								
21	1.21 [2] (1.21-1.21)	10.9 [10] (7.0-15.9)	3.74 [10] (2.72-6.26)	3.5 [1]	1.2 [3] (1.1-1.3)	26.2 [5] (11.5-47.1)	22.6 [5] (9.8-37.5)	5.9 [1]

and the point where it cuts the abscissa suggests that after the concentration of phosphorus rose above 2.5 mg./100 ml., approximately 1 atom of magnesium accompanied every 2.5 atoms of phosphorus. The relationship between magnesium and phosphorus in the pig's amniotic fluid was somewhat different, and there was less variation in the concentration of either element. The atomic ratio of the total magnesium to the total phosphorus was about 1:1.7 (Table 2).

Membranes

Table 3 gives the quantities of solids, calcium, magnesium and total phosphorus in various foetal membranes of man, pig and rabbit. Figures are

Fig. 1. Relationship between calcium and citric acid in the allantoic fluid of the pig. x, Allantoic fluid at 46 days; ●, allantoic fluid at 19-21 days.

Fig. 2. Relationship between magnesium and inorganic P in the allantoic fluids of the pig. x, Allantoic fluid at 46 days; ●, allantoic fluid at 19-21 days.

also given for the endometrium of the pig at two stages of pregnancy, for Wharton's jelly from the inner surface of the amnioallanto chorion at the forty-sixth day, and for some umbilical cords. No published figures for magnesium and phosphorus have been found, and the only analytical information about calcium concerns human membranes and cord near term. The present figures for the calcium concentrations in such membranes are much lower than those reported by Burg & Csabay (1930) unless these authors expressed their findings in terms of 100 g. of ash. If they did, the present findings agree with theirs. Our figures agree with those of Lévy, Sapir, Walter, Vellay & Mignon (1951), who found 6-18 mg. of calcium/100 g. of fresh cord tissue.

The figures for the pig indicate that there was a considerable build-up of calcium in these membranes between the twentieth and forty-sixth days of pregnancy. At first there was no significant difference between the endometrium and the membranes, but later the membranes contained much more than the endometrium. A certain amount of histochemical work has been done previously on this animal. Höpping (1937) found a deposition of calcium in the foetal membranes of the pig beginning at 32-35 days and ending at 90-100 days of foetal age. This deposition occurred regularly, and it was diffuse throughout the allanto chorion. The calcium was laid down mainly in the 'ground substance' but also inside the cells and around the vessels. Höpping (1937) considered that it constituted a depot for foetal needs. Wislocki & Dempsey (1946b), using the Bodian protargol reaction, found calcium in the cells of the chorionic villi, chorionic fossae and the stroma of the chorion adjacent to the allantoic membrane of the pig. They stated that calcium was deposited throughout early and middle pregnancy, but that it later disappeared. They thought that the process in the pig was a physiological one, probably associated with foetal ossification. On the contrary, however, they considered that the calcification in the placenta of rodents, cats and human beings was an aspect of ageing (Wislocki & Dempsey, 1945, 1946a; Wislocki, Deane & Dempsey, 1946). Wislocki & Dempsey (1946b) also found that the uterine glands of the pig gave strong reactions for calcium.

Brambell (1933) also described calcium deposits infiltrating the foetal membranes of the pig between the allantois and the chorion. He regarded them as storage places for excess of salts not used by the foetus. The calcification began at about 35 days and rose to a peak at 35-55 days of gestation.

The present results support in a very general way these earlier findings. Note, however, the high

Table 3. *Concentration of solids, calcium, magnesium and total phosphorus in the foetal membranes and adjoining tissues of man, pig and rabbit*

Figures denote the average and the range.

Species and foetal age (days)	Tissue	Solids (g. %)	Ca (mg./100 g. of tissue as dissected)	Mg (mg./100 g. of tissue as dissected)	Total P (mg./100 g. of tissue as dissected)	No. of estimations
Human						
105	Amniochorion	9.6	143	8.2	101	1
182	Amniochorion	13.6	21.5	6.9	109	1
At term	Amnion	10.4 (3.9-22.9)	18.3 (7.5-41.0)	2.6 (1.45-4.8)	47.3 (12.7-115)	12
	Chorion	14.8 (14.3-15.8)	14.2 (13.4-15.6)	7.2 (5.9-7.9)	101 (74.5-120)	5
	Amniochorion	14.3 (11.3-16.6)	14.2 (10.2-19.9)	8.3 (5.8-14.6)	90.4 (67.7-113)	5
Pig						
19-21	Endometrium	10.0	9.3	5.5	61.0	1
	Allantochoion	4.27 (3.15-5.4)	9.95 (9.8-10.1)	5.4 (3.95-6.8)	68.0 (44.0-92.0)	2
46	Endometrium	13.3 (12.9-13.7)	10.6 (8.8-12.4)	6.9 (6.8-7.0)	115 (108-122)	2
	Amnioallantochoion	5.6 (5.3-6.05)	123 (67.0-164)	8.4 (1.8-11.8)	115 (87.0-140)	4
	Amnion	5.6	26.0	5.4	46.4	1
	Allantochoionic tips	15.5 (12.9-17.3)	46.2 (26.8-58.4)	3.1 (2.54-3.5)	99.1 (25.6-170)	5
At term	'Placenta'	7.07 (6.34-7.74)	60.8 (44.5-68.6)	9.2 (8.95-11.3)	68.0 (62.3-76.0)	4
46	Wharton's jelly	2.7 (2.25-3.34)	17.9 (15.2-20.7)	3.5 (3.3-3.8)	21.3 (18.1-27.8)	4
	Cord	5.4 (5.2-5.7)	15.8 (13.5-17.4)	7.4 (5.5-8.9)	44.6 (35.0-54.0)	3
At term	Cord	10.1 (7.8-12.5)	20.0 (14.6-25.5)	9.0 (6.5-11.6)	—	2
Rabbit						
21	Inverted yolk sac	16.6 (14.1-18.9)	28.3 (20.9-37.4)	14.9 (12.2-18.7)	179 (158-211)	3
	Amnion	7.7 (6.9-8.8)	99.7 (98.0-101)	17 (15.8-18.2)	84.4 (79.7-89.0)	2
	Allantois	4.7 (2.7-6.9)	28.1 (18.5-35.9)	12.7 (7.5-19.7)	44.0 (39.1-47.7)	4

concentration of calcium at the twenty-first day of pregnancy in the rabbit's amnion, which is not as in humans in contact with chorion. The rabbit's amnion appears to differ considerably in chemical composition, but not in total solids, from the human amnion at term. Other points in Table 3 which seem to justify mention are the following. (a) All the results require consideration in terms of total solids, for the latter varied considerably and unpredictably. Contrast, for example, the findings for the pig's allantochoionic and amnioallantochoionic structures. The large amount of solid matter in the inverted yolk sac of the rabbit was probably partly responsible for the high concen-

trations of calcium, magnesium and phosphorus in it. (b) Calcium and phosphorus varied more or less independently. (c) There was a high concentration of magnesium in the amnion of the rabbit, and a very low one in the human amnion at term. These differences were not accompanied by similar differences in the amounts of solids, but they were accompanied by corresponding differences in the concentration of magnesium in the amniotic fluids and sera, those of the rabbit having high and those of the human low values. (d) The figures for Wharton's jelly were unlike those for any of the membranes. The concentration of calcium, however, was much the same as in the cord.

DISCUSSION

Skeletal muscle

The tissue analysed consisted of a mixture of intracellular and extracellular material. These differ in chemical composition, and adult muscle contains less extracellular material than foetal muscle. It is safe to say that the low concentrations of magnesium and phosphorus in foetal muscle were partly due to this, for extracellular fluid at all ages always contains much less of these two elements than skeletal-muscle cells. Figures for the composition of extracellular fluid can be derived from an analysis of serum; and by making certain assumptions about the exclusively extracellular position of chlorides it is possible to derive the composition of the cells. This is less exact, however, for calcium, because some of the calcium in the serum is bound to protein, and consequently the concentration in the interstitial fluids within the muscle may be expected to be lower than that in the serum. How much lower is roughly known for the adult but not for the foetus, and the differences in the relative concentrations of the various proteins in the serum make it difficult to approximate. When, however, it was assumed that all the chloride in the muscle samples was extracellular and that the extracellular fluids of the foetus contained as great a concentration of calcium as the serum, whereas those of the adult contained only 50 % as much, the figures shown in Table 4 were obtained. If the figures given by Toribara, Terepka & Dewey (1957) for ultrafiltrable calcium in adult human serum are correct, 50 % is perhaps too low a figure to have taken, but some of the calcium in the foetal serum must be bound to proteins and no allowance has been made for this. The calcium values for the cells are probably the lowest which it is reasonable to take, and it is probable that, unless calcium is bound in some insoluble form outside the muscle cells of the foetus, the foetal-muscle cells contain more, possibly considerably more, calcium than those of adults. This

may be explained by the observation that much of the calcium within the cell is in the nucleus (Scott, 1932; Gulick, 1944; Chirman, 1946) and the histological indications are that the skeletal-muscle cells of the foetus contain relatively more nuclei and less cytoplasm than those of the adult.

When the concentration of magnesium within the cells was calculated on similar assumptions, it was found that the cells in foetal muscle contain at least as much magnesium as those in adult muscle.

Membranes

The high concentrations of calcium at certain stages of pregnancy invite further investigation, as do the changing concentrations of magnesium and phosphorus, although these may not be so great (cf. results from the pig). There is reason to suppose that the high concentrations of calcium are functional (Höpping, 1937; Wislocki & Dempsey, 1946b), but there is little information as to its state of combination. This subject requires reinvestigation by new techniques in the light of the association between calcium and citric acid in the fluids. Pathological deposits of calcium are often finite granules composed of calcium and phosphorus in a ratio of about 2:1 by weight. These are visible microscopically and often radiologically as, for instance, are the deposits in the placenta in the late stages of human pregnancy (Dawson, 1953; Fleming, 1943; Linsman & Chalek, 1952; Masters & Clayton, 1940; Simon, 1951; Teacher, 1935; Wehefritz, 1925), which have been stated to be a hereditary characteristic (Solomons & Falkner, 1946; Solomons, 1949). X-rays do not show up the calcium in the membranes or in the placenta in the early and mid stages of pregnancy, when this organ contains about 28 mg./100 g. of fresh material (Masters & Clayton, 1940).

From the figures given in Table 3 it is clear that there is always enough phosphorus to provide both for deposits in a 2:1 ratio and for its other functions in the cell, but there is no evidence that calcium is so deposited.

Table 4. *Concentrations of calcium and magnesium to be expected within the cells of adult and foetal skeletal muscle*

It is assumed that 50 % of the calcium and 60 % of the magnesium in adult serum and 100 % of the calcium and magnesium in foetal serum was ultrafiltrable and that all chlorides were extracellular.

Species and foetal age (days)	Calcium (mg./100 g. of cells)		Magnesium (mg./100 g. of cells)	
	Foetus	Adult	Foetus	Adult
Human				
105-154	20.4 (12.8-44.4)	5.7 (4.6-6.9)	25.9 (14.2-33.2)	26.6 (23.8-31.5)
Pig				
43-46	9.6 (4.0-17.9)	4.3 (3.2-6.6)	26.0 (18.3-46.4)	30.1 (25.9-33.3)

Serum and fluids

Although the concentration of calcium in the foetal serum was found to be higher than that in the maternal serum only at 46 days' gestation in the pig, it must be accepted from the literature on the rabbit and other animals that this is quite a usual finding during foetal life. Determinations of citrates in foetal serum up to date do not suggest that they play much part in maintaining this calcium in solution. The concentration of magnesium in foetal serum may also frequently exceed that in maternal serum, and the concentrations of calcium and magnesium in the other foetal fluids are often so different from those in either the foetal or maternal serum that these differences must be created and maintained by active cellular metabolism. A case has been made out for supposing that the allantoic fluid of the rabbit is largely the product of the foetal mesonephros (Davies & Routh, 1957), but until some functional studies of this organ have been made it will be impossible to be sure. The allantoic fluid of the pig, at least in the earlier stages of its development, is probably formed in some other way (McCance & Widdowson, 1954), but, even if the organ concerned and its location were known, the problem would have only been defined.

SUMMARY

1. Calcium, magnesium and phosphorus have been determined in the serum, skeletal muscle, membranes and fluids of the rabbit at 21 days' gestation, of the pig at 19 and 46 days, and of the human foetus at several stages of gestation.

2. In the foetal muscles the concentrations of calcium were higher, and of magnesium and phosphorus lower, than in those of adults. The calcium was probably intracellular.

3. Foetal serum may contain more calcium, magnesium and inorganic phosphate per unit volume than the maternal serum.

4. The amniotic membranes and the allantoic fluids may contain high concentrations of calcium.

5. When there was much calcium in the allantoic fluid of the pig it was accompanied and probably maintained in solution by high concentrations of citrates.

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Phosphorylserine and Cerebral Phosphoprotein

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It has been shown previously that the phosphoprotein fraction of brain is implicated in the rapid changes in energy-rich phosphates which occur when electrical pulses are passed through cerebral slices *in vitro* (Heald, 1957a). This result was obtained when the cerebral tissues were initially fixed in trichloroacetic acid and the phosphoprotein fraction was determined by the method of Strickland (1952). Further work, in which brain slices were first fixed in a lipid extractant and later extracted with dilute alkali, gave no clear indication of the presence of such a phosphoprotein fraction in the tissue (Heald, 1957b). It was suggested that possibly the phosphoprotein, as originally measured, was a stable acid-degradation product of a more labile phosphate which was split to yield inorganic phosphate under alkaline conditions. To pursue this work it seemed necessary to try to characterize the phosphoprotein by means of a specific component and also to try to resolve the discrepancy mentioned above.

This paper describes experiments showing that the phosphoprotein fraction, when obtained from cerebral tissues which had first metabolized radioactive phosphorus and were subsequently fixed in trichloroacetic acid, contains radioactive phosphorylserine. When this work was almost completed the author became aware of a paper of Vladimirov, Ivanova & Pravdina (1956) also describing the isolation of phosphorylserine from

brain. Identification of the product by these authors does not appear to have been entirely unequivocal.

MATERIALS AND METHODS

Tissues and media. Slices of guinea-pig cerebral cortex were incubated with radioactive phosphate in media containing glucose and buffered with 2-amino-2-hydroxy-methylpropane-1:3-diol (Heald, 1956b). Generally six slices of 100–150 mg. wet wt. were obtained from each cortex. For short-term experiments, in which electrical pulses were applied for brief periods, the apparatus and techniques used were those described previously (Heald, 1956b). In such experiments, since the radioactivity of the phosphorylserine finally isolated was low, slices from two or three animals were pooled for each experiment. In all cases slices from one cerebral hemisphere served as controls for slices from the other hemisphere of any one animal.

Longer experiments, in which slices were incubated with radioactive phosphate for up to 90 min., were carried out in conventional manometric apparatus. All incubations were at 37.5° in an atmosphere of O₂.

Electrical pulses. These were condenser pulses supplied by the apparatus of Ayres & McIlwain (1953) at 50 cyc./sec. and were of 15 v peak potential and a duration of 0.4–0.5 m-sec.

Radioactive phosphate. This was obtained as carrier-free orthophosphoric acid and was treated as described by Ennor & Rosenberg (1954) before use.

Preparation of the phosphoprotein fraction. After incubation, tissues were fixed either in 10% (w/v) trichloroacetic acid or in CHCl₃-methanol (2:1, v/v). From tissues