

Severe undernutrition in growing and adult animals

4.* The impact of severe undernutrition on the chemical composition of the soft tissues of the pig

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The outstanding changes that take place in the chemical structure of the body of an undernourished man or animal are a loss of fat and protein and a gain in the proportion of water. Bischoff & Voit (1860) commented on this situation 100 years ago, and Voit (1881) wrote along the same lines again 20 years later. He stated that the composition of the body was not fixed and invariable, and that the tissues of the very young and of the undernourished contained more water than those of normal adults. This was partly because the undernourished body contained less fat than the well-nourished one, but Voit also noted that the undernourished muscles contained more water than well-nourished ones. Mendel & Rose (1911–12) described some experimental work on rabbits in which they showed that during starvation the percentage of water in the muscle increased from 75 to 79. Lipschütz found that the same sort of changes took place in the muscle of undernourished carp (Lipschütz, 1911), and also of eels (Lipschütz, 1915). Terroine, Brenckmann & Feuerbach (1922–3) and Terroine, Feuerbach & Brenckmann (1924) considered that their own experiments showed that the composition of the fat-free body of an adult mammal was almost unalterable by undernutrition or by high- and low-protein diets, and that any changes detected by analysis were due to fluctuations in the fat and carbohydrate reserves, but at almost the same time the evidence that the undernourished body and its tissues contained an excessive amount of water was again summarized by Morgulis (1923).

Later it was shown that the water retained in undernutrition is accompanied by sodium (Weech, Snelling & Goettsch, 1933), and investigations made on man during and after the Second World War made it clear that in chronic undernutrition there is a great increase in the percentage of the body occupied by extracellular fluid (Cachera & Barbier, 1943; Henschel, Mickelsen, Taylor & Keys, 1947; Beattie, Herbert & Bell, 1948; Widdowson & McCance, 1951). Undernutrition increases the proportion of extracellular fluids in mammals other than man (Kerpel-Fronius & Kovach, 1948), and the demonstration that it does so in muscle as well as in the body as a whole (Dicker, 1949; Waterlow & Mendes, 1957; Frenk, Metcoff, Gómez, Ramos-Galván, Cravioto & Antonowicz, 1957) rings back to the observations of Voit (1881).

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Many of the observations cited above were made on men or animals that were already mature or nearly mature when undernutrition began. It is well known that underfeeding a young child or animal retards its growth (Waters, 1908, 1909; Aron, 1911; Drummond, 1916; Jackson, 1932), but few studies have included investigations of the composition of the organs and tissues. Lowry, McCay, Hastings & Brown (1942) analysed the skeletal muscle of rats which were about one-third of normal size after being underfed from weaning for 611 or 752 days, and concluded that 'retardation results in the maintenance in muscle of youthful muscle fibres and a youthful proportion of fibres and extracellular tissue'. More recently, Waterlow & Mendes (1957) and Frenk *et al.* (1957) have analysed muscle obtained by biopsy from young children who were both undernourished as regards calories and malnourished as regards protein; they found that the proportion of water and sodium was high before treatment and fell as the infants recovered.

The retardation of growth brought about in the pigs described by McCance (1960) was greater than that of the children or of Lowry *et al.*'s (1942) rats, and was therefore expected to produce considerable differences in the composition of the body and of skeletal muscle. How much the composition of the 'vital' organs might be affected was less certain. Some of the pigs described by McCance (1960) provided the material for the present paper, which is concerned with the effects of severe undernutrition of young animals and of its relief on the composition of skeletal muscle, skin, heart, liver, kidney and brain. The composition of muscle from undernourished cockerels has already been described (Dickerson & McCance, 1960), and it is important in considering any of this work to remember that the deficiency has been one of all the dietary constituents except water. The results should not be expected to be exactly the same as those found after specific, single, deficiencies such as those of protein, or in kwashiorkor (Frenk *et al.* 1957; Stanier, 1957; Widdowson & McCance, 1957; Waterlow & Mendes, 1957; Mendes & Waterlow, 1958; Smith & Waterlow, 1960). Nor should they be expected to be identical with those produced by underfeeding adult animals.

EXPERIMENTAL

Tissues have been analysed from eight pigs whose growth was retarded by severe undernutrition so that they weighed 3.4–13 kg at the age of 5–15 months (McCance, 1960), and skeletal muscle alone from four pigs whose growth was similarly retarded, and which were then given a plentiful supply of food so that they gained weight rapidly. Seven of the undernourished pigs had died suddenly and unexpectedly during the night. Death may have been due to hypoglycaemia (Lhermitte & Sigwald, 1942) or cold (McCance, 1960), or to some mild infection that would have passed unnoticed in normal pigs, but no cause of death was found at post-mortem examination, which was always carried out in the laboratory first thing the following morning. The eighth undernourished pig was killed with pentobarbitone sodium (60 mg/kg body-weight). Two of the four animals that were undernourished and then allowed to grow were also killed at the laboratory with pentobarbitone sodium; the others were killed at the slaughterhouse and they therefore lost a large amount of blood before

death, which has been shown to affect the composition of skeletal muscle (Widdowson & Southgate, 1959). Small samples of blood were removed for analysis from the other pigs that were killed, but this operation probably did not make any significant difference to the composition of their muscle.

As in other aspects of this study of undernutrition two kinds of controls have been used. One consisted of well-nourished pigs of the same body-weight as the undernourished animals, and these were of a much younger age; the others were animals of the same age but a much greater body-weight. The 'weight' controls were killed at the laboratory with pentobarbitone sodium, but all but one of the 'age' controls were killed at the slaughterhouse, as were two of the rehabilitated animals, and they again

Table 1. *Description of the experimental pigs whose tissues were analysed*

Group no.	Pig		Undernourished			
	No.	Sex	Age at beginning of undernutrition (weeks)	Weight at beginning of undernutrition (kg)	Age at death (months)	Weight at death (kg)
1	1	♀	1.5	3.0	10	3.6
	2*	♂	1.5	3.2	6	3.4
2	3†	♂	2	4.2	14	8.0
	4†	♀	2	4.0	10	6.7
	5	♀	3	5.8	15	12.7
3	6‡	♀	4	7.7	8	10.5
	7‡	♂	4	5.5	8	12.3
	8	♂	5	9.6	5	13.1

Pig	Undernourished then rehabilitated					
	No.	Sex	At end of period of undernutrition		At end of period of rehabilitation	
			Age (months)	Weight (kg)	Age (months)	Weight (kg)
9*	♂	9	4.4	13	36.5	
10†	♀	14	8.0	23	209	
11	♀	12	6.1	16	51.0	
12	♂	12	6.2	16	55.5	

*†‡ Litter-mates.

lost much blood. In presenting the results for muscle, comparisons have been made between animals that were killed in a similar way. The tissues of these well-nourished control animals formed part of the material used to study the effect of development on the composition of soft tissues (Dickerson & Widdowson, 1960; Widdowson & Dickerson, 1960).

The undernourished animals fell naturally into three groups. The first consisted of two that weighed 3.4 and 3.6 kg. They lived 6 and 10 months. The 'weight' controls for these two consisted of two well-nourished piglets aged 2-3 weeks, weighing 3.5 and 3.7 kg. Two of the three animals in the second group were maintained at a body-weight of 6-8 kg for 10 and 14 months and the third lived for 15 months and never

weighed more than 12.7 kg. The third group included the three animals with which this investigation began, which were maintained at a body-weight of 10–13 kg for 5–8 months. A well-nourished piglet reaches a weight of 7 kg in 4 weeks and 12 kg in about 6 weeks, and the 'weight' controls used for these undernourished animals consisted of two aged 4 weeks weighing 7 kg, and one aged 6 weeks weighing 12 kg.

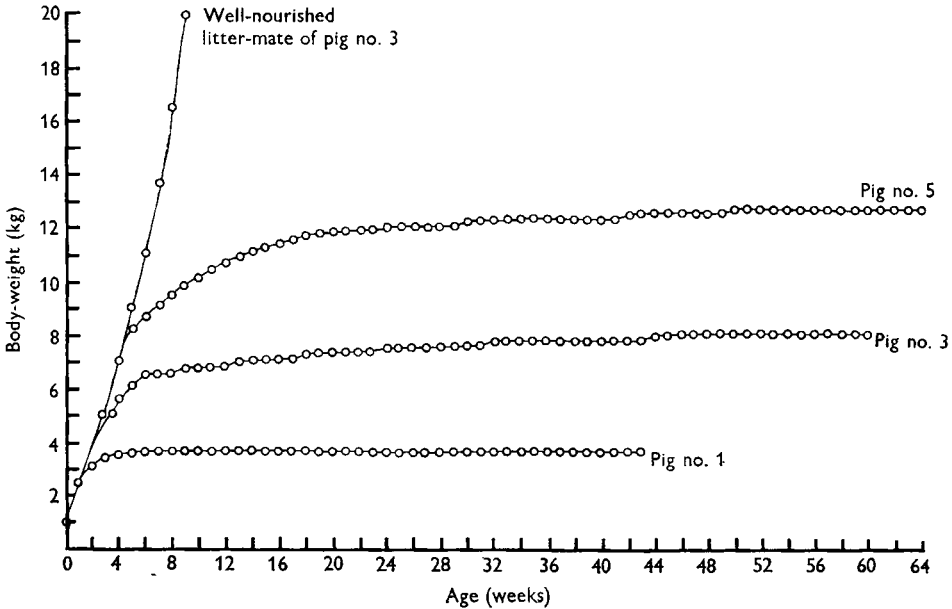


Fig. 1. Body-weights of three undernourished pigs and one well-nourished litter-mate.

Table 1 sets out the history of the undernourished pigs, the length of the period of undernutrition and the weights of the animals at death, and Fig. 1 illustrates the changes in body-weight of one in group 1 and of two in group 2. It must be emphasized that the undernourished animals gained very little weight after the food intake was reduced, which was always during the suckling period. They had never weighed more than their weight at death.

The sampling of the organs and tissues, and the chemical determinations were made as described by Dickerson & Widdowson (1960) and Widdowson & Dickerson (1960). Iron in the livers was determined by means of thioglycollic acid (McCance, Widdowson & Shackleton, 1936). Calculations of the distribution of water in skeletal muscle and skin and of the relationships of the various cellular constituents to each other were made as described by Dickerson & Widdowson (1960) and Widdowson & Dickerson (1960).

RESULTS

Skeletal muscle

Effect of undernutrition. The effect of normal development on the composition of pig muscle has been described elsewhere (Dickerson & Widdowson, 1960), and may be summarized as follows. Postnatal development is associated with a fall in the pro-

portion of total water, and in that of the extracellular electrolytes sodium and chloride. These changes are accompanied by an increase in the amount of total nitrogen and in the amounts of the intracellular proteins of the sarcoplasm and fibrils. At the same time, the proportion of extracellular protein falls. As the cell mass increases, the amounts of the cellular minerals potassium, phosphorus and magnesium also increase, whilst the amount of calcium falls. Calculated values for the composition of the cells indicate that the amount of protein N per unit of water increases whereas the amount of total intracellular cations per unit of protein N decreases.

Table 2. *Effect of undernutrition on the composition of the skeletal muscle of pigs*

(Mean values expressed per kg of fresh muscle)

Constituent	Undernourished			Well-nourished	
	Group 1 (6 and 10 months, 3·4 and 3·6 kg) (2 pigs)	Group 2 (10-15 months, 6·7- 12·7 kg) (3 pigs)	Group 3 (5-8 months, 10·5- 13·1 kg) (3 pigs)	'Weight' controls (18-42 days, 3·5-12·3 kg) (5 pigs)	'Age' control (‘adult’, c. 200 kg) (1 pig)
Water (g)	838	828	817	784	735
Total N (g)	21·9	23·7	28·6	27·8	31·1
Non-protein N (g)	1·8	2·6	—	3·9	4·0
Sarcoplasmic protein N (g)	1·6	4·4	—	5·0	7·6
Fibrillar protein N (g)	6·9	13·1	—	16·2	18·7
Extracellular protein N (g)	14·4	3·6	—	2·5	1·0
Na (m-equiv.)	84·5	61·2	44·2	30·0	24·2
K (m-equiv.)	52·5	73·7	87·9	106	92·5
Cl (m-equiv.)	63·8	43·6	32·4	26·9	21·1
P (m-moles)	44·5	48·5	53·5	76·2	68·0
Mg (m-equiv.)	11·5	13·0	14·6	19·6	23·2
Ca (m-equiv.)	7·7	2·6	2·4	2·4	2·3
	Derived values*				
Chloride space (g)	527	360	268	222	174
Intracellular water (g)	311	468	549	562	561
Intracellular protein N (g/kg intracellular water)	18·3	37·4	—	37·7	47·0
Total intracellular cations (m-equiv./g intracellular protein N)	12·2	5·4	—	5·9	4·4

* See p. 460.

Table 2 shows the composition of skeletal muscle from the three groups of undernourished pigs, and of that from the well-nourished ‘weight’ and ‘age’ controls. As already stated, the weights of the animals which comprised the ‘weight’ controls corresponded to those of the undernourished animals and varied from 3·5 to 12 kg. The composition of their muscle was, however, sufficiently similar for it to seem justifiable, for the sake of simplicity, to average the figures. The last column shows values for a well-nourished ‘adult’ pig of approximately the same age as the undernourished ones were at death.

Comparison of the undernourished animals with the 'weight' controls shows that underfeeding caused an increase in the amount of water and a decrease in the amount of total N per kg of muscle. The values for the nitrogenous fractions show that there was a change in the distribution of N, for the non-protein fraction and the two intracellular protein fractions, those of the sarcoplasm and fibrils, were reduced to a greater extent than the total N, whereas there was a large relative increase in the amount of extracellular protein. There was also a change in the distribution of the water, for the extracellular electrolytes, Na and Cl, were higher, whereas the intracellular constituents, K, P and Mg, were lower. The concentration of Ca was higher. Thus, the changes induced by underfeeding in the composition of muscle were the opposite of those that take place during normal growth, and the composition of the muscle was similar to that normally found in animals even younger than the 'weight' controls. For instance, the concentration of Cl, and hence in all probability the volume of extracellular fluid, was considerably larger in the muscle of the undernourished pigs in group 1 than it was in that of pig foetuses at 90 days' gestation (Dickerson & Widdowson, 1960).

Table 3. *Composition of skeletal muscle from rehabilitated pigs*

(Mean values expressed per kg of fresh muscle)

	Unbled before death*		Bled before death*	
	Undernourished then rehabilitated (2 pigs)	Well-nourished adult (1 pig)	Undernourished then rehabilitated (2 pigs)	Well-nourished adult (4 pigs)
Water (g)	737	735	772	749
Total N (g)	29.8	31.1	31.1	35.6
Collagen N (g)	—	1.5	0.8	0.8
Na (m-equiv.)	25.0	24.2	19.4	19.1
K (m-equiv.)	96.5	92.5	110	102
Cl (m-equiv.)	26.6	21.1	14.2	13.2
P (m-moles)	64.2	68.0	71.5	72.5
Mg (m-equiv.)	12.6	23.2	24.0	21.8
Ca (m-equiv.)	2.8	2.3	2.7	2.1

* See p. 458.

The magnitude of the departure from the normal appeared to vary with the body-weight of the animals, and thus with the stage of development at which underfeeding began. This relationship is further shown by the derived values. In the smallest undernourished pigs as compared with the 'weight' controls the ratio of protein N to water in the cells was low and that of total intracellular cations to protein was high. These changes provide evidence that the muscle cells of these animals were overhydrated. No evidence of overhydration was found in the muscle cells of the larger undernourished pigs.

Effect of rehabilitation. Table 3 shows the composition of muscle from pigs which were undernourished for periods varying from 8 to 13 months and then rehabilitated for 4-9 months. The rehabilitated animals have been divided into two pairs according to whether the animals were bled or not before the sample of muscle was taken. The

values for each of the pairs of rehabilitated animals are set out by the side of those for corresponding animals well nourished for the whole of their lives.

The findings indicate that the muscle of the animals that had once been under-nourished still contained more extracellular fluid than that of the well-nourished controls. An unexplained low value for magnesium was found in both the rehabilitated animals that had not been bled, and when the analyses were repeated the same answers were obtained.

Skin

The effect of development on the composition of pig skin has been described by Widdowson & Dickerson (1960) and some of the changes are again evident from Table 4. The later development of skin is associated with a fall in the proportion of water and an increase in the proportion of N. Over the age range of the 'weight'

Table 4. *Composition of the skin of pigs*

(Mean values expressed per kg of fat-free skin)

Constituent	Under-nourished, group 1 (6 and 10 months, 3.4 and 3.6 kg) (2 pigs)	Well-nourished 'weight' controls (18-19 days, 3.5-3.7 kg) (3 pigs)	Under-nourished, group 2 (10-15 months, 6.7-12.7 kg) (3 pigs)	Well-nourished 'weight' controls (4-6 weeks, 6.9-12.3 kg) (3 pigs)	Well-nourished 'age' controls (8-18 months, c. 200 kg) (6 pigs)
Water (g)	813	782	817	778	685
Total N (g)	28.8	36.5	28.0	36.5	53.2
Collagen N (g)	25.4	18.8	20.1	30.9	48.5
Na (m-equiv.)	95.2	90.2	107	86.8	81.6
K (m-equiv.)	39.7	44.5	33.1	41.9	26.2
Cl (m-equiv.)	74.5	74.7	78.5	75.5	74.4
P (m-moles)	25.3	38.8	27.0	34.4	17.2
Mg (m-equiv.)	6.2	7.8	5.7	7.8	4.4
Ca (m-equiv.)	11.9	10.8	9.7	8.4	9.2

Derived values* (as percentage of total N or water)

Collagen N	88.2	51.5	71.8	84.6	91.1
Fibre water	30.0	23.2	23.6	38.0	68.0
Non-fibre water	43.8	56.5	54.2	43.0	23.4
Total extracellular water	73.8	79.7	77.8	81.0	91.4
Cell water	26.2	20.3	22.2	19.0	8.6

* See p. 460.

controls, however, small though it is, the amount of collagen in pig skin increases greatly, both absolutely and relative to the amount of total N, and the distribution of the extracellular water between the fibrous and non-fibrous proteins of the extracellular phase changes accordingly. As the amount of water falls materially during later growth, there is a corresponding rise in the amount of total N and collagen N, but not much change in the ratio between the two. The rapidity with which the changes take place during suckling makes it essential to compare the composition of the skin of the undernourished animals with that of their appropriate 'weight' controls

rather than with mean values for the whole group of 'weight' controls which were satisfactory for skeletal muscle.

Table 4 shows the composition of the skin of the undernourished animals in groups 1 and 2, each set out alongside the corresponding 'weight' controls. The last column again shows the composition of skin from animals of about the same age as the undernourished ones, but which had been well nourished for the whole of their lives. When the animals weighed about 3 kg at the time their food was reduced the amount of collagen/kg skin increased, and it accounted for a greater proportion of the total N than it did in the well-nourished younger animals of the same body-weight. When underfeeding began at a body-weight of 4-5 kg, however, the amount of collagen/kg skin was lower, and it accounted for a smaller proportion of the total N. Thus the effect of undernutrition on the amount of collagen/kg skin depended upon whether the rapid deposition of collagen that takes place in the normal animal between 3 and 4 to 6 weeks had already occurred before underfeeding began. Underfeeding appeared to cause a slight increase in the concentration of Na, but it had no consistent effect on the concentration of Cl. Undernutrition did not prevent a considerable reduction from taking place in the cellular constituents, K and P. The reduction during normal development was, however, always greater.

From the mean values, the distribution of water in the skin has been calculated as described by Widdowson & Dickerson (1960). The percentage of the total water accounted for by the extracellular fluid appeared to change little during underfeeding. As was to be expected from the collagen values already discussed, the distribution of the extracellular water was different in the two groups of undernourished animals, for the percentage of the total water associated with the collagen fibres increased with the increase in the amount of collagen in the smaller animals and decreased in the others.

Heart

Table 5 sets out the effect of undernutrition on the size of the heart and on its chemical composition. The three groups of undernourished animals are shown separately with a single group of 'weight' controls and also 'age' controls as for skeletal muscle in Table 2. The effect of development on the weight of the heart is naturally to increase it, but the heart weight falls as a percentage of the body-weight. Undernutrition did not prevent the weight of the heart continuing to increase to some extent, for the hearts of the undernourished pigs were heavier than those of well-nourished animals of similar weight. The normal increase was, however, prevented to a greater extent in body-weight than in the weight of the heart, and consequently undernutrition reversed the normal effect of development on the weight of the heart expressed as a percentage of the body-weight.

As in skeletal muscle, undernutrition also reversed some of the changes in normal chemical development, for it increased the proportion of water, Na and Cl and decreased that of total N, K, Mg and P to values resembling those at birth (Widdowson & Dickerson, 1960). In other respects it allowed the normal processes of development to go forward—the proportion of collagen N increased, and this increase was very striking when the collagen N was reckoned as a percentage of the total N. The

chloride space was calculated and used as a measure of the extracellular fluid, but the values for the composition of intracellular fluid thus derived indicated that the cardiac muscle cells, at any rate of the smallest undernourished animals, had become permeable to Cl. The values have therefore not been included in the table.

Table 5. *Composition of whole heart of pigs*

(Mean values expressed per kg of fresh tissue)

Heart weight and composition	Undernourished			Well-nourished	
	Group 1 (6 and 10 months, 3.4 and 3.6 kg) (2 pigs)	Group 2 (10-15 months, 6.7-12.7 kg) (3 pigs)	Group 3 (5-8 months, 10.5-13.1 kg) (3 pigs)	'Weight' controls (18-42 days, 3.5-12.3 kg) (5 pigs)	'Age' controls (8-18 months, c. 200 kg) (2 pigs)
Weight: g	25, 40	45-86	56-75	15-58	410, 430
as percentage of body-weight	0.68, 1.16	0.60-0.67	0.45-0.68	0.40-0.73	0.21
Composition:					
Water (g)	831	832	825	814	825
Total N (g)	20.9	21.3	25.7	23.6	26.2
Non-protein N (g)	2.3	2.0	—	2.3	2.5
Collagen N (g)	1.4	1.2	—	0.8	1.7
Na (m-equiv.)	68.0	56.6	47.5	46.2	45.8
K (m-equiv.)	66.2	71.4	85.0	94.6	87.5
Cl (m-equiv.)	56.4	41.5	36.9	36.0	32.0
P (m-moles)	64.9	59.6	63.5	76.8	68.2
Mg (m-equiv.)	14.8	11.6	17.0	17.8	19.2
Ca (m-equiv.)	4.5	3.8	5.8	4.5	3.3

Liver

Table 6 gives values for the weight of the liver and for its weight as a percentage of the body-weight. It also shows the chemical composition of the livers of the undernourished animals compared with that of the 'weight' and 'age' controls. The undernourished animals in groups 2 and 3 had livers which accounted for approximately the same proportion of their body-weight as those of their 'weight' controls. The livers of the smallest undernourished animals formed a larger proportion of their body-weight. The weight of the liver increases during normal development, but decreases relative to the weight of the body so, as in the heart, the changes brought about in the weight of the liver by undernutrition were the reverse of those during normal development.

From the values for water and protein ($N \times 6.25$) the livers of the undernourished animals could not have contained any appreciable amount of fat. Undernutrition prevented the concentration of Na and Cl from falling to their proper level. The livers of the undernourished animals in all three groups contained a higher proportion of water and of Na and Cl than those of the 'weight' or 'age' controls, suggesting that they had more extracellular fluid in them, and in fact the undernourished liver, like the heart, resembled the organ of the newborn in chemical composition.

The 'weight' controls for the undernourished animals were killed while they were still suckling and, as was expected from previous work (Venn, McCance & Widdowson,

1947), their livers contained very little Fe. Since undernutrition began during the suckling period, the undernourished animals no doubt also had low concentrations of Fe in their livers at the beginning of the experiment. The results show that the amounts of Fe in the liver had reached normal concentrations during the period of undernutrition. The undernourished animals were, moreover, not anaemic (McCance, 1960).

Table 6. *Composition of liver of pigs*

(Mean values expressed per kg of fresh tissue)

	Undernourished			Well-nourished	
	Group 1 (6 and 10 months, 3.4 and 3.6 kg) (2 pigs)	Group 2 (10-15 months, 6.7-12.7 kg) (3 pigs)	Group 3 (5-8 months, 10.5-13.1 kg) (3 pigs)	'Weight' controls (18-42 days, 3.5-12.3 kg) (4 pigs)	'Age' controls (8-18 months, c. 200 kg) (5 pigs)
Liver weight and composition					
Weight: g	122, 165	141-379	284-285	92-382	2250-3100
as percentage of body-weight	3.4, 4.9	2.1-3.5	2.3-2.7	2.6-3.2	1.1-1.6
Composition:					
Water (g)	783	771	769	759	716
Total N (g)	27.0	27.5	31.1	26.0	34.5
Na (m-equiv.)	43.0	52.7	45.6	39.7	35.8
K (m-equiv.)	88.1	82.5	82.5	77.9	82.2
Cl (m-equiv.)	43.5	42.7	36.9	31.8	29.2
P (m-moles)	104	94.0	91.0	109	119
Mg (m-equiv.)	17.8	14.5	16.9	17.5	19.4
Ca (m-equiv.)	2.5	3.7	3.6	2.2	2.6
Fe (mg)	227	362	391	38.0	282

Table 7. *Composition of kidneys of pigs*

(Mean values expressed per kg of fresh tissue)

	Undernourished			Well-nourished	
	Group 1 (6 and 10 months, 3.4 and 3.6 kg) (2 pigs)	Group 2 (10-15 months, 6.7-12.7 kg) (3 pigs)	Group 3 (5-8 months, 10.5-13.1 kg) (3 pigs)	'Weight' controls (18-42 days, 3.5-12.3 kg) (5 pigs)	'Age' controls (8-18 months, c. 200 kg) (5 pigs)
Kidneys weight and composition					
Weight: g	25, 30	35-64	50-85	22-62	396-474
as percentage of body-weight	0.68, 0.87	0.46-0.52	0.41-0.65	0.47-0.76	0.22-0.23
Composition:					
Water (g)	829	813	825	810	812
Total N (g)	20.3	23.2	23.7	21.7	23.7
Na (m-equiv.)	67.1	75.4	63.1	64.6	62.9
K (m-equiv.)	66.3	69.7	78.7	70.7	60.1
Cl (m-equiv.)	56.5	55.5	50.6	53.0	57.0
P (m-moles)	79.0	80.0	78.0	84.2	69.0
Mg (m-equiv.)	11.2	12.0	14.2	14.1	8.9
Ca (m-equiv.)	5.6	5.5	5.3	6.0	4.4

Kidneys

Table 7 shows the effect of development and undernutrition upon the absolute weight of the kidneys and upon the weight expressed as a percentage of that of the body. The absolute weight of this organ increases with the weight of the body during normal development but, as with the heart and the liver, the weight as a percentage of the body-weight falls. Undernutrition did not alter the ratio of kidney weight to body-weight, and in all three groups the absolute weight of the kidneys was about the same as in the appropriate 'weight' control. The parallelism between the rate of growth of the body and that of the kidneys was also reported in rats by Widdowson & McCance (1960).

Undernutrition did not make much difference to the gross composition of the kidneys, which is also set out in Table 7. These results are in keeping with the fact that, whether an animal is well nourished or undernourished, it must maintain functionally normal and active kidneys if it is to survive at all.

Brain

This organ shows the mixed effects of undernutrition more perfectly than any other and the findings are set out in Table 8. The first point to note is that undernutrition did not prevent the absolute weight of the brain from rising. It may have

Table 8. *Composition of brain of pigs*

(Mean values expressed per kg of fresh tissue)

	Undernourished			Well-nourished	
	Group 1 (10 months, 3.6 kg) (1 pig)	Group 2 (10-15 months, 6.7-12.7 kg) (3 pigs)	Group 3 (5-8 months, 10.5-13.1 kg) (3 pigs)	'Weight' controls (18-42 days, 3.5-12.3 kg) (5 pigs)	'Age' controls (8-18 months, c. 200 kg) (5 pigs)
Brain weight and composition					
Weight: g	58	77-95	78-88	41-62	83-117
as percentage of body-weight	1.6	0.74-1.3	0.60-0.75	0.50-1.3	0.04-0.06
Composition:					
Water (g)	793	789	789	816	758
Total N (g)	15.4	16.3	17.3	14.8	19.1
Na (m-equiv.)	77.6	58.2	51.7	62.7	60.7
K (m-equiv.)	55.8	78.5	86.5	70.2	76.0
Cl (m-equiv.)	54.7	43.3	38.3	47.0	41.1
P (m-moles)	100	107	115	87.0	125

gone up more slowly than in normal animals, but in those weighing 7-13 kg the brain reached its mature dimensions or not far short of them. Since, however, the body-weights of these animals scarcely increased during the period of investigation, the undernourished animals had brains that formed a larger and therefore more immature percentage of their body-weights than the brains of either the 'weight' or the 'age' controls. Undernutrition also reversed some of the normal effects of development on the composition of the brain; this fact is best shown by the concentrations of Na, K

and Cl in the smallest undernourished animals. On the other hand, even this degree of undernutrition did not prevent some of the normal decrease in the percentage of water from taking place over the age period concerned, nor did it prevent some of the normal increase of total N and P.

DISCUSSION

The impact of undernutrition upon an animal still in the period of active growth may alter the composition of the body in ways that are very difficult to understand unless they are interpreted in the light of certain general principles which are themselves not widely recognized. Undernutrition, even if severe enough to hold a pig at a weight of 3–4 kg for a year, does not prevent some of the developmental processes going forward—to some extent at any rate. At the same time undernutrition completely holds up some of the changes associated with development and may reverse them, so that at the end of the year the chemical structure of the organs may resemble that in an animal much younger than the one taken originally for the experiment on undernutrition. Undernutrition naturally tends to reverse some processes more easily than others and in some organs more easily than in others, and the effects produced also depend upon the degree of development that has been reached before the experiment began. Some of the effects are only understandable, moreover, by reference to the normal or abnormal processes going on in other parts of the body or in the body as a whole.

These considerations make it necessary in an investigation of this kind to have two sets of control animals, namely animals of the same size and animals of the same age as the undernourished ones. Without these it is impossible to distinguish those changes that are part of a normal ageing process from those that are an apparent reversal of the normal process.

It is a well-known fact that the underfeeding of animals, like the normal process of growth, affects the weights of different organs to a different extent (Aron, 1911; Jackson, 1925), and that the pattern of the effects is influenced by the period of growth at which undernutrition began (Jackson, 1915*b*). During the normal growth of the pig, McMeekan (1940) found that those parts of the body essential to life processes and body function are relatively well developed at birth, and make a smaller increment in postnatal life than the body as a whole, whereas those organs primarily connected with movement or the storage of reserves appear ill-developed at birth, but grow more during postnatal life. The differences between the rate of the chemical development of different organs and tissues during foetal life have also been explained on a functional basis (Widdowson & Dickerson, 1960). The organs that function and undergo most of their chemical development during foetal life, and whose continued function during postnatal life is vital to the existence of the organism, for example the heart, kidneys and liver, are the ones that in the present investigation have been found to change least in chemical composition as the result of undernutrition. Skeletal muscle, on the other hand, which undergoes substantial changes in chemical composition after birth, changes most in chemical composition as the result of underfeeding.

It is well known that when adult animals are undernourished the liver becomes

smaller. If the animal is completely deprived of food, the loss in weight of the liver is relatively greater than that of the rest of the body (Jackson, 1925; Addis, Poo & Lew, 1936; Widdowson & McCance, 1956); if the undernutrition is less severe and more prolonged the findings may be similar (Jackson, 1915*a*; Widdowson & McCance, 1956), but the difference between the loss in weight of the liver and that of the rest of the body tends to be less. In the pigs of the present experiments the liver did not lose weight, and in the smallest undernourished pigs even increased in weight. Jackson (1915*b*) also found an increase in weight of the liver in rats held at a constant body-weight by underfeeding from 3 weeks of age, whereas when underfeeding of a similar severity was begun at 6 weeks of age, only 3 weeks later, the liver lost weight. The response of the liver to underfeeding thus depends on the stage of growth at which undernutrition begins. Another factor that may influence the response of the organs to underfeeding is the hormonal status of the animal. McCance (1960) reported that the adrenal glands of these undernourished pigs were enlarged, and Goodlad & Munro (1959) have recently shown that the breakdown of muscle that results from the injection of cortisone into rats is accompanied by an increase in the amount of protein in the liver, provided that sufficient energy is supplied by the food.

Complete starvation in some species (Mottram, 1909) and a deficiency of dietary protein (Trowell, Davies & Dean, 1954) have been shown to produce fatty livers; at death the livers of the pigs in our experiments did not contain excessive amounts of fat, so this is another way in which chronic undernutrition from early in life seems to differ from other types of dietary deficiency.

The changes in chemical composition of pig muscle as the result of underfeeding are essentially similar to those described by Dickerson & McCance (1960) in the pectoral muscle of cockerels undernourished from an early age. These authors pointed out that changes in the composition of a unit weight of muscle may result from certain constituents being deposited faster than others, or from the greater loss of some constituents. From measurements of the total muscle mass it was also demonstrated that there might be a real increase in the extracellular constituents and a decrease in the cellular ones. Underfeeding cockerels did, in fact, almost completely arrest cellular growth in their pectoral muscles. It seems reasonable to suppose that the changes in the muscle of the larger undernourished pigs in groups 2 and 3 resulted from a similar suppression of cell growth relative to that of the connective tissue. In the pigs in group 1, which were undernourished from a slightly less advanced age and size, the changes in the composition of the muscle were greater and there was evidence that the composition of the cells themselves had been altered by underfeeding. Similar evidence of changes in cellular composition were found in the muscle of the undernourished cockerels.

The effect of the stage of development at the onset of undernutrition is clearly seen in the skin. This organ normally changes very quickly in chemical composition during the latter half of the suckling period and the effect of underfeeding on its composition was found to depend upon whether this normal change in composition had already taken place when underfeeding began. McCance (1960) has described the macroscopical and histological appearance of the skin of undernourished pigs. It is

difficult as yet to correlate the structure of the skin with its chemistry in any great detail, but the histological finding that the corium was oedematous is in agreement with the chemical finding that the skin of the undernourished animals contained a higher proportion of water than that of the well-nourished 'weight' controls, and this is another of the changes that might have been due to an excess of cortisone.

There seems little doubt that the brains of the undernourished animals continued to grow during underfeeding, as has been noted many times over during the past 100 years (see Jackson, 1925). The findings of Ford & McCance (to be published) on the size of the endocranial cavity of the skull are in agreement with this observation. It has been pointed out on p. 467 that the changes in chemical composition of the brain due to underfeeding are mixed ones—certain aspects of its composition appearing to be a reversal of the process of normal development, whereas in other respects its composition appears to have changed as it should have done during the normal ageing process. The brain is a composite structure and the changes in its gross composition may be due to different parts being affected to a different degree by the undernutrition. Nevertheless, it is probable that functional maturation of the brain is necessary for the survival of the animal and the changes in composition during the normal ageing process may be part of the further development of essential parts. This, with many of the other aspects of undernutrition revealed by a study of its effects on immature chemical structure, must be left for future work.

SUMMARY

1. The chemical composition of the skeletal muscles, skin, heart, liver, kidneys and brain of eight pigs has been examined after prolonged periods of undernutrition which began during the suckling period and prevented any material gain in weight. Skeletal muscle was also studied from four pigs whose growth was similarly retarded and which were then given a plentiful supply of food so that they gained weight rapidly.
2. The effects varied with (*a*) the age and size of the animal when the food intake was reduced, (*b*) the functional and anatomical maturity of the organ, (*c*) the growth priority of the organ or part of the organ.
3. In skeletal muscle undernutrition reversed the normal process of chemical development; in some organs, e.g. the liver, it delayed the normal changes of maturation; in others, e.g. the skin and brain, both effects could be observed simultaneously. The gross composition of the kidneys was little changed.

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REFERENCES

- Addis, T., Poo, L. J. & Lew, W. (1936). *J. biol. Chem.* **115**, 111.
- Aron, H. (1911). *Philipp. J. Sci. (B, Med.)*, **6**, 1.
- Beattie, J., Herbert, P. H. & Bell, D. J. (1948). *Brit. J. Nutr.* **2**, 47.
- Bischoff, T. L. W. & Voit, C. (1860). *Die Gesetze der Ernährung des Fleischfressers*. Leipzig and Heidelberg: Winter.
- Cachera, R. & Barbier, P. (1943). *Paris méd.* **125**, 341.
- Dicker, S. E. (1949). *Biochem. J.* **44**, 274.
- Dickerson, J. W. T. & McCance, R. A. (1960). *Brit. J. Nutr.* **14**, 331.
- Dickerson, J. W. T. & Widdowson, E. M. (1960). *Biochem. J.* **74**, 247.
- Drummond, J. C. (1916). *Biochem. J.* **10**, 77.
- Freng, S., Metcoff, J., Gómez, F., Ramos-Galván, R., Cravioto, J. & Antonowicz, I. (1957). *Pediatrics, Springfield*, **20**, 105.
- Goodlad, G. A. J. & Munro, H. N. (1959). *Biochem. J.* **73**, 343.
- Henschel, A., Mickelsen, O., Taylor, H. L. & Keys, A. (1947). *Amer. J. Physiol.* **150**, 170.
- Jackson, C. M. (1915a). *Amer. J. Anat.* **18**, 75.
- Jackson, C. M. (1915b). *J. exp. Zool.* **19**, 99.
- Jackson, C. M. (1925). *The Effects of Inanition and Malnutrition upon Growth and Structure*. London: Churchill.
- Jackson, C. M. (1932). *Amer. J. Anat.* **51**, 347.
- Kerpel-Fronius, E. & Kovach, S. (1948). *Pediatrics, Springfield*, **2**, 21.
- Lhermitte, J. & Sigwald, J. (1942). *Bull. Acad. Méd., Paris*, **126**, 384.
- Lipschütz, A. (1911). *Z. allg. Physiol.* **12**, 118.
- Lipschütz, A. (1915). *Zur allgemeinen Physiologie des Hungers*. Braunschweig: Wieweg.
- Lowry, O. H., McCay, C. M., Hastings, A. B. & Brown, A. N. (1942). *J. biol. Chem.* **143**, 281.
- McCance, R. A. (1960). *Brit. J. Nutr.* **14**, 59.
- McCance, R. A., Widdowson, E. M. & Shackleton, L. R. B. (1936). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 213.
- McMeekan, C. P. (1940). *J. agric. Sci.* **30**, 276.
- Mendel, L. B. & Rose, W. C. (1911-12). *J. biol. Chem.* **10**, 255.
- Mendes, C. B. & Waterlow, J. C. (1958). *Brit. J. Nutr.* **12**, 74.
- Morgulis, S. (1923). *Fasting and Undernutrition*. New York: Dutton and Co.
- Mottram, V. H. (1909). *J. Physiol.* **38**, 281.
- Smith, R. & Waterlow, J. C. (1960). *Lancet*, **i**, 147.
- Stanier, M. W. (1957). *Brit. J. Nutr.* **11**, 206.
- Terroine, E. F., Brenckmann, E. & Feuerbach, A. (1922-3). *Arch. int. Physiol.* **20**, 466.
- Terroine, E. F., Feuerbach, A. & Brenckmann, E. (1924). *Arch. int. Physiol.* **22**, 233.
- Trowell, H. C., Davies, J. N. P. & Dean, R. F. A. (1954). *Kwashiorkor*. London: Arnold.
- Venn, J. A. J., McCance, R. A. & Widdowson, E. M. (1947). *J. comp. Path.* **57**, 314.
- Voit, C. (1881). *Physiologie des allgemeinen Stoffwechsels und der Ernährung*. In Hermann, L., *Handbuch der Physiologie*, Vol. vi. Leipzig: F. C. W. Vogel.
- Waterlow, J. C. & Mendes, C. B. (1957). *Nature, Lond.*, **180**, 1361.
- Waters, H. J. (1908). *Proc. Soc. for the Promotion of Agric. Sci. 29th Annu. Mtg, Washington, D.C.*, p. 71.
- Waters, H. J. (1909). *Proc. Soc. for the Promotion of Agric. Sci. 30th Annu. Mtg, Portland, Oregon*, p. 70.
- Weech, A. A., Snelling, C. E. & Goettsch, E. (1933). *J. clin. Invest.* **12**, 193.
- Widdowson, E. M. & Dickerson, J. W. T. (1960). *Biochem. J.* **77**, 30.
- Widdowson, E. M. & McCance, R. A. (1951). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 275, p. 165.
- Widdowson, E. M. & McCance, R. A. (1956). *Brit. J. Nutr.* **10**, 363.
- Widdowson, E. M. & McCance, R. A. (1957). *Brit. J. Nutr.* **11**, 198.
- Widdowson, E. M. & McCance, R. A. (1960). *Proc. roy. Soc. B* **152**, 188.
- Widdowson, E. M. & Southgate, D. A. T. (1959). *Biochem. J.* **72**, 200.