

## The effect of over-feeding newborn rabbits on somatic and visceral growth, body composition and long-term growth potential

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1. Newborn rabbits were over-fed by encouraging them to suck from two lactating does. These double-fed animals were compared with single-fed litter-mate controls.
2. Single- and double-fed rabbits were compared with respect to body-weight, bone length and weights of heart, kidney, lung, liver, spleen, stomach, brain and adipose lobes at ages 7, 14 and 21 d and at 25 weeks. Body composition with respect to fat, protein and water was also analysed in animals killed during the first month of life.
3. Double feeding had a profound effect on body-weight, such that by 21 d of age these animals weighed 65% more than controls. Longitudinal growth was also increased, but to a lesser extent. Organ weights were largely determined by body-weight with the exception of the brain which was unaffected by double feeding and the heart and adipose lobes which were represented to excess. Body composition studies demonstrated a marked increase in body fat and a decrease in body water as a percentage of body-weight in the double-fed.
4. When mature, double-fed rabbits weighed a little more than controls and had accumulated significantly more fat. No other difference between them was demonstrated.
5. Other experiments on rats, mice and pigs, along with these experiments on rabbits, suggest that young mammals respond to over-feeding with a limited increase in real growth associated with excess deposition of adipose tissue. Studies on infants of diabetic mothers have demonstrated that the human fetus responds to over-nutrition in a very similar way. Therefore we should be cautious about over-feeding human infants, especially premature ones, as it seems unlikely that excessive deposition of fat will be beneficial.

Widdowson & McCance (1960) investigated the effect of over- and under-feeding rats from birth by reducing or increasing the litter size. The rats reared in small litters not only gained weight more rapidly during the pre-weaning period, but continued to maintain a growth advantage into adult life. This enhancement of growth potential is dependent on high-plane nutrition in the immediate postnatal period (Widdowson & McCance, 1963). The relevance of these studies to the plane of nutrition in early life in man is difficult to assess for two reasons. First, the effect of nutrition on long-term growth potential is highly dependent on its timing in relation to the period when rapid brain growth occurs (Dobbing, 1981). In this respect rats are born in a relatively immature state (Dobbing, 1981). Second, epiphyseal fusion never occurs in rats, allowing growth to continue into adult life (McCance, 1962). However, the possible effect of indiscriminate feeding of the immature mammal during the early phases of development has become a clinical issue now that many infants of under 32 weeks gestation survive. It is common practice to feed such infants via a nasogastric tube, and to give them as much milk as they will tolerate. Human breast milk is often used when it is available but a number of highly modified infant feeds are being prepared; some with high energy and protein contents. There have been a number of reports indicating that with such milks the infants grow more rapidly (Calvert *et al.* 1983).

The present study was instigated to examine the effect of over-feeding early in life on the subsequent growth and development of newborn rabbits. The rabbit is a more appropriate animal to study than the rat for two reasons. First, the brain of the rabbit undergoes rapid growth in the pre- and postnatal periods (Harel *et al.* 1972), a similar situation to that in man (Dobbing & Sands, 1973), whereas in the rat this occurs only after birth (Dobbing

& Sands, 1979). Second, when the rabbit matures the epiphyses fuse (Khermosh *et al.* 1972). The precise objectives of the study were to see what effect accelerated weight gain in the first 3 weeks of life had on somatic and visceral growth, body composition and long-term growth potential.

#### EXPERIMENTAL

##### *Animals, diets and experimental design*

The experiments were conducted on the young from a colony which originated from New Zealand White and Californian cross-bred rabbits. Nursing does normally feed their young only once daily. Therefore the young can be separated after their first feed and nursed in an incubator, provided they are returned to the mother for approximately 30 min each morning for feeding.

In these experiments over-feeding was achieved by introducing the young rabbits to a second lactating doe 8–12 h after they had fed from their own mothers, as first described by Hardman *et al.* (1970). Rabbits fed in this way will take twice as much milk as their normally fed litter-mates and are termed double-fed. Litter size was kept to a maximum of six animals and each double-fed rabbit was paired with a litter-mate of similar birth weight which acted as a single-fed control. In the first series of experiments the double-fed rabbits were killed after either 7, 14 or 21 d. The single-fed animals were killed at 7, 14, 21 or 28 d. The last group were of similar weight to the double-fed at 21 d and permitted a weight-for-weight comparison. Six animals in each of the identified groups from eleven litters were analysed. In a second series of experiments, twelve double-fed rabbits from ten litters, each with single-fed litter-mate controls, were reared to maturity at 25 weeks. Only single-sex pairs were used for the study, and therefore a certain selection took place at 3 weeks when sexing first became possible. Six pairs of each sex were then reared to maturity.

##### *Experimental procedure*

Rabbits were killed at ages 0, 7, 14, 21 and 28 d and at 25 weeks. Those killed during the first 28 d were stunned with a sharp blow to the skull followed by bleeding from the jugular vein and carotid artery. The blood was retained. The carcasses were shaved using soap and water as a lubricant and an open razor. After shaving, the body was thoroughly washed and dried. Photographs were taken of a selection of shaven animals in a standard lateral position under controlled-lighting conditions. Animals killed at 25 weeks were killed using a fatal intravenous dose of Euthanal (sodium pentobarbitone) (May & Baker Ltd).

The heart, lung, liver, kidney, spleen, stomach, brain and adipose tissue lobes were dissected out and weighed. The carcass weight was taken to be the weight of the body minus the gut contents and minus the fur in shaven animals. The right humerus was dissected out and measured with calipers to 0.1 mm.

All the rabbits killed in the first 28 d of life were subject to carcass analysis of water, fat and protein. Loss of tissue fluid during dissection was less than 2.5% of the body-weight. Each animal carcass was homogenized with twice its weight of water in a Waring blender. Samples of approximately 30 g were freeze-dried so that the carcass water content could be estimated. The water estimations were performed in triplicate and the results obtained were subjected to an analysis of variance. The 95% confidence limit about the mean of each set of observations was 0.73%, which indicates that the homogenate was adequately mixed and that the freeze-drying process had been effective. The freeze-dried homogenate was used for analysis of fat and protein.

##### *Analytical methods*

The nitrogen content of the freeze-dried homogenates was measured using a modified Kjeldahl–Gunning method. Samples were analysed in duplicate and the results were subjected to an analysis of variance. The 95% confidence limit for the mean of each pair

of results was 0.19%. The protein content of the homogenate was estimated by multiplying the mean of each pair of results by a factor of 6.25.

Total triglycerides were measured in the homogenate. This was done by extracting the triglycerides with chloroform-methanol (2:1, v/v), hydrolysing the lipid with alcoholic potassium hydroxide and estimating the glycerol released using the micromethod of Boehringer (Biochemia Test Combination).

#### *Statistical methods*

Differences between groups of single- and double-fed rabbits were tested for statistical significance using Student's paired *t* test.

### RESULTS

The double-fed rabbits gained weight more rapidly than the single-fed. When carcass weights were compared (Table 1), double-fed rabbits advanced their weight attainment by about 1 week. As rabbits aged their body proportions changed (Fig. 1). The ears, neck and legs elongated and the head and trunk became proportionately smaller. Despite the weight attainment of the double-fed animals, their body proportions remained immature. They appeared fuller in the body and neck and the overall appearance was of a more stocky animal.

Longitudinal growth was evaluated by measurement of the left humerus. Double-fed animals had increased longitudinal growth for their age (Table 2) but their bones were short in comparison with single-fed animals of similar weight (Fig. 2).

The weight of the left kidney and heart in relation to body-weight is shown in Fig. 3. The relation between kidney and body-weight was linear and was the same for single- and double-fed rabbits. Therefore kidney weight was dependent on body-weight irrespective of growth rate. The relation between heart weight and body-weight was linear but it was not the same for single- and double-fed animals. The heart was relatively larger in the animals with rapid weight gains.

The weights of the liver, spleen, lung and stomach did not show a clear linear relation with body-weight and so they have been plotted as a percentage of body-weight at different ages (Fig. 4). In the single-fed rabbits, liver growth lagged behind body growth so that the liver became proportionately smaller. In the double-fed this lag was significantly reduced and the liver represented a larger percentage of body-weight during the first 2 weeks. Subsequently the liver came to occupy a similar percentage of body-weight in single- and double-fed animals.

Spleen weight in relation to body-weight was variable and there was no consistent difference between single- and double-fed animals.

In both groups of animals the lungs grew less quickly than the body and so became proportionately smaller. Single- and double-fed animals were similar in this respect.

In the single-fed there appeared to be no consistent change in the relation of stomach weight to body-weight with time, although there was a great deal of individual variation. In the double-fed, stomach weight did not increase in proportion to body-weight, so that the stomachs were proportionately smaller.

The cervical adipose tissue weight (cervical plus interscapular lobes) and the perirenal adipose tissue weight as a percentage of body-weight are presented in Fig. 5. Double feeding caused expansion of the cervical adipose lobes in the first week. These lobes are composed of brown adipose tissue. Subsequently energy excess had its major effect on the white adipose tissue in the perirenal lobes.

The mean brain weights of single- and double-fed rabbits were similar at each age (Fig. 6), indicating that double feeding had little effect on brain growth.

Table 1. *The effect of age and double feeding\* on carcass weight in rabbits*  
(Mean values and standard deviations for six rabbits)

Age (d)...	0		7		14		21		28	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Single-fed	46.9	4.3	95.6	14.9	166.4	20.9	261.2	44.4	429.3	73.5
Double-fed			158.5	19.6	288.7	31.8	429.7	60.5	—	—
Statistical significance of difference between groups: <i>P</i>			< 0.005		< 0.001		< 0.001			

\* For details of feeding regimen, see p. 390.

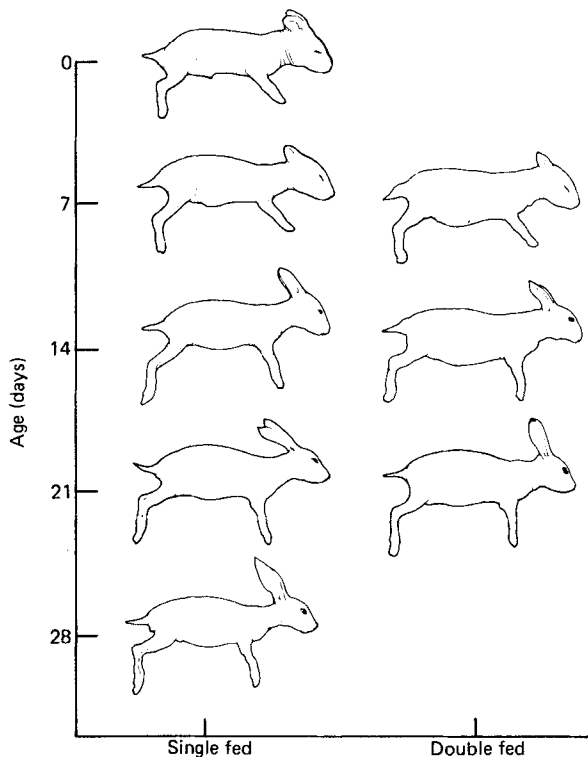


Fig. 1. A representative from each group of single- and double-fed rabbits was photographed in a lateral position. The photographs were reproduced to give a constant nose-to-tail measurement so that the body proportions could be easily compared. The outlines of these photographs have been drawn for presentation.

The results of the body composition analysis for water, protein and fat are presented in Table 3. During normal growth there was a trend towards a decrease in body water and an increase in body fat. Body protein also tended to increase as the animals matured. Double-fed animals at 7, 14 and 21 d of age contained less water and more fat than their single-fed counterparts. These changes were greater in magnitude than those associated with normal maturation and were statistically significant. At first sight it would appear that body protein was appropriate in the double-fed for the size of the animals. However, it is clear

Table 2. The effect of age and double feeding\* on humerus length (mm) in rabbits  
(Mean values and standard deviations for six rabbits)

Age (d)...	0		7		14		21		28	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Single-fed	1.7	0.1	2.2	0.1	2.7	0.1	3.2	0.1	3.8	0.1
Double-fed	—	—	2.4	0.1	3.0	0.1	3.6	0.1	—	—
Statistical significance of difference between groups: <i>P</i>			< 0.005		< 0.005		< 0.005			

\* For details of feeding regimen, see p. 390.

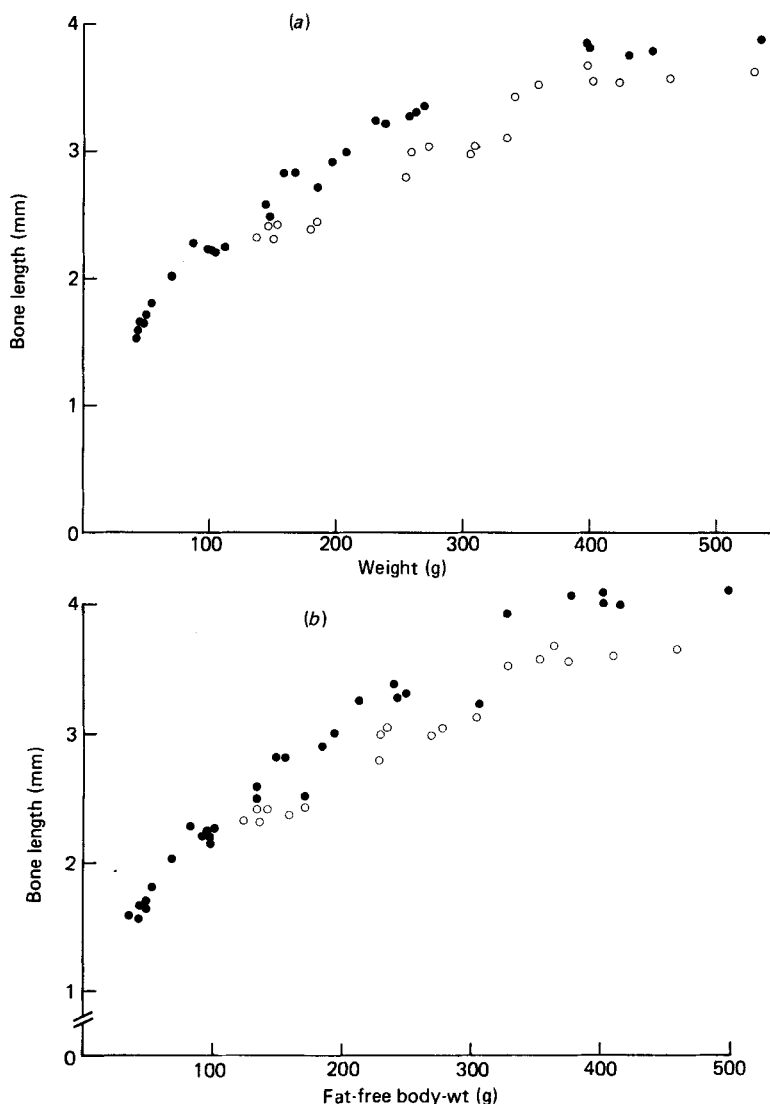


Fig. 2. The relation between bone length (mm) and (a) body-weight (g) and (b) fat-free body-weight (g) in single- (●) and double-fed (○) rabbits.

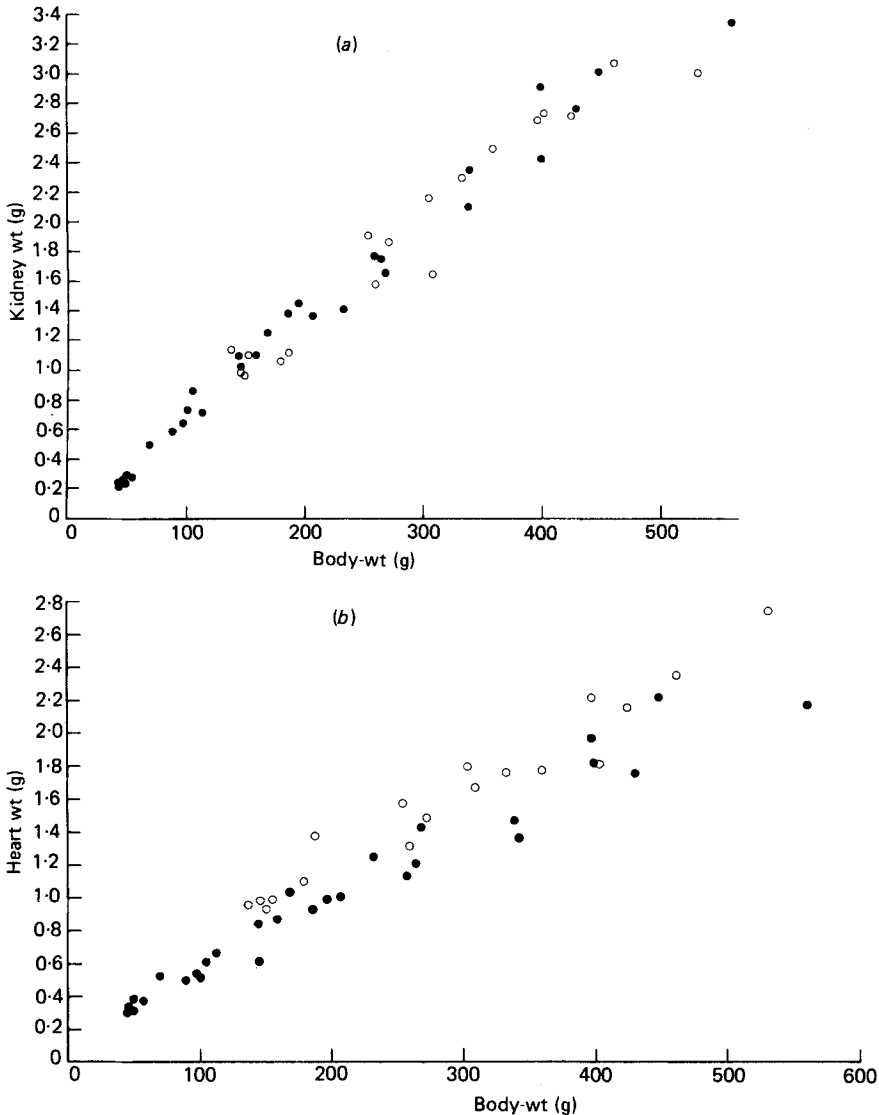


Fig. 3. The relation between (a) kidney weight (g) and body-weight (g) and (b) heart weight (g) and body-weight (g) in single- (●) and double-fed (○) rabbits.

from Fig. 2 that double-fed rabbits were short in relation to fat-free body-weight. This indicated that the protein content of the body was excessive for the size of the skeleton.

The single- and double-fed rabbits reared to 25 weeks of age were comparable with those previously described in the growth and body composition study in terms of birth weight and growth pattern during the pre-weaning period. One pair of female rabbits had to be excluded from the study because one of the pair developed overgrown teeth, which severely interfered with her ability to feed. The pattern of growth in the male and remaining female rabbits is shown in Fig. 7. It was apparent from the mean weights that single-fed rabbits never fully caught up. The weight difference between single- and double-fed rabbits was more pronounced in males than females. However, in both sexes this difference became a smaller percentage of body-weight as the animals grew larger. The difference was further obscured

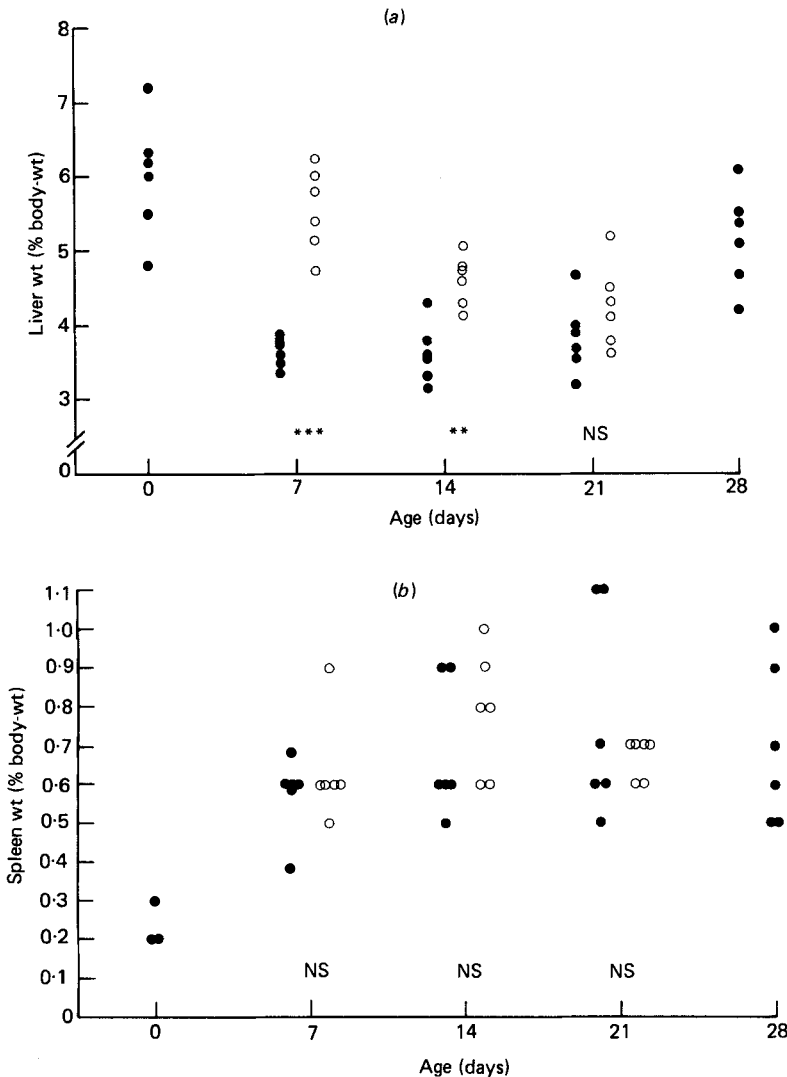


Fig. 4. The effects of age and double feeding on (a) liver weight and (b) spleen weight as a percentage of body-weight. (●), Single-fed; (○), double-fed. NS, not significant; \*\*  $P < 0.005$ ; \*\*\*  $P < 0.001$ .

by the age-related increase in distribution of body-weights seen in each group. At 25 weeks of age the difference in body-weight between single- and double-fed male rabbits was still statistically significant, but this was not the case for females (Table 4).

Further study demonstrated that although the mean bone lengths for male and female double-fed animals tended to be longer than the means of the single-fed, this was not statistically significant. There was, however, a significant difference in the combined weight of the subscapular, cervical, inguinal and perirenal adipose lobes, the double-fed animals containing more of this tissue (Table 4).

There was no difference in weight between the organs of single- and double-fed rabbits when expressed as a percentage of body-weight. Absolute brain weights were expressed and again no difference was found.

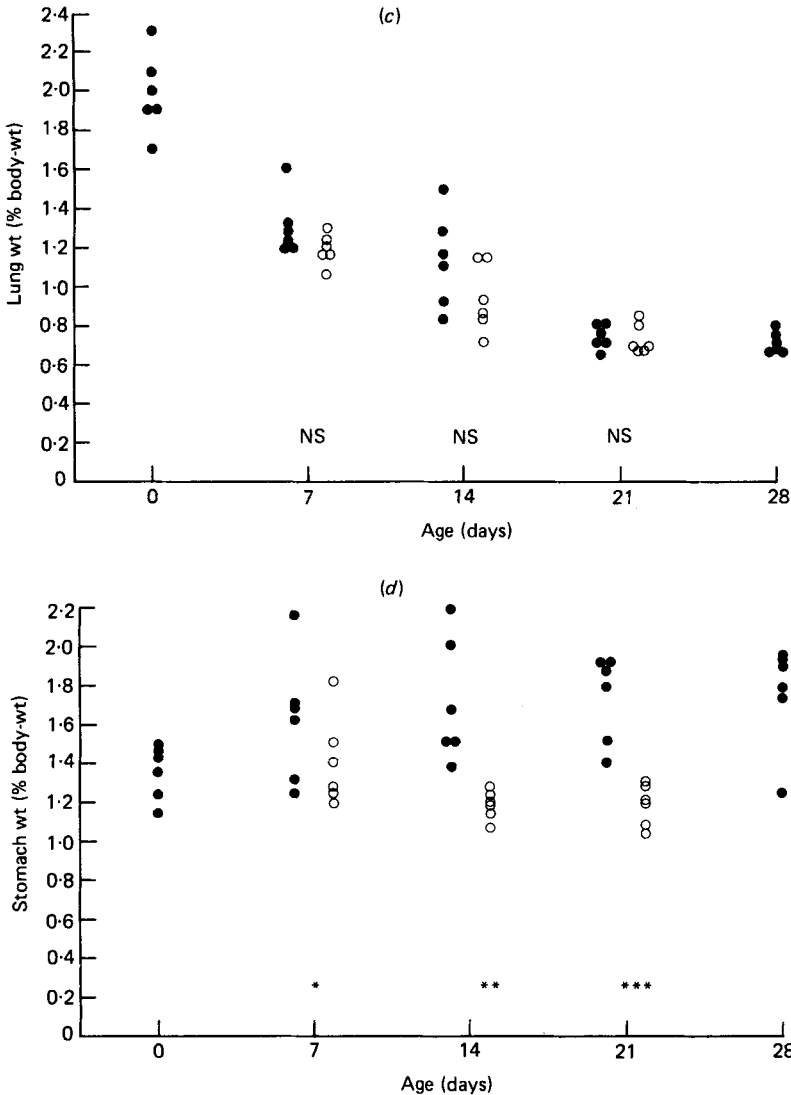


Fig. 4 *cont.* The effects of age and double feeding on (c) lung weight and (d) stomach weight as a percentage of body-weight. (●), Single-fed; (○), double fed. NS, not significant; \* $P < 0.05$ ; \*\* $P < 0.005$ ; \*\*\* $P < 0.001$ .

#### DISCUSSION

The acceleration of weight gain in newborn rabbits gave rise to an abnormal pattern of growth. Whilst the kidneys continued to grow in proportion to body-weight, the brain weight was determined by the age of the animals. Longitudinal growth and stomach weight followed an intermediate pattern. Adipose tissue and heart weights were represented to excess. Furthermore, body composition was altered with respect to water content, which was decreased, and fat content, which was increased.

As the rabbits developed beyond weaning to maturity, the differences between single- and double-fed animals became less pronounced. At 25 weeks of age, double-fed rabbits still tended to be a little heavier and contained a little more adipose tissue, but otherwise there was little difference between them.



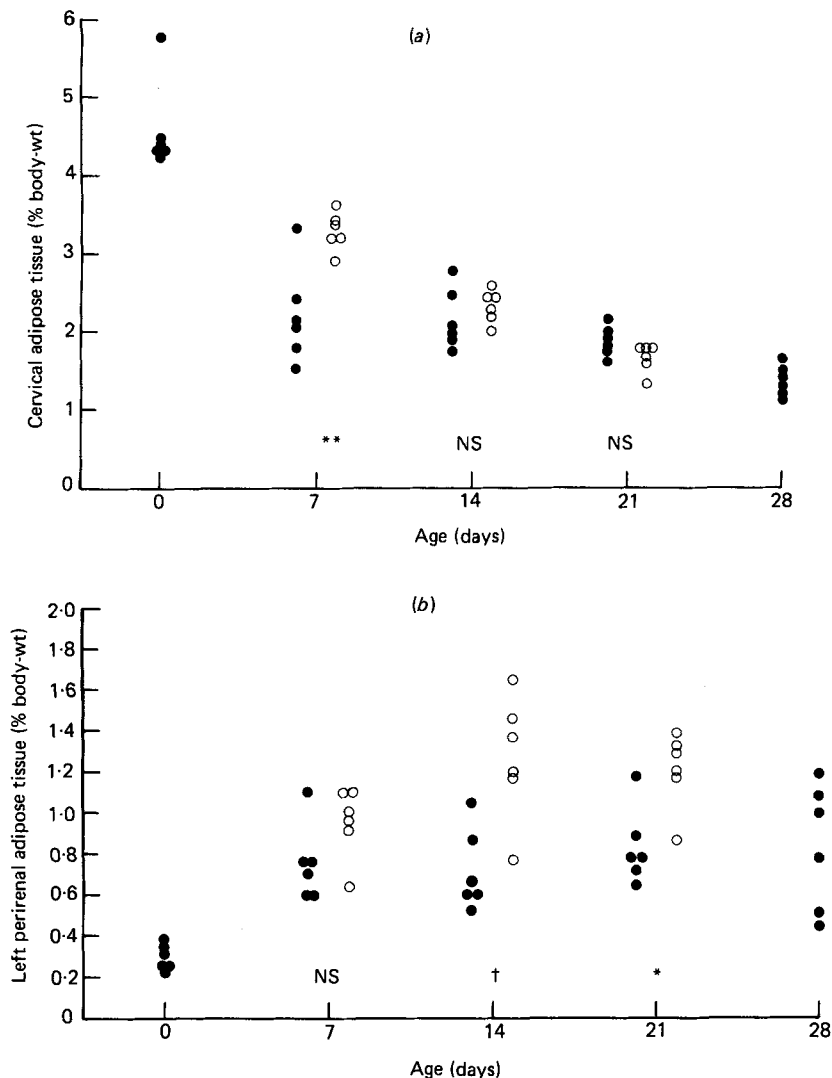


Fig. 5. The effects of age and double-feeding on the weights of (a) cervical and (b) perirenal lobes of adipose tissue as a percentage of body-weight. (●), Single-fed; (○), double-fed. NS, not significant; \* $P < 0.05$ , † $P < 0.02$ , \*\* $P < 0.01$ .

The pattern of growth that occurred early in life merits further discussion. The results indicated that young rabbits can respond to excess energy by increasing longitudinal or skeletal growth, but much of the excess energy is stored as fat.

The internal organs probably grow partly in response to the functional demands that are made on them and, in most cases, this will be related to body-weight. The liver of the double-fed was proportionately very large at the end of the first week. Kennedy (1957) and Widdowson & McCance (1953) have demonstrated that liver growth is dependent on energy intake. Therefore a very high milk intake per unit body-weight during the first week would explain this finding. The proportionately large heart seen in the double-fed can also be explained on a functional basis, as rapidly growing tissue has an increased metabolic demand and therefore requires an increased blood flow. The pattern of growth observed by

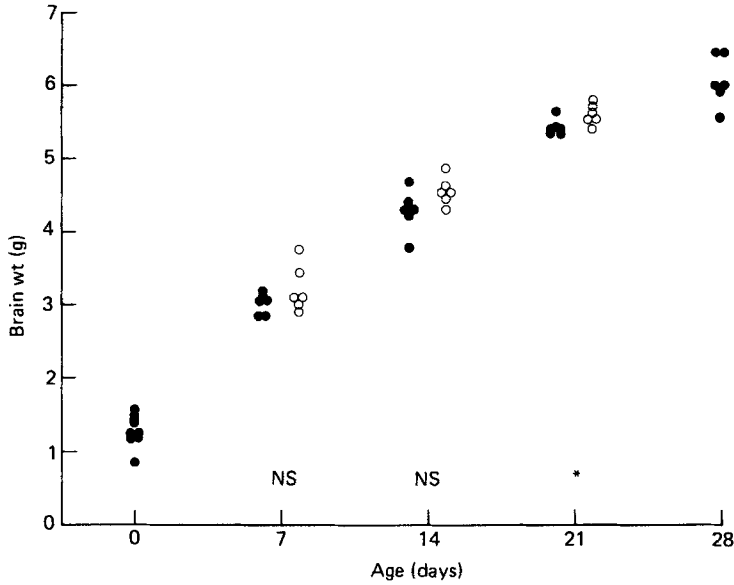


Fig. 6. The effects of age and double feeding on brain weight. (●), Single-fed; (○), double-fed. NS, not significant; \* $P < 0.05$ .

Table 3. *The effect of age and double feeding\* on body composition*  
(Mean values and standard deviations for six rabbits)

Age (d)...	0		7		14		21		28	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Body water (% body-wt)									
Single-fed	79.6	1.4	75.5	2.1	73.5	1.4	71.0	1.9	69.6	2.1
Double-fed	—	—	71.3	3.5	69.5	1.9	67.2	1.3	—	—
Statistical significance of difference between groups: $P$			< 0.01		< 0.005		< 0.005			
	Body protein (% body-wt)									
Single-fed	12.0	0.7	13.3	0.3	13.7	0.4	14.6	0.7	14.9	1.3
Double-fed	—	—	13.7	1.5	13.4	1.0	14.3	0.3	—	—
$P$			NS		NS		NS			
	Body fat (% body-wt)									
Single-fed	3.3	0.7	5.5	2.0	6.7	1.4	7.3	1.8	6.5	2.4
Double-fed	—	—	8.8	1.3	10.8	1.7	11.0	1.9	—	—
$P$			< 0.002		< 0.001		< 0.01			

NS, not significant.

\* For details of feeding regimen, see p. 390.

Widdowson & McCance (1960) in over-fed rats during the pre-weaning period is very similar to our observations in rabbits with respect to skeletal growth, fat deposition and growth of internal organs.

Single-fed rabbits demonstrated the changes in body composition that are characteristic of the young of many species (Stanier *et al.* 1979; Rucklidge, 1981), namely a progressive rise in body fat content associated with reciprocal changes in body water. This change was

Over-feeding newborn rabbits

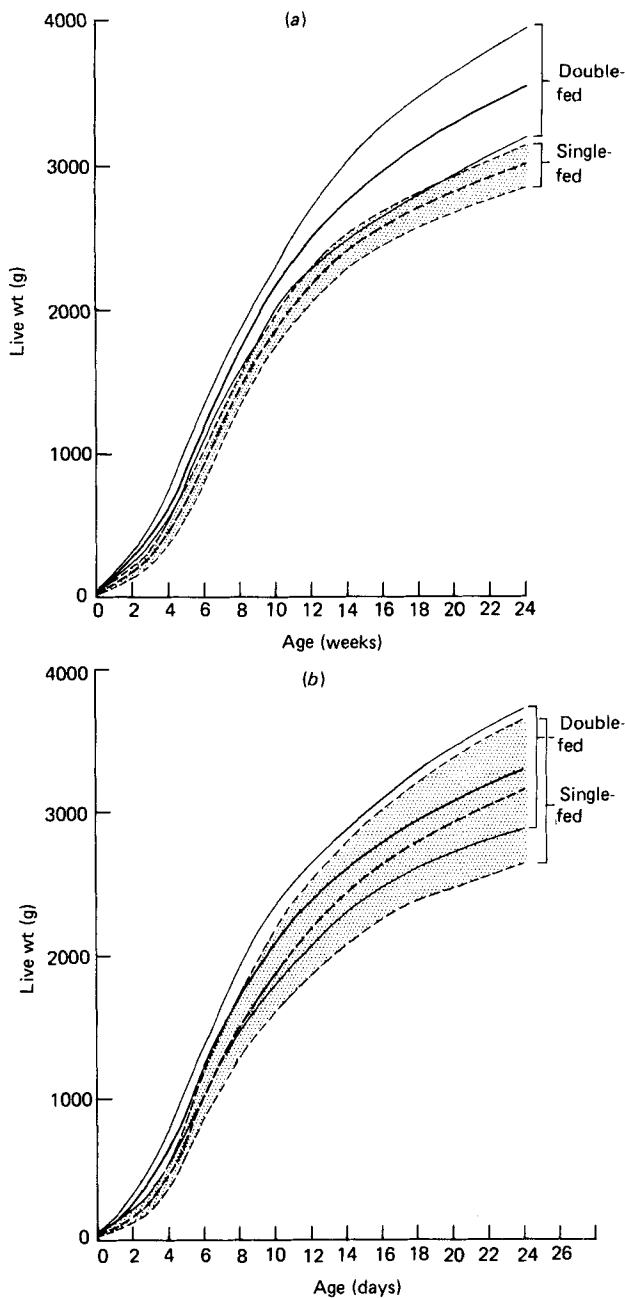


Fig. 7. Weight charts for single- (-----) and double-fed (—) (a) male (n 6) and (b) female (n 5) rabbits showing mean body-weights and standard deviations during the first 6 months of life. (The middle line is the mean and the outer lines are drawn through  $\pm 1$  SD for the group.)

exaggerated in the double-fed beyond the point where it could be explained on the basis of advancing maturity. In other words, double-fed rabbits were fat. Rapid growth in young rats (Widdowson & McCance, 1960), mice (Rucklidge, 1981) and pigs (Stanier *et al.* 1979) also leads to an increase in fat deposition irrespective of whether the feeds are artificial or species-specific milk, or whether the rapid growth is effected by over-feeding or by genetic

Table 4. *The effect of double-feeding\* on body-weight, bone length and adipose tissue in mature male (n 6) and female (n 5) rabbits*

(Mean values and standard deviations)

Group	All		Male		Female	
	Mean	SD	Mean	SD	Mean	SD
	Body-weight (g)					
Single-fed	2904	296	2918	141	2887	440
Double-fed	3268	431	3453	372	3045	422
Statistical significance of difference between groups: <i>P</i>	< 0.001		< 0.05		NS	
	Humerus length (mm)					
Single-fed	7.23	0.32	7.19	0.23	7.28	0.42
Double-fed	7.35	0.19	7.35	0.22	7.35	0.16
<i>P</i>	NS		NS		NS	
	Adipose tissue weight (g)					
Single-fed	210	43	213	27	208	61
Double-fed	288	89	308	101	265	77
<i>P</i>	< 0.01		< 0.05		NS	

NS, not significant.

\* For details of feeding regimen, see p. 390.

selection. Normal growth is also associated with a gradual increase in protein concentration in the body. This is probably due to the maturation of skeletal muscle which changes its composition as growth occurs (Widdowson, 1981); extracellular fluid occupies a smaller proportion of muscle mass whereas intracellular protein concentration increases. Rapid growth does not alter the protein concentration in rabbits, mice (Rucklidge, 1981) or pigs (Stanier *et al.* 1979). However, it may not be appropriate for protein concentrations to remain unaltered in animals whose weight gain has outstripped their longitudinal growth. If bone length can be used as a guide to over-all skeletal size, then it is clear that double-fed rabbits have excess protein for the size of the skeleton. This may be due to an advance in maturation of skeletal muscle resulting from rapid growth, similar to that which has been described in fast-growing rats (Widdowson & McCance, 1960).

When the long-term effects of nutritional excess early in life are examined it is apparent that there is a difference between rabbits and rats. Whereas over-fed rats (Widdowson & McCance, 1960) show a 25% increase in adult body-weight, double-fed rabbits show only a 12.5% increase, which is largely obscured by normal variation. This endorses the theory that the presence of early nutritional influences depends on its relation to the critical periods of brain growth (Dobbing, 1981).

The weights of the adipose lobes in 25-week-old rabbits indicated that the weight difference between single- and double-fed animals was likely to be due to excess fat deposition. Similar results were obtained by Widdowson & McCance (1960) on rats; fast-growing rats contained 25% fat compared with 15% in slow-growing rats. Mice suckled in small and large litters (Lemonier *et al.* 1973) also show similar long-term results. Therefore in several species there does seem to be a link between early nutritional experience and body fat content in adult life.

Infants of diabetic mothers have many features in common with double-fed rabbits. These infants experience an acceleration of weight gain during the last trimester (Cardell, 1953).

The cause of this excessive weight gain is a matter of some debate, but there is no doubt that its severity is related to the mother's degree of hyperglycaemia during pregnancy (Pedersen, 1977*a*). Organ weights remain in proportion to body-weight with the exception of the brain – which is unaffected by rapid weight gain, and the heart – which is hypertrophied (Driscoll *et al.* 1960). An increase in body length occurs. There is some debate as to whether the increase in body length is appropriate for the increase in body-weight. The consensus of opinion (Pedersen, 1954) affirms that it is, although Osler (see Pedersen, 1977*b*) reported bone lengths that were short in relation to body-weight. The latter is consistent with the results for rabbits. Body composition studies also demonstrate parallels between infants of diabetic mothers and double-fed rabbits. Infants of diabetic mothers have an increase in body fat content and a decrease in body water (Fee & Weil, 1963; Pedersen, 1977*b*).

A number of long-term follow-up studies of infants of diabetic mothers have been published. White *et al.* (1953) reported on the measurement of these infants at various ages during childhood and adolescence. An excessive number of children were above the standard height-for-age, and an even larger number were above the standard weight-for-age. An Australian follow-up study (Breidahl, 1966) showed that 7% of these children were above the 90th percentile for height and 21% above the 90th percentile for weight. Farquhar (1969), studying Edinburgh-born infants of diabetic mothers, showed that an excessively large number had a high weight:height index during childhood and adolescence. A retrospective Canadian study (Verdy *et al.* 1974) concluded that the risk of developing adult obesity was greater for infants of diabetic mothers. All the available evidence suggests that infants of diabetic mothers have an altered weight distribution later in life. They tend to be heavier than normal children both for age and for height. The long-term effect of double-feeding rabbits is very similar, again demonstrating an increase in weight not matched by an increase in length.

In conclusion, it is clear that young mammals have a capacity for increased growth when subject to an excessive energy intake, and this is associated with excessive deposition of adipose tissue. Early high-plane nutrition also enhances growth potential, which is most readily demonstrated in species that are born in an immature state with regard to brain growth. It should be a matter of some concern that the main long-term effect in double-fed rabbits, and infants of diabetic mothers, is an increase in adult weight, a finding that is partially obscured by the normal mature weight distribution. The fact that the nutritional consequences of being an infant of a diabetic mother are similar to the nutritional consequences observed in double-fed rabbits suggests that premature infants would respond to over-feeding in a similar fashion. With present technology many pre-term infants thrive well, and we must be cautious about developments that may lead to excessive weight gains.

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