J. Physiol. (1948) 107, 355–364 612.261:612.647

AN INTEGRATION OF THE TOTAL OXYGEN CONSUMPTION OF THE SHEEP FOETUS FROM THAT OF THE TISSUES

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(*Received* 14 *October* 1947)

Since the literature relating to the respiratory intensity of the mammalian embryo was reviewed by Needham (1931), Barcroft and his co-workers have investigated this problem, working with the goat and sheep, and have obtained values for the foetal oxygen uptake by measuring the oxygen tension and the blood flow through the foetal placental bed (Barcroft, Flexner & McClurkin, 1934; Barcroft, Kennedy & Mason, 1939). Their results tended to show that the values obtained earlier by Cohnstein & Zuntz (1884) were too low. More recent values for placental blood flow, by means of improved methods (Barcroft & Torrens, 1946), gave even higher figures for oxygen consumption (Barcroft & Elsden, 1946). It was felt that, although the improved experimental procedure justified reliance in the later and higher values, it was desirable to check them by an independent method since there was the possibility that experimental interference might have led to unphysiological results.

From figures obtained by Krebs (Krebs, H. A., unpublished), it seemed likely that an integration of the total oxygen consumption of the foetus from the results of tissue slice experiments might yield values comparable with those obtained by direct determination on the whole animal. The present paper describes experiments carried out in an attempt to obtain such integrated values for the sheep foetus over a wide range of ages. The results extend and, in some cases, modify those previously reported in summary form (Carlyle, 1945 *a*, *b*, *c*).

MATERIAL AND GENERAL METHODS

Foetal material was obtained from pregnant ewes of the same Welsh stock as used by Barcroft for previous investigations of this nature. The sheep were tupped at known dates. Pregnancy was interrupted by Caesarian operation under spinal anaesthesia (duracaine) as previously described (Barcroft & Mason, 1938). Where an attempt was made to estimate foetal blood volume, the foetus was killed by bleeding from the carotid arteries; in all other cases death was caused by ligation of the cord. Tissues were removed immediately after death.

The age of the foetus was always checked at operation by reference to standard growth curves for body weight and crown-rump length (Barcroft, 1945, 1946).

Material was obtained from foetuses ranging in age from 51 to 144 days (normal gestation lasts approximately 147 days), also from the newly born lamb and the adult non-pregnant ewe. In all cases, the values given are the mean of duplicate determinations on two animals, the number of animals available making larger groups impracticable. In addition, we were fortunate to obtain values from one ewe whose reputed age was 21 yr. These latter figures are included for purposes of comparison.

For an integration of total oxygen consumption it is necessary to determine, for each tissue, its oxygen utilization rate per unit weight, its total weight as a proportion of the body weight, and the percentage of dry matter in the fresh material. While it would be desirable to deal with every tissue or organ unit, practical considerations have limited us to those which, by virtue of their high oxygen utilization or large mass, seemed likely to contribute significantly to the final value, namely skin, cartilage, bone, muscle, liver, gut, lung, brain and blood.

Dry-weight determinations

It has long been generally believed that, in the mammal, there is a tendency towards a progressive dehydration throughout adult life with increasing age. Numerous data are available to show that such a change occurs during prenatal life (Needham, 1931). Figures for the foetal lung at various ages (Fauré-Frémiet & Dragoiu, 1923), and for various tissues at one age (Davidson & Weymouth, 1944), show that the dry-matter content of sheep foetal tissues is usually lower than that of the adult and may vary considerably according to the foetal age and the particular tissue. It was therefore necessary to obtain fresh values for each tissue at each age.

Methods

The general procedure was to remove a sample of suitable size (usually 0·5–1·0 g.) from the freshly killed animal, wash it quickly in tap water to remove surface contaminant, remove surface moisture with filter paper, place in a weighed watch-glass, weigh immediately, and put into a drying oven at 105° C. Vessels were subsequently reweighed at 24 hr. intervals until constant weight was attained. Except for gut, duplicate samples were taken. In some of the early experiments, additional samples were weighed in closed Thunberg tubes and dried under reduced pressure after treatment with acetone. As no significant difference was observed in the results obtained, or in the time taken to reach constant weight, subsequent determinations were carried out by the simpler method. We are satisfied that, when an open watch-glass was used, no significant error was caused either by evaporation during the initial weighing or resorption of water during the final weighing. The following samples require special note:

Skin. Whole skin, stripped clean of subcutaneous tissue, but including, in animals older than the 97-day foetus, the wool.

Bone. In all cases two samples of midshaft femur.

Cartilage. Suprascapular cartilage.

Gut. The whole gut including oesophagus and rectum was taken as one sample after removal of the contents.

Results

The results are shown in Fig. 1. The values for dry-matter content of the whole body are calculated from those of the individual tissues and the figures for proportionate weight of the tissues are given in Fig. 2. Both individual and total figures confirm the general picture of increasing dry-matter content with age during foetal life, and this trend apparently continues after birth.

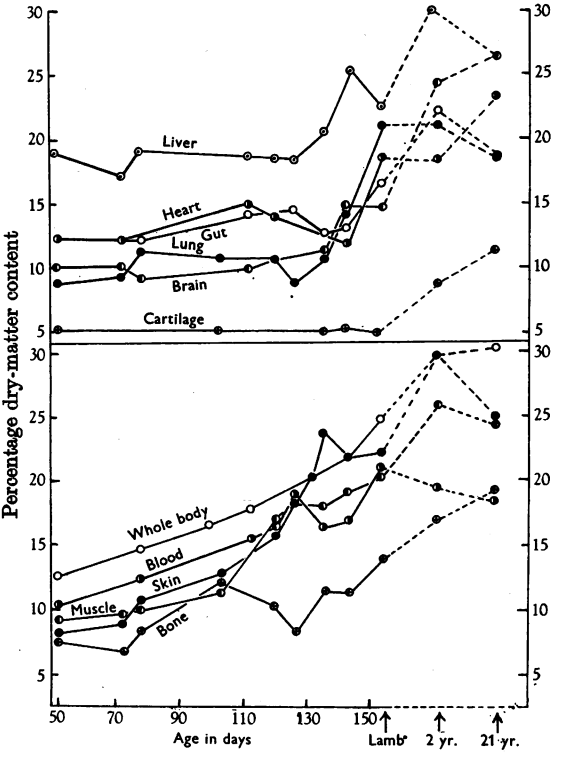

Fig. 1. Dry-matter content of tissues of the sheep foetus, lamb and adult sheep. Cartilage and bone are plotted at $\frac{1}{4}$ scale along the ordinate.

Comparison of the values for the 2 yr. old ewe with foetal values on the one hand, and those of the 21 yr. old ewe on the other, shows a general increase in dry-matter content during the period from birth to maturity, but there is no evidence of a further increase in extreme age, except in the skeletal tissues.

Although there is a general tendency throughout foetal life towards loss of tissue water, all the tissues or organs do not behave alike. There is a considerable variation both as between dry-matter content of different tissues at the same age, and the change in dry-matter content of individual tissues over a given age range.

Reference to Fig. 1 shows that the tissues or organs appear to fall into two main groups: those in which the dry-matter content rises throughout foetal life, and those in which it remains relatively constant until the end of gestation, when it rises rapidly. The first group contains mainly supporting structures, the second the organs. Rather surprisingly, bone shows a progressive water loss, but cartilage maintains the same content until after birth. The dry matter in each tissue varies independently, and that in the whole body is the sum of that in the parts, there being no common causal factor operating equally on all tissues.

Weights of tissues as a proportion of total body weight

Methods

The material was taken as previously described. The following tissues require special note:

Skin. The whole integument was dissected off the animal, freed from adherent subcutaneous tissue, and weighed. In foetuses older than 100 days the wool became wet owing to immersion in the saline bath when Caesarian section was performed. Before removing the skin, the wool was dried as far as possible by rubbing with dry towels. In all cases it was necessary for subsequent calculations that the weight of the actual skin as distinct from that of the wool should be known. As shaving the whole animal was impracticable, two representative areas of skin were taken, and weighed before and after shaving. Comparative figures for whole skin and shaved skin are given in Fig. 2.

Gut. The entire gut was treated as described in the preceding section, and weighed in two fractions: the stomach, including oesophagus, and intestines.

Blood was collected by bleeding out the live animal from both carotids into a weighed vessel.

Inert material. Included in this category are the wool, and the contents of the gut.

Muscle. After removal of the skin, blood, brain and viscera, the carcass was then weighed. This weight was taken to represent muscle plus skeletal structures. The muscle was then dissected away from the skeleton as cleanly as possible. This process took some time. Any part of the carcass not being worked on was kept in a damp cloth to minimize loss by evaporation. After cleaning had been completed, the skeleton was reweighed and the weight of muscle obtained by difference.

Skeleton. This presented two difficulties. It proved impossible to remove the last traces of adherent ligament and tendon by physical methods. An attempt was made to complete the cleaning by partial maceration, but this was not successful as it affected the cartilage. The figures presented for skeletal weight are thus slightly too high, but the error involved is not great. The more important problem is that of determining the relative proportions of bone and cartilage. Though in the adult animal these two constituent parts are well defined, zones of transition being small, this is not so in the foetus. Here it is difficult to speak of bone or cartilage in the sense of separate uniform tissues, the major part of the skeleton being in the process of transition, and mechanical separation impossible. But, because the skeleton forms such a large proportion of the whole organism, and since an error in the computation of its oxygen uptake would materially affect the final figure, it is necessary to know not only the relative weight of the skeleton as a whole but, also, how much is bone and how much cartilage. Here, and subsequently, we have used the term cartilage in a special sense, loosely to connote all such tissues, permeating or closely adherent to bone, having approximately the same water and calcium content and Q_{O_2} as the definitely cartilaginous structures. The procedure involved a preliminary maceration of the skeleton in water for 14 days. The resultant loss in weight is shown in Table 1 (column 1).

From the appearance of the macerated skeleton it was apparent that not all the cartilage had been removed. To determine the amount of cartilage unmacerated, each skeleton, after maceration, was divided into three parts: (*a*) femur (=bone); (*b*) suprascapular cartilage (=cartilage); and (*c*) the rest of the skeleton. Each of these was estimated for dry-matter content and for calcium.

From these results the ratio of bone to cartilage in the macerated skeleton was calculated. Combination of these results gives an estimate of the cartilage and bone in the fresh skeleton (Table 1, columns 4 and 5).

In two animals (51-day foetus and 21 yr. old ewe), a simpler procedure was adopted. A dry-weight determination was carried out on the fresh skeleton by means of three samples as before, and the ratio in the fresh skeleton calculated directly. These results are incorporated in Table 1, where the average dry weights for the whole skeleton are also given (column 6).

TABLE 1. The proportion of bone and cartilage in the fresh and macerated skeleton (expressed as percentage of total body weight)

	Loss on maceration (cartilage)	Macerated skeleton		Fresh skeleton		
		Cartilage	Bone	Cartilage	Bone	Dry weight %
	1	2	3	4	5	6
Foetus 51 days	—	—	—	5·9	5·8	25
109 „	18·4	3·3	4·3	22	4·3	28
112 „	15·3	4·9	5·8	19	5·8	29
136 „	12·9	2·8	7·4	16	7·4	28
143 „	10·3	5·8	6·9	16	7·0	28
Lamb 6 days	8·8	6·1	10·1	15	10	36
Adult 2 yr.	3·9	1·7	5·0	5·6	5·0	49
Adult 21 yr.	—	—	—	4·4	9·1	67

Results

The results are given in Fig. 2. Owing to the small number of animals represented by each value, there is a certain irregularity due to individual

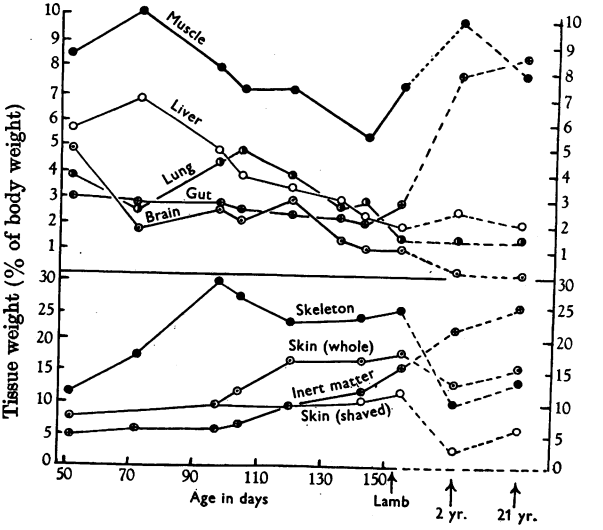

Fig. 2. The weight of tissues of the sheep foetus, lamb and adult sheep, as a percentage of the total body weight. Muscle is plotted at ¼ scale along the ordinate.

variation. In spite of this, two points stand out clearly: the different organs do not all develop at the same rate; while some are growing faster than

the animal as a whole, e.g. skeleton and skin, others are growing more slowly, e.g. brain, muscle and gut. As in the previous section, there appears to be considerable tissue independence.

Oxygen uptake of the tissues

Methods

The oxygen consumption was measured in Warburg manometers by the simplest method, i.e. oxygen uptake in glucose-phosphate-buffer with pure oxygen as the gas phase, carbon dioxide being absorbed with caustic potash. The preparation of the different tissues is described below.

Owing to the limited number of animals available, it was desirable that, if possible, values for all the tissues studied should be obtained from each animal and, as only six experimental manometers could be utilized at any one time, only three of the tissues could be studied in the normal state, that is immediately after death of the animal, and the respiration of the others was examined after a lapse of anything up to 2 hr. Fuhrman & Field (1943) had reported that certain tissues in the rat could be kept at 2° C. for an hour without alteration in their oxygen uptake as subsequently measured at 37° C. Preliminary experiments with this technique for foetal tissues of the sheep indicated that, with the exception of brain, the oxygen consumption after such treatment was not significantly different from that of the fresh tissue. Accordingly, for oxygen uptake of brain, liver and blood, material from the freshly killed animals was used. Other material was taken at the same time and kept in Ringer phosphate at 2° C. until required. Throughout the experiment, the second series of determinations was made after 1 hr. on muscle, stomach and intestine, and the third series on lung, cartilage and bone after 2 hr. After temperature equilibration of the flasks, readings were taken at 15 min. intervals, duplicate samples of each tissue being used. From liver, lung, cartilage and skin, tissue slices were cut in the ordinary way. Treatment of the other tissues was as follows.

Bone. A small piece of bone was broken up into fragments of approximately 1 mm. cube.

Muscle. The standard sample was a small square of diaphragm. In some cases a third flask containing teased skeletal muscle was used. Where this was done, the oxygen uptake agreed with that of the other two flasks.

Gut. It was not found possible to slice this material. Small squares were taken and teased into smaller pieces. In the young foetus it was sufficient to strip the mucosa from the muscularis.

Brain. A sample of cerebral cortex, similar to that taken for dry-weight determinations, containing both grey and white matter, was used. The dry matter was determined by drying the whole contents of the flask, weighing, and subtracting the dry weight of 3 c.c. of the buffer as determined separately. Later an adjustment was made to allow for the fact that 3 c.c. of suspension contained less than that volume of the aqueous medium.

It is realized that the treatment of some of these tissues is open to the objection that the thickness of the particles was greater than that considered desirable for free diffusion of oxygen. We regard this as unavoidable.

Results

As will be seen from Fig. 3, the tissues fall into two main groups: those in which O_2 uptake increases with increasing age (brain, intestine, stomach and possibly lung), and others in which it falls.

It would be interesting to speculate as to the significance of the sharp peak at birth shown by the curve for brain. Also interesting is the fact that the values for the 21 yr. old sheep show no general fall, although this animal appeared senile.

Integration of the total oxygen uptake

From the results described above it is possible to estimate the oxygen consumption for the whole animal from the uptake of the isolated tissues. Total oxygen consumption is expressed as c.c. O_2/kg. body weight/min. From the percentage dry matter of the tissues or organs and their percentage of the total body weight, the dry matter of each in g. can be calculated for each kg. of body weight. The expected oxygen uptake of that weight of dry matter can then be

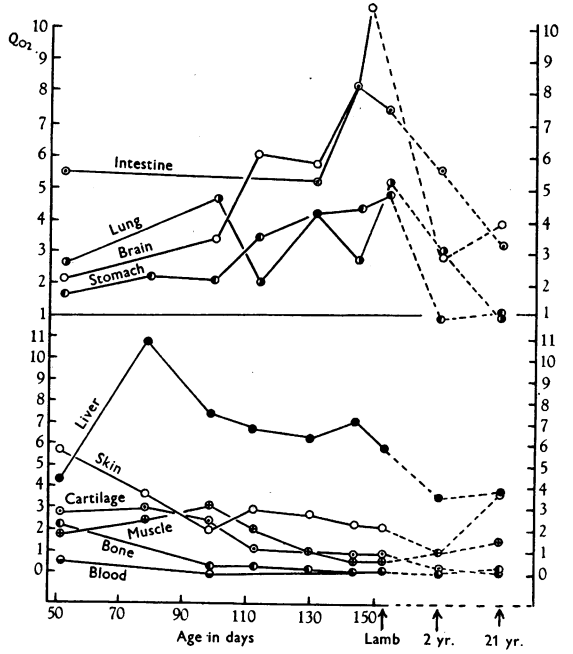

Fig. 3. Q_{O_2}'s of tissues of the sheep foetus, lamb and adult sheep. The value for brain in the lamb (13·6) is not shown in the figure.

obtained. By doing this for each of the selected tissues, a certain oxygen uptake, x c.c., corresponding to tissues which constitute y% of the total body weight is found. Of this total body weight a certain fraction, z%, is inert matter having no oxygen consumption. Since we have only dealt with certain tissues, $y < (100-z)$. It is assumed that the average oxygen consumption of the tissues not accounted for is not materially different from the average of those measured, and, accordingly, that the figure given by $\frac{x(100-z)}{y}$ will approximate to the oxygen uptake of the whole. It will be seen from the tables that the selected tissues constitute, in all cases, not less than 88% of the total body weight. Details of these calculations at one selected age are given in Table 2. The final figures are summarized in Table 3.

TABLE 2. Detailed calculation for integrated oxygen uptake of whole foetus at 51 days

Tissue	Body weight (%)	Dry matter (D.M.) (%)	g. D.M./kg. body weight	Q_{O_2}	Oxygen uptake c.c./min./kg. body weight
Skin	7·8	8·2	6·4	5·7	0·61
Cartilage	5·9	21·7	12·8	2·8	0·60
Bone	5·8	29·4	17·0	2·2	0·62
Muscle	35·3	9·1	32·2	1·8	0·97
Liver	8·2	19·4	15·9	4·3	1·14
Intestine	1·6	12·6	2·0	5·5	0·18
Stomach	1·6	12·6	2·0	2·6	0·09
Lung	4·3	9·1	3·9	1·7	0·11
Brain	5·2	10·4	5·4	2·1	0·19
Blood	8·0	10·4	8·3	0·5	0·07
	83·7		105·9		4·58
Dead matter	5·0				
Total	88·7				

Scaled total = 4·58 × 95/83·7 = 5·2 c.c./min./kg.
Total on dry matter = 4·58 × 1000/105·9 = 43·3 c.c./min./kg.
Total dry weight % = 12·7.

TABLE 3. Summary of oxygen uptake by whole tissues (c.c./kg. body weight/min.)

	Foetal age in days						Lamb	Adult	
Tissue	51	78	99	112	130	144	6 days	2 yr.	21 yr.
Skin	0·61	0·60	0·41	0·74	1·05	0·92	0·98	0·18	0·87
Skeleton	1·22	1·76	2·36	0·54	0·35	0·54	0·57	0·10	0
Muscle	0·97	1·77	1·71	1·34	0·77	0·50	0·62	2·1	2·0
Liver	1·14	1·83	1·16	0·83	0·63	0·73	0·47	0·47	0·33
Gut	0·27	0·28	0·32	0·25	0·28	0·39	0·56	1·0	0·65
Lung	0·11	0·14	0·29	0·30	0·21	0·20	0·28	0·16	0·05
Brain	0·19	0·09	0·15	0·30	0·18	0·26	0·45	0·04	0·004
Blood	0·07	0·06	0·05	0·04	0·05	0·03	0·04	0·05	0·09
Other tissues	0·62	0·30	0·06	0·32	0·56	0·47	0·14	0·50	0·19
Total	5·2	6·9	6·5	4·7	4·1	4·0	4·1	4·6	4·2

DISCUSSION

It is necessary now to consider the validity of the final integrated figures, and to compare them with values obtainable for the actual oxygen uptake of the whole animal. Differences might be found on both technical and physiological grounds. The indirectness of the method favours the introduction of additive errors at each stage, producing an error of large magnitude in the final figure. A particularly large error might occur in the determination of oxygen uptake, owing to the difficulties of handling some of the tissues. Furthermore, in respect to oxygen uptake, the conditions under which the values were obtained differed in important respects from those present in the intact animal. These include the difference in partial pressure of oxygen and carbon dioxide, the absence of bicarbonate from the medium, and, in some cases, mechanical disturbance of the tissue. At best, there is reason to suppose that the integrated figures would be somewhat lower than those obtaining in vivo since the tissues would be under basal conditions; in particular we have to consider the effect

of loss of voluntary muscle tone, cessation of such bodily activities as the heart-beat and the changed conditions of secretory activities.

In view of these a priori arguments, the agreement shown, when the figures are compared with those obtained on the whole animal, is remarkable. It will be seen that although the figures obtained in vivo are substantially higher at the earlier ages, they are of the same order of magnitude, and show the same variation with respect to age. Agreement at the later ages is very close. It may be noted that, in so far as the integrated figures are less than those obtained by Barcroft, and higher than those of Cohnstein & Zuntz, they support the view that the earlier figures are too low.

In view of this agreement we feel that the integrated values are sufficiently reliable to justify consideration of their possible significance.

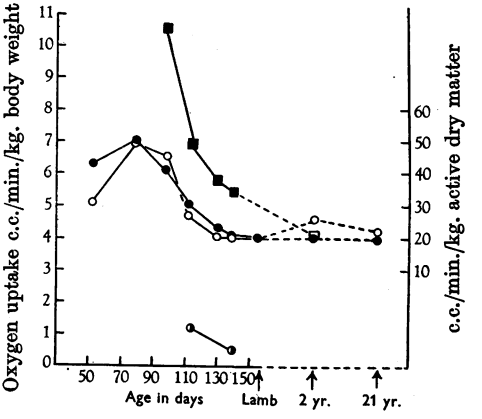

Fig. 4. A comparison of values of total oxygen consumption, in c.c./min./kg. body weight, obtained in vivo for the sheep foetus (■—■ Barcroft & Elsden, 1946; ◑—◑ Cohnstein & Zuntz, 1884), and for the resting adult sheep (□ Carlyle, unpublished indirect calorimetry results), with those obtained by integration (○—○). The integrated values are also expressed as c.c./min./kg. active dry matter (●—●).

The decreasing oxygen consumption per unit of body weight with increasing body weight as shown in Fig. 4 agrees with similar results in the earlier literature (Needham, 1931) and later work for the goat (Barcroft, *et al.* 1934).

In so far as oxygen consumption is a measure of energy utilization, this indicates a falling metabolic rate with increasing body weight, as is implied in the formulae widely used for predicting metabolic rates in adult animals from the body weight, such as that put forward by Brody, Procter & Ashworth (1934):

$$Q = 70{\cdot}5 \times M^{0{\cdot}734},$$

where Q = cal./day, and M is the body weight in kg. The integrated figures for oxygen consumption obtained here suggest that this correlation holds for the sheep foetus. We have examined the data presented to see if they elucidate this relationship. They indicate that four changes are likely to affect the total oxygen consumption per unit of fresh weight: (1) progressive loss of tissue water, (2) increase of inert material, (3) change in body composition in favour of tissues of low oxygen uptake, (4) tendency in some tissues at least towards a lowered oxygen utilization rate per unit of dry matter.

Comparison of the curves for oxygen uptake per unit of total body weight, and per unit of active dry matter (Fig. 4), suggests that the change in total uptake is not determined by the simple operation of the first two factors, but is to be explained by the change in body composition in favour of tissues of a relatively lower Q_{O_2}, together with the decrease in the Q_{O_2} of certain tissues.

The tendency, noted above, in certain tissues for Q_{O_2} to fall with increasing age is interesting. A possible explanation would be that it was consequent upon biophysical changes in intracellular environment resulting from the decrease in water content, but the available data tend to disprove such a contention.

SUMMARY

1. Figures are presented for the dry-matter content, oxygen uptake, and relative weight of certain tissues of the sheep foetus, lamb and adult ewe.

2. Considerable differences were found between the tissues in each series.

3. On the basis of these figures, integrated values for the total oxygen consumption of the whole animal are presented.

4. The values so obtained show considerable agreement with those of other workers for the intact animal.

5. The validity of these results and their possible significance are discussed.

The author wishes to acknowledge his very great indebtedness to the late Sir Joseph Barcroft, under whose direction the work was carried out, for his advice and interest throughout these experiments.

He is also indebted to the Agricultural Research Council for a research grant during the initial period of the work.

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