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BROWN ADIPOSE TISSUE AND THE RESPONSE OF NEW-BORN RABBITS TO COLD

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Adipose tissue is present in mammals in two forms, brown and white. The brown adipose tissue cell contains multiple small fat droplets with a central nucleus, whereas the white adipose tissue cell has a single large fat droplet and an eccentric nucleus. Brown adipose tissue is especially prominent in hibernating, cold-adapted and most new-born mammals. Many speculations as to its function have been made, especially in the hibernating animal (Johansson, 1959). Smith (1962) proposed that brown adipose tissue might be a site of thermogenesis in the cold-adapted rat because of the high *in vitro* oxygen consumption of the tissue. Smith & Hock (1963) have also shown that the subcutaneous temperature close to brown fat rose above the rectal temperature in the marmot during the early stages of arousal from hibernation.

The new-born rabbit resembles the cold-adapted rat in two respects. When exposed to cold both can increase their oxygen consumption even when totally paralysed (Cottle & Carlson, 1956; Dawes & Mestyán, 1963). They also respond to intravenous infusion of noradrenaline with a large increase in oxygen consumption and a rise in body temperature (Hsieh & Carlson, 1957; Scopes & Tizard, 1963). In this paper the participation of brown adipose tissue in the metabolic responses to cold and noradrenaline has been examined in new-born rabbits. A preliminary report of this work has been published (Dawkins & Hull, 1964).

METHODS

The oxygen consumption of new-born rabbits was measured by a closed-circuit method. Temperatures were recorded from copper-constantan thermocouples and displayed continuously on a Cambridge slow recorder. The subcutaneous thermocouples were inserted a distance of 1 cm under the skin. The deep colonic thermocouple was inserted a distance of 2–3 cm from the anus. The tip always lay well within the abdominal cavity. The position of all thermocouples was confirmed by dissection at the end of each experiment. For intravenous infusion, a polyethylene catheter was inserted into a branch of the external jugular

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vein under ether anaesthesia and the animal was allowed to recover. Drugs were infused at a rate of 0·02 ml./min by a motor driven syringe. Noradrenaline bitartrate (Winthrop Laboratories) solution was made up immediately before use and doses were expressed as μg base/kg body weight. Doses of pronethalol (Imperial Chemical Industries Ltd.) were expressed as mg salt/kg body weight.

For examination of the blood chemistry during exposure to cold or after I.V. injection of noradrenaline, rabbits were delivered by Caesarean section at term. Only animals weighing more than 40 g were used. Immediately after delivery the litter was placed in an incubator at 35° C. Two hours after delivery animals were exposed in individual containers to an ambient temperature of 20° C and killed at varying intervals. Blood samples were taken by cutting both jugular veins and collecting the blood in a tube containing 0·05 ml. of 0·4 % wt./vol. heparin with 8 % sodium flouride. A sample of blood was deproteinized immediately for estimation of blood glucose and lactate and the plasma separated for glycerol and free fatty acid analyses. Control litter mates kept at 35° C were killed simultaneously. Noradrenaline was given in a single I.V. injection (25 μg/kg) and animals killed at varying intervals after injection. Control litter mates injected with 0·15 M-NaCl were killed simultaneously. Blood glucose was measured by the method of Huggett & Nixon (1957), blood lactate by the method of Barker & Summerson (1941), plasma free fatty acids by the method of Dole & Meinertz (1960) and plasma glycerol by the method of Hagen & Hagen (1962) and in some instances also by the method of Vaughan (1962), which gave good agreement.

Tissue studies. Care was taken to dissect all fragments of muscle from brown adipose tissue in studies on its composition and metabolism. Interscapular white adipose tissue from pregnant adult rabbits was used to compare with brown adipose tissue from new-born rabbits. No differences in composition and behaviour were found between the interscapular white adipose tissue from pregnant and non-pregnant adult rabbits.

Total fat was estimated by the method of Folch, Lees & Sloane-Stanley (1954), phospholipid by the method of Kennedy (1953), cholesterol by the Lieberman–Burchard reaction as described by Stadtman (1957), and chromatographic separation of fats was performed on columns of silicic acid (Mallinckrodt 100 mesh), by the method of Börgstrom (1952). Tissue fatty acids were measured by the method of Dole & Meinertz (1960), tissue glycerol by the enzymatic method of Vaughan (1962), tissue nitrogen by the micro-Kjeldahl method, glycogen by the method of Caroll, Longley & Roe (1956), succinoxidase activity by the method of Schneider & Potter (1943) and glycerol kinase activity by the method of Bublitz & Kennedy (1954).

Oxygen consumption of slices was measured by standard manometric techniques in Krebs–Ringer-phosphate solution containing NaCl (0·15 M) 100 parts; KCl (0·154 M) 4 parts; $CaCl_2$ (0·11 M) 3 parts; $MgSO_4.7H_2O$ (0·154 M) 1 part; 0·1 M phosphate buffer pH 7·4, 21 parts and glucose 200 mg/100 ml., and the release of glycerol and free fatty acids from slices was measured according to the system described by Vaughan (1962) in Krebs–Ringer bicarbonate solution containing NaCl (0·15 M) 100 parts; KCl (0·154 M) 4 parts; $CaCl_2$ (0·11 M) 3 parts; KH_2PO_4 (0·154 M) 1 part; $NaHCO_3$ (1·3 %) 21 parts; glucose, 0·2 % and bovine albumen 3 %. Tissue fractionation of subcellular particles was performed as described by Dawkins (1959).

RESULTS

Distribution and composition of brown adipose tissue

Brown adipose tissue in new-born rabbits is situated between the scapulae and around the neck (Fig. 1). Deposits of adipose tissue are also present in these regions in the adult rabbit but are then composed of white adipose tissue. White adipose tissue is found in new-born rabbits in small

amounts in the axillae, inguinal regions and around the kidneys. Scattered islands of a few cells of white adipose tissue are also found in the deeper layers of the skin.

Total fat and phospholipid contents of individual tissues of new-born rabbits weighing 50–60 g are shown in Table 1. Nearly half the wet weight of brown adipose tissue and about a third of the wet weight of white adipose tissue was fat. The low fat content of white adipose tissue of new-

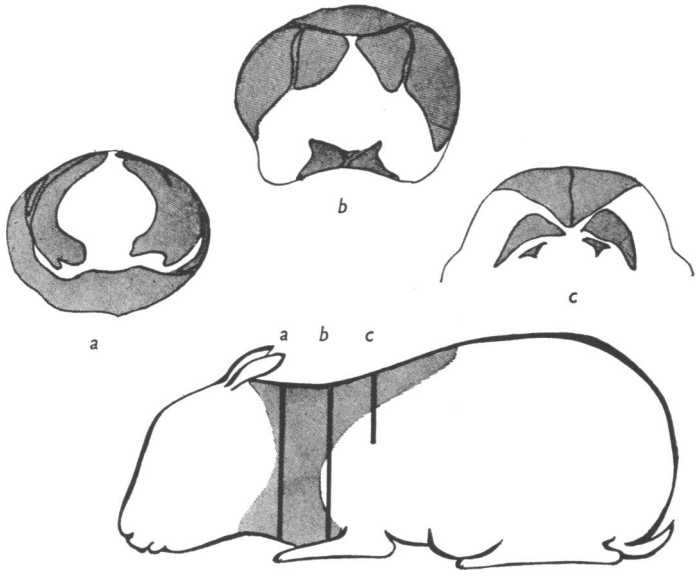

Fig. 1. Site and extent of brown adipose tissue in a new-born rabbit. The sections *a*, *b* and *c* were traced from transverse sections of a whole new-born rabbit cut on a freezing nicrotome and stained for fat with Sudan IV.

born rabbits was in striking contrast to the high fat content of white adipose tissue of adult rabbits (Table 2). In brain, thoracic and abdominal viscera, carcass and skin, phospholipids formed a much larger amount of the fat present. Brown adipose tissue contained about one half of the total body fat. The carcass (the remainder of the animal after removal of the skin, brain, thoracic and abdominal viscera), liver, skin and white adipose tissue were the other major contributors. Histological examination of skin stained for fat showed scattered islands of a few white adipose tissue cells, especially around the nose. If the fat content of the skin was mainly due to the presence of white adipose tissue, then with the recognizable deposits in axilla, inguinal region and perirenal areas, white adipose tissue accounted for less than 30 % of all identifiable adipose tissue in the new-born rabbit.

The third column of Table 1 shows the phospholipid content of individual tissues. Phospholipids are important components of cell membranes and subcellular structures and are probably not available as substrate for immediate oxidation. The phospholipid contents of brown adipose tissue,

TABLE 1. Distribution of fat in individual tissues of eight new-born rabbits weighing 50–60 g. Results are expressed as the mean ± S.E.

	Tissue as % of body wt.	Total fat content (% of wet wt.)	Phospholipid content (% of wet wt.)	Mean total fat content of tissue (g/kg body wt.)
Brown adipose tissue	5·5 ± 0·2	49 ± 0·9	1·8 ± 0·2	27
White adipose tissue	1·4 ± 0·2	35 ± 1·8	0·8 ± 0·2	4·8
Liver	5·5 ± 0·4	16 ± 1·3	0·7 ± 0·1	8·8
Skin	19 ± 0·5	2·7 ± 0·5	0·2 ± 0·1	5·1
Heart and lungs	3·1 ± 0·4	2·5 ± 0·5	0·6 ± 0·2	0·8
Alimentary tract	6·2 ± 0·2	1·2 ± 0·2	0·2 ± 0·1	0·9
Brain	2·4 ± 0·2	2·3 ± 0·6	0·9 ± 0·2	0·6
Carcass (remainder of animal)	50 ± 1·3	2·0 ± 0·3	0·3 ± 0·1	10
Whole body	100	5·8	0·4	58

white adipose tissue and liver formed only a small proportion of the total fat.

Analysis of interscapular adipose tissue from new-born and adult rabbits (Table 2) showed that the fat content of adult adipose tissue (white) was much higher than in the new-born (brown). The glycogen content in both was very low, although greater in the new-born. Nitrogen and water

TABLE 2. Tissue analysis of interscapular adipose tissue from eight new-born and eight adult rabbits. Results are given as the mean ± S.E.

	Lipid (mg/g wet wt.)	Glycogen (mg/g wet wt.)	Nitrogen (mg/g wet wt.)	Water (mg/g wet wt.)
Adult	845 ± 80	0·05 ± 0·01	2·9 ± 0·8	117 ± 9
New-born	482 ± 24	0·36 ± 0·08	8·4 ± 0·6	415 ± 16

contents of interscapular adipose tissue from the new-born rabbit were considerably higher when compared with the adult. This is consistent with the histological appearance of a much greater amount of cytoplasm relative to fat in new-born interscapular adipose tissue.

Analysis of the composition of the fats which could be extracted from interscapular adipose tissue of new-born and adult rabbits is shown in Table 3. In both, the major fraction was triglyceride. However, fat from the new-born contained a significant proportion of monoglyceride, which was not found in the adult. There were also greater amounts of free cholesterol, phospholipids and free fatty acids in the fat from new-born

TABLE 3. Analysis of distribution of lipids of interscapular fat from six new-born and six adult rabbits. Results are given as the mean ± S.E. Free fatty acids are expressed as mg palmitic acid

Lipid fraction	Adult % of total lipid	New-born % of total lipid
Triglyceride	96 ± 3·0	89 ± 2·0
Diglyceride	Nil	Nil
Monoglyceride	Nil	6·0 ± 1·4
Cholesterol free	0·17 ± 0·03	0·44 ± 0·14
Cholesterol esterified	Nil	Nil
Phospholipid	0·7 ± 0·08	3·7 ± 0·7
Free fatty acids	0·07 ± 0·03	0·19 ± 0·04

interscapular adipose tissue than from the adult. These fractions made up only a small proportion of the total fat in both cases.

Changes in the amount and fat content of brown adipose tissue were examined in rabbits before and after birth (Fig. 2). Brown adipose tissue was dissected *en bloc* from between the scapulae and around the neck and weighed. Histological examination revealed that only small amounts of muscle were included in this block of tissue. The weight of brown adipose tissue relative to body weight and the fat content reach a maximum at birth. Most of the fat appears in the tissue during the last week of gestation (term = 31 days). After birth the weight of the tissue relative to body

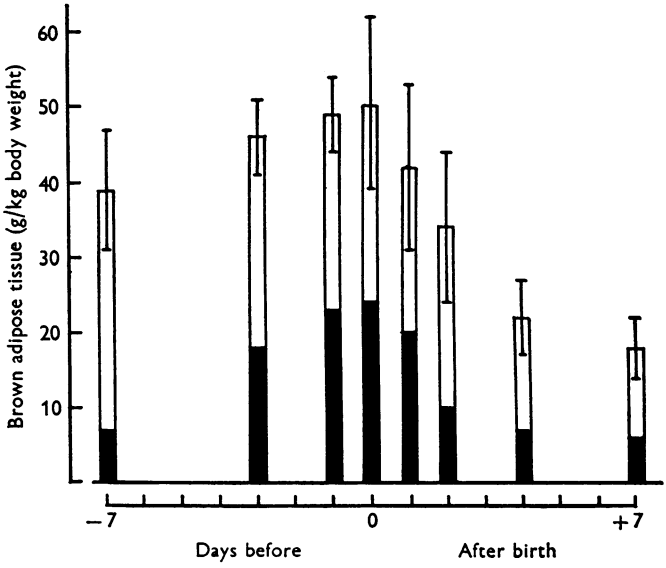

Fig. 2. Amount and composition of brown adipose tissue in rabbits before and after birth. The height of the columns represents the mean wet weight/kg body weight, the vertical bars are the standard deviation and the black area indicates the fat content.

weight falls, partly as a result of the fall in fat content. From a week after birth, brown adipose tissue is gradually replaced by white adipose tissue over a period of three months.

At birth the scatter of values for brown adipose tissue per kilogram body weight is large (mean 50 ± 12 g/kg body weight S.D.). Regression analysis of measurements on sixty-one individual new-born rabbits (Fig. 3)

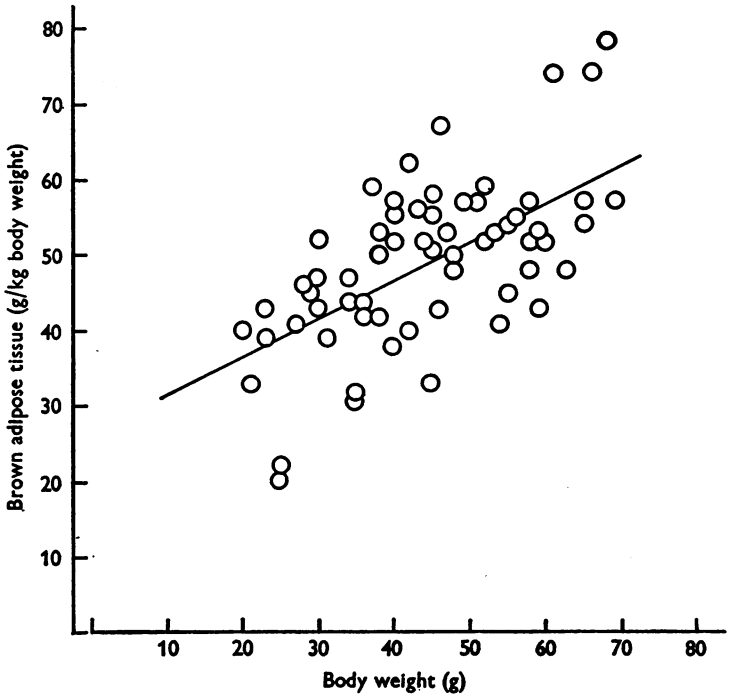

Fig. 3. Relation between body weight and the amount of brown adipose tissue in g/kg body weight in sixty-one new-born rabbits. The regression line is given by the equation $Y = 0{\cdot}52X + 26{\cdot}25$ where Y is amount of brown adipose tissue in g/kg body weight and X is the body weight in g. (Regression coefficient $0{\cdot}52 \pm 0{\cdot}09$ S.E.)

showed that the amount of brown adipose tissue was significantly related to body weight ($P < 0{\cdot}001$). Runts are therefore less well endowed with brown adipose tissue per kg body weight than their larger litter mates.

Local heat production by brown adipose tissue

To investigate the participation of brown adipose tissue in the response of the new-born rabbit to cold (31 rabbits) and noradrenaline (12 rabbits) continuous temperature recordings were made from subcutaneous thermocouples. One was placed between the scapulae over the brown adipose tissue and another in the lumbar region over the sacrospinalis muscle.

In 5 experiments a subcutaneous thermocouple was placed in the anterior abdominal wall over the liver. In all experiments the deep colonic temperature was recorded.

At 35° C the subcutaneous temperatures over brown adipose tissue and lumbar muscles and the deep colonic temperature were similar (Fig. 4). When the ambient temperature was reduced to 25° C, both subcutaneous

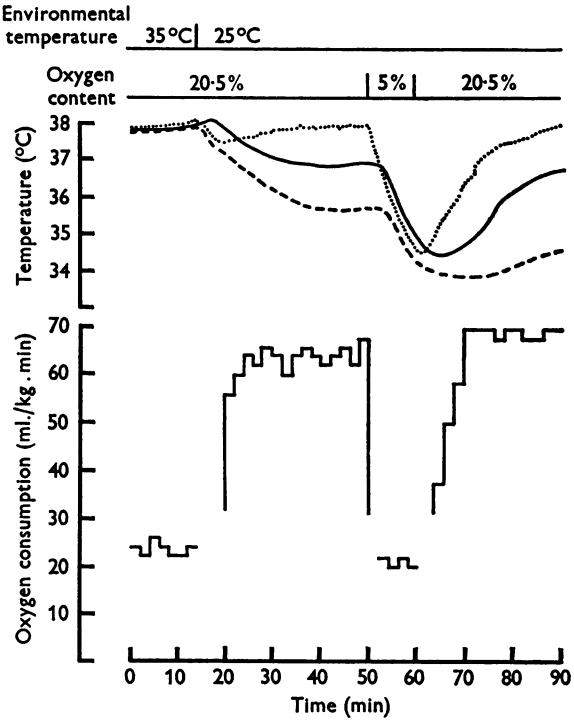

Fig. 4. The effect of cold and hypoxia on oxygen consumption and temperatures in a rabbit weighing 57 g, 12 hr after natural delivery (full line, deep colonic temperature interrupted line, subcutaneous lumbar temperature and dotted line, subcutaneous temperature over brown adipose tissue).

temperatures fell, followed shortly by a fall in a deep colonic temperature. However, the subcutaneous temperature over brown adipose tissue rose again slightly and maintained a steady level, although the lumbar subcutaneous and colonic temperatures continued to fall. After 30 min a steady state was reached, in which there was more than 1° C difference in temperature between the subcutaneous temperature over brown adipose tissue and the deep colonic temperature. The subcutaneous temperature over the lumbar muscle was more than 2° C lower than the subcutaneous

temperature over brown adipose tissue. During this period of exposure to cold the oxygen consumption was maintained at a level approximately three times higher than the minimal oxygen consumption. After 40 min exposure at 25° C the oxygen content of the circuit was reduced to 5% and the oxygen consumption fell. All three temperatures fell, and the subcutaneous temperature over the brown adipose approached the subcutaneous temperature in the lumbar region. When air was again circulated, the temperature over brown adipose tissue immediately increased while the deep colonic and subcutaneous lumbar temperatures continued to fall. The deep colonic temperature started to increase before the subcutaneous lumbar temperature. The oxygen consumption rose again to a level slightly higher than before the exposure to hypoxia.

TABLE 4. Oxygen consumption and subcutaneous and deep colonic temperatures in new-born rabbits at different ambient temperatures. Results are given as the mean ± S.E.

Ambient temperature (°C)	No. expts.	Oxygen consumption (ml./kg body weight/min)	Temperature °C		
			Subcutaneous lumbar	Subcutaneous interscapular	Deep colonic
35	31	23·4 ± 0·46	38·2 ± 0·13	38·6 ± 0·11	38·6 ± 0·11
32·5	11	33·3 ± 2·2	37·3 ± 0·27	38·3 ± 0·29	38·0 ± 0·24
30	13	42·6 ± 2·0	36·3 ± 0·33	37·9 ± 0·27	37·2 ± 0·33
27·5	11	51·5 ± 2·3	35·4 ± 0·38	37·6 ± 0·31	36·3 ± 0·37
25	31	57·5 ± 1·4	34·5 ± 0·25	37·2 ± 0·20	35·9 ± 0·26
At 25° after hypoxia (5–7% oxygen for 10 min).	15	15·0 ± 0·8	32·6 ± 0·43	33·4 ± 0·37	33·3 ± 0·36

In thirty-one new-born rabbits (Table 4) the temperature of the bath was reduced below the neutral range by steps every 30 min. This period was long enough for stabilization of body temperatures and oxygen consumption. The three temperatures gradually separated and the oxygen consumption increased. The differences between the three temperatures were greater and the oxygen consumption higher at lower environmental temperatures. The maximal consumption in this group of new-born rabbits weighing 35–74 g was reached at an ambient temperature between 30 and 22·5° C. All three body temperatures fell in parallel when the maximal oxygen consumption was reached and the ambient temperature was further reduced. The temperature in the anterior abdominal wall close to the liver was lower than the deep colonic temperature at all stages.

The intravenous infusion of noradrenaline 2 μg/kg.min for 10 min in twelve unanaesthetized new-born rabbits caused a large rise in oxygen consumption. The subcutaneous temperature over the brown adipose tissue rose more rapidly and reached a higher temperature than the deep colonic or subcutaneous temperature over the lumbar muscles (Fig. 5). After administration of pronethalol (10 mg/kg), infusion of noradrenaline

did not affect the body temperatures, nor did it produce a rise in oxygen consumption. At the end of infusion of noradrenaline the mean rise in the subcutaneous temperature over brown adipose tissue was $1{\cdot}8 \pm 0{\cdot}1°$ C S.E.; in deep colonic temperature $0{\cdot}9 \pm 0{\cdot}1°$ C S.E. and in subcutaneous temperature over lumbar muscles $0{\cdot}6 \pm 0{\cdot}1°$ C S.E.

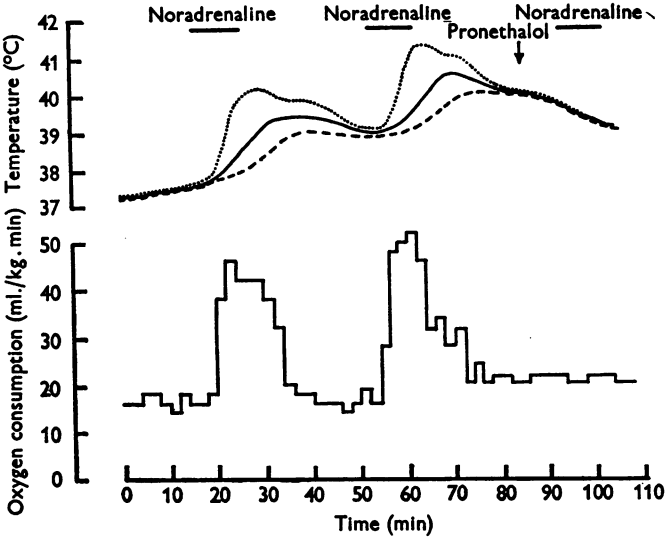

Fig. 5. The effect of intravenous infusion of noradrenaline (2 μg/kg.min for 10 min) before and after pronethalol (10 mg/kg) on oxygen consumption and body temperature (full line, deep colonic temperature, interrupted line, subcutaneous lumbar temperature and dotted line, subcutaneous temperature over brown adipose tissue).

Blood changes during the metabolic response to cold and to noradrenaline

Changes in blood glucose, lactate, glycerol and free fatty acids were measured during exposure to cold or after I.V. injection of noradrenaline in order to investigate the metabolic processes underlying the increase in oxygen consumption. Since removal of blood has been shown to reduce the metabolic response to cold in the new-born rabbit (Dawes & Mestyán, 1963) animals were killed at 5, 10 and 20 min and compared with litter mates killed at the same time and kept at 35° C. Litter mates of those injected with noradrenaline were injected with the same volume of 0·15 M NaCl.

Blood glucose values in unfed rabbits 2 hr after delivery showed a considerable scatter (Fig. 6*a*). Exposure to 20° C produced a gradual increase in blood glucose. The blood glucose after 20 min was significantly higher ($P < 0{\cdot}01$) than in control litter mates. This ambient temperature

always evoked the maximal oxygen consumption in new-born rabbits. Blood lactate levels (Fig. 6*b*) were low in the control animals and rose within five minutes of exposure to cold. This increase is highly significant ($P < 0{\cdot}001$). Administration of noradrenaline by single I.V. injection (25 μg/kg in 0·1 ml.) produced a small but just significant rise ($P < 0{\cdot}05$)

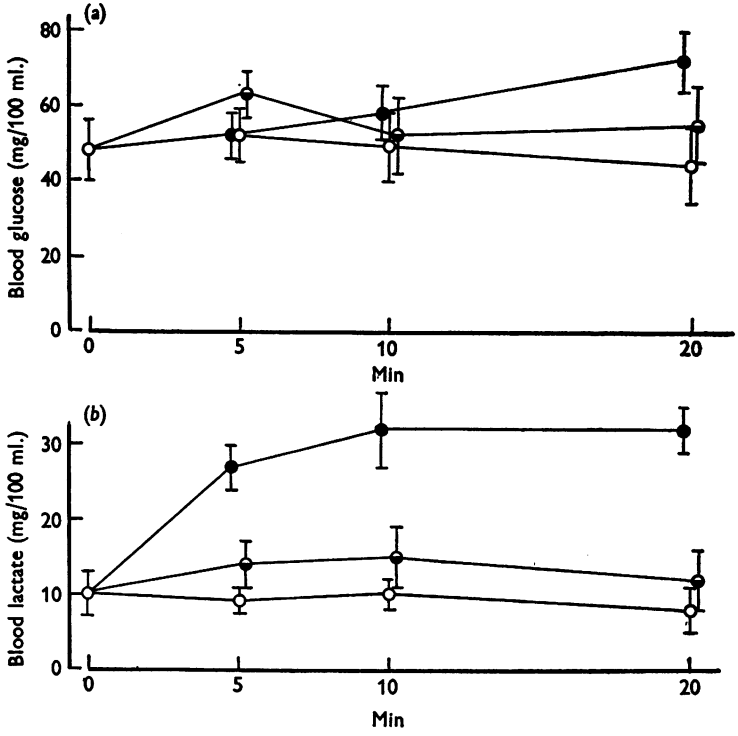

Fig. 6. Changes in blood glucose and blood lactate levels during exposure to 20° C (●) or after injection of noradrenaline (25 μg/kg I.V. ◒) in unfed new-born rabbits two hours after delivery. Control animals (○) were kept at 35° C. Each point and vertical bar represent the mean ± S.E. of at least six rabbits.

in blood glucose (Fig. 6*a*), but no significant change in blood lactate (Fig. 6*b*). This amount of noradrenaline in a single injection produced an initial depression and then an increase in oxygen consumption of 100%, maximal at 6 min and returning to the basal level at 15 min. Control litter mates injected with 0·1 ml. of 0·15 M-NaCl showed no difference from non-injected litter mates.

Exposure to 20° C produced an immediate large and sustained rise in plasma glycerol (Fig. 7*a*) which was highly significant ($P < 0{\cdot}001$). Plasma

free fatty acids also showed a considerable scatter in new-born rabbits kept at 35° C for 2 hr after delivery (Fig. 7*b*). Exposure to 20° C produced a small but just significant rise in plasma free fatty acids ($P = 0{\cdot}05$). The

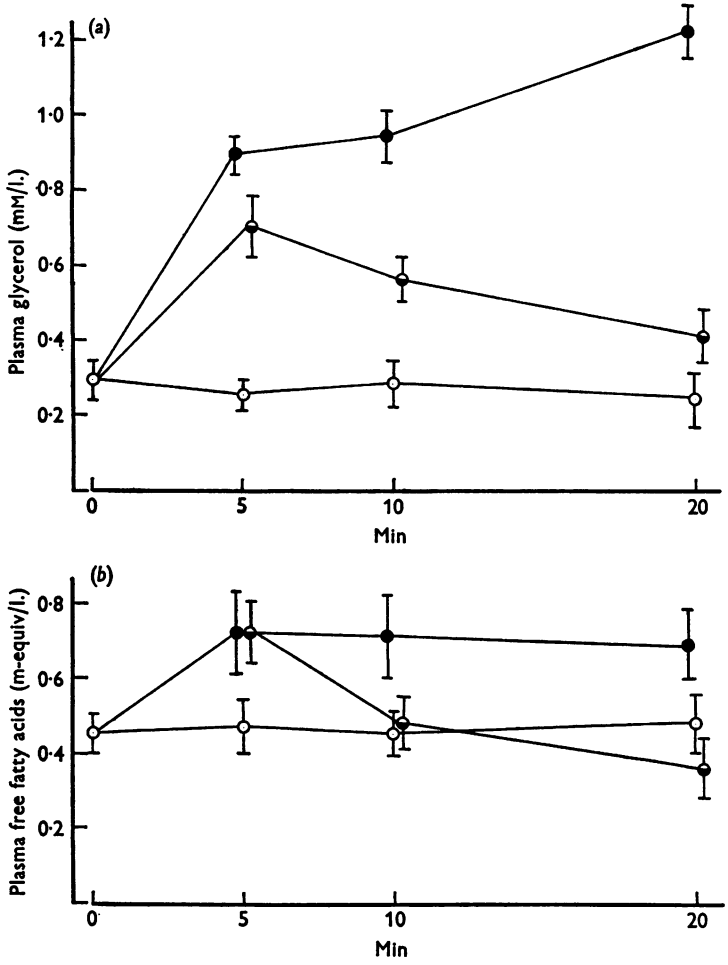

Fig. 7. Changes in plasma glycerol and plasma free fatty acids during exposure to 20° C (●) or after injection of noradrenaline (25 μg/kg I.V. ◒) in unfed new-born rabbits 2 hr after delivery. Control animals (○) were kept at 35° C. The vertical bars represent the S.E. of the mean values which are plotted.

small rise in plasma free fatty acids suggested that most of the free fatty acids liberated were not released from the cell. During exposure to cold there was also a small but significant ($P < 0{\cdot}01$) rise in brown adipose tissue free fatty acids (control $3{\cdot}6 \pm 0{\cdot}3$ μ-equiv/g mean ± S.E., cold exposed

$4{\cdot}8 \pm 0{\cdot}4$ μ-equiv/g mean $\pm$ S.E., $n = 8$). Glycerol levels in brown adipose tissue showed no significant change (control $4{\cdot}9 \pm 0{\cdot}3$ μM/g mean $\pm$ S.E., cold exposed $5{\cdot}4 \pm 0{\cdot}4$ μM/g mean $\pm$ S.E., $n = 8$).

Noradrenaline (I.V. 25 μg/kg in 0·1 ml). also caused a highly significant ($P < 0{\cdot}001$) rise of plasma glycerol (Fig. 7*a*) and a smaller rise in plasma free fatty acids (Fig. 7*b*) which was also significant ($P < 0{\cdot}01$).

Behaviour of brown adipose tissue in vitro

Tissue analysis (Table 2) indicated that the proportion of cytoplasm relative to lipid was much higher in new-born interscapular adipose tissue than in the adult. The oxygen consumption of slices of interscapular

TABLE 5. Oxygen consumption of slices of interscapular adipose tissue in Krebs–Ringer phosphate supplemented with 0·2 % glucose. Results are given as the mean $\pm$ S.E. ($n = 10$). Noradrenaline and insulin were added to the flasks to give final concentrations of 2·5 μg/ml. and 0·1 I.U./ml. medium, respectively.

Tissue from	Oxygen Uptake (ml./g wet wt/hr)
Adult	$0{\cdot}04 \pm 0{\cdot}01$
New-born	$0{\cdot}95 \pm 0{\cdot}10$
New-born + noradrenaline	$1{\cdot}60 \pm 0{\cdot}20$
New-born + insulin	$0{\cdot}97 \pm 0{\cdot}16$
New-born + noradrenaline and insulin	$2{\cdot}16 \pm 0{\cdot}24$

adipose from new-born rabbits was more than twenty times greater than those from adult rabbits (Table 5). Oxygen consumption could be further increased by the *in vitro* addition of noradrenaline. Insulin alone did not alter the oxygen consumption, but potentiated the effect of noradrenaline.

Succinoxidase activity represents the maximum *in vitro* oxidation rate of any of the intermediates of the tricarboxylic acid cycle by a tissue homogenate. It is a useful index of comparative oxidative potential of tissues. When succinoxidase activity of various tissues of new-born and adult rabbit were compared (Table 6) interscapular adipose tissue in the new-born was found to be as active as new-born cardiac muscle and more

TABLE 6. Succinoxidase activity of various tissues from ten new-born and ten adult rabbits Results are given as the mean $\pm$ S.E.

	Adult (ml. oxygen/g wet wt./hr)	New-born (ml. oxygen/g wet wt./hr)
Interscapular adipose tissue	$0{\cdot}2 \pm 0{\cdot}07$	$12{\cdot}3 \pm 1{\cdot}9$
Cardiac muscle	$19{\cdot}5 \pm 1{\cdot}3$	$12{\cdot}0 \pm 0{\cdot}8$
Skeletal muscle (Quadriceps)	$3{\cdot}2 \pm 0{\cdot}4$	$3{\cdot}9 \pm 0{\cdot}7$
Liver	$14{\cdot}0 \pm 0{\cdot}5$	$8{\cdot}2 \pm 0{\cdot}5$
Kidney	$16{\cdot}1 \pm 1{\cdot}3$	$5{\cdot}3 \pm 0{\cdot}6$

active than new-born liver, kidney and skeletal muscle. Succinoxidase activity of interscapular adipose from adult rabbits was very low. Cardiac muscle, liver and kidney had a lower succinoxidase activity in the new-born than in the adult.

Succinoxidase activity is entirely located in the mitochondrial fraction of the cell homogenates. The products of tissue fractionation of interscapular adipose tissue from new-born and adult rabbits were compared (Table 7). The nitrogen content of brown adipose tissue from the new-born was three times higher than that of white adipose tissue from the

TABLE 7. Tissue fractionation of interscapular adipose tissue from eight new-born and eight adult rabbits. Results are given as the mean ± S.E. Wet weight refers to that of the whole homogenate.

	Adult		New-born	
Cell fraction	Nitrogen (mg/g wet wt.)	Phospholipid (mg/g wet wt.)	Nitrogen (mg/g wet wt.)	Phospholipid (mg/g wet wt.)
Homogenate	2·9 ± 0·8	7·1 ± 0·7	8·4 ± 0·6	17·8 ± 1·8
Nuclei	0·7 ± 0·1	1·7 ± 0·1	1·4 ± 0·2	2·1 ± 0·3
Mitochondria	0·4 ± 0·1	1·7 ± 0·1	2·5 ± 0·3	9·5 ± 0·2
Endoplasmic reticulum	0·4 ± 0·2	1·8 ± 0·2	0·9 ± 0·2	4·4 ± 0·6
Supernatant	1·1 ± 0·3	1·3 ± 0·2	3·5 ± 0·5	2·1 ± 0·4

adult. The major differences between new-born and adult lay in the amount of nitrogen in the mitochondrial and supernatant fractions. Phospholipid analysis of cell fractions showed that in both new-born and adult interscapular adipose tissue, most of the phospholipid content was contained in the subcellular organelles and not in the fat droplets which separate in the supernatant fraction.

When slices of white adipose tissue are suspended in a suitable medium, glycerol and free fatty acids are released (Vaughan, 1962). Glycerol cannot be metabolized by white adipose tissue since the necessary glycerol kinase is absent (Margolis & Vaughan, 1962). Glycerol kinase was also found to be absent in homogenates of brown adipose tissue from new-born rabbits but was present in the liver. The rate of release of glycerol in both brown and white adipose tissue is therefore directly related to the rate of hydrolysis of triglyceride.

Rates of release of both glycerol and free fatty acids from slices of adult and new-born interscapular adipose tissue were compared (Fig. 8). Adult white adipose tissue released more free fatty acids but less glycerol than new-born brown adipose tissue. Addition of noradrenaline increased rates of release of glycerol and free fatty acids, but the differences between brown and white adipose tissue remained. The over-all *in vitro* rate of lipolysis in brown adipose tissue was about three times greater than in

white adipose tissue. Theoretically, for every mole of triglyceride hydrolysed three moles of free fatty acid and one mole of glycerol could appear in the medium. From Fig. 8 it was calculated that with white adipose tissue from adult rabbits 1·3 moles of free fatty acid appeared in the medium for every mole of glycerol. However, with brown adipose tissue from new-born rabbits only 0·2 moles of free fatty acids appeared in the medium for every mole of glycerol.

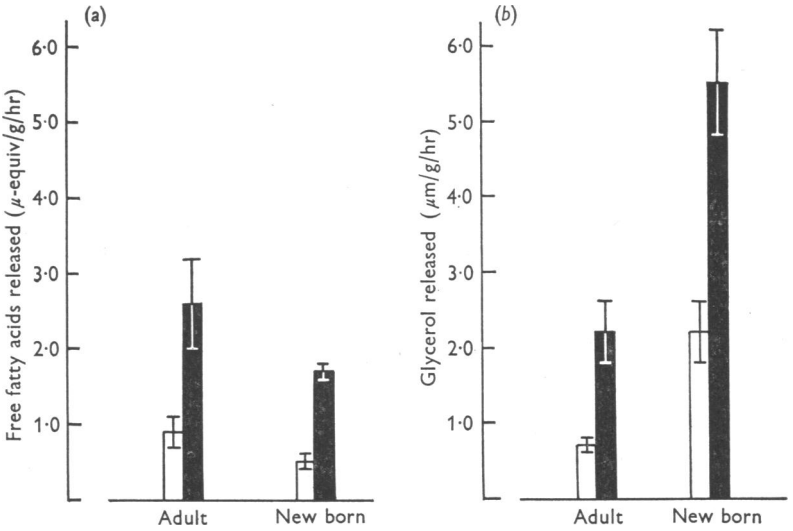

Fig. 8. Release of glycerol and free fatty acids by slices of interscapular adipose tissue suspended in Krebs–Ringer-bicarbonate solution containing 3% bovine albumin and 0·2% glucose, in the presence (solid columns) and absence (open columns) of noradrenaline at a concentration of 2·5 μg/ml. of medium. Results are given as the mean ± standard error ($n = 6$).

DISCUSSION

Composition and distribution of brown adipose tissue

Brown adipose tissue can be found in the interscapular region in most new-born animals. It persists into adult life in the hibernating animal and in some rodents. With cold-adaptation the amount of brown adipose tissue increases (Smith, 1962), but even in the cold-adapted rat the amount is small compared with the new-born rabbit, in which it represented on average 5% of the body weight.

Brown adipose tissue seems to be embryologically quite distinct from white adipose tissue, but whether brown adipose tissue is a stage in the development of white adipose tissue or not is a matter of dispute (Barnett, 1962). Comparative studies of new-born mammals (M. J. R. Dawkins &

D. Hull, unpublished) have shown considerable variation of the amount and distribution of both brown and white adipose tissue. Brown adipose tissue was especially prominent in the interscapular region in the new-born rabbit, guinea-pig and coypu. In the kitten and the lamb the interscapular pad of brown adipose tissue was small, but substantial amounts were found in thin sheets between the trunk muscles and around the kidneys. In the human new-born infant, there were thin sheets of brown adipose tissue deep to the white subcutaneous adipose tissue in the neck and between the scapulae. The new-born rat had only small amounts of interscapular brown adipose tissue. The new-born pig had virtually no adipose tissue of either kind.

Brown adipose tissue from hibernating animals and adult rats is similar to brown adipose tissue from the new-born rabbit. In the adult rat it has a much richer blood supply (Fawcett, 1952) and a higher noradrenaline content (Sidman, Perkins & Weiner, 1962) than white adipose tissue. Histological examination and injections of radio-opaque-material studies have shown a rich nerve and blood supply in brown adipose tissue of new-born rabbits (M. J. R. Dawkins & D. Hull, unpublished). Electron microscopic examination of brown adipose tissue from new-born rats and mice has also revealed a very rich nerve and blood supply and many mitochondria in each cell (Napolitano & Fawcett, 1958). The high *in vitro* oxygen consumption reported for slices of brown adipose tissue from the rat (Joel & Shackney, 1962), and the hedgehog (Fleischmann, 1929) was also found with brown adipose tissue from new-born rabbits. The brown colour of brown adipose tissue in the rat is due to the presence of large amounts of mitochondrial cytochromes (Joel & Ball, 1960). The large proportion of total nitrogen found in the mitochondrial fraction on subcellular fractionation of brown adipose tissue and the high succinoxidase activity relative to nitrogen content suggest that brown adipose tissue of new-born rabbits also contains large amounts of cytochrome pigments. Brown adipose tissue from adult rats has a lower fat content and higher glycogen content than white adipose tissue (Fawcett, 1952). Interscapular adipose tissue from the new-born rabbit also had a lower fat and higher glycogen content than interscapular adipose tissue from the adult. The total body fat of new-born rabbits was found to be 5·8% of the body weight. This figure differs considerably from the figure of 2% reported by Widdowson (1950). About half of the total body fat was found in brown adipose tissue.

Sites of heat production

Possible major sites of heat production in the new-born rabbit are skeletal muscle, liver and heart. New-born rabbits exposed to cold did not shiver readily, but some new-born rabbits shiver when the oxygen con-

sumption is near the maximum. The rise in blood lactate during cold exposure might suggest an increase in glycogenolysis in muscle. However, muscle bulk is considerably less in the new-born rabbit (H. J. Shelley & M. Young, personal communication) than it is in older rabbits. The subcutaneous temperature close to the lumbar muscles in new-born rabbits exposed to cold was always considerably lower than the deep colonic temperature, even in animals observed to be shivering.

The liver has been proposed as an important site of heat production in the cold-adapted rat exposed to cold (Donhoffer, Szegvar, Varga-Nagy & Jarai, 1957) or infused with noradrenaline (Hannon, Evonuk & Larson, 1963). The reduction in oxygen consumption at the neutral temperature in young kittens subjected to functional evisceration and the reduction of the increase in oxygen consumption during intravenous infusion of noradrenaline led Scopes & Tizard (1963) to suggest that the liver was a site of heat production in the young kitten. Blood glycerol and lactic acid levels rose considerably and plasma free fatty acid levels rose slightly during cold exposure. All these substrates could be oxidised by the liver. The increased cardiac output in new-born rabbits exposed to cold (Dawes & Mestyán, 1963) must also contribute to the increase in oxygen consumption and the heat produced. The *in vitro* oxygen consumption of cardiac muscle is as high as brown adipose tissue (Table 6) but it only represents 0·5% of the body weight.

Adipose tissue has generally been regarded as a storage tissue and as an insulating material. However, the recent demonstration of considerable metabolic activity in white adipose tissue prompted Cahill (1962) to suggest that adipose should be thought of 'not merely as a simple insulating blanket, but perhaps as an electric blanket'. Clearly brown adipose tissue would be more important in this respect because of its much higher metabolic activity. Smith (1962) has suggested that brown adipose tissue may be a site of thermogenesis in the cold-adapted rat and in hibernating animals during arousal from hibernation (Smith & Hock, 1963). In the new-born rabbit exposed to 25° C (Fig. 4) at the steady state the subcutaneous temperature over interscapular brown adipose tissue was 2·7° C higher than the subcutaneous temperature over the lumbar muscles and 1·3° C higher than the temperature deep inside the abdomen. The higher temperature recorded from the more superficial site could only be due to local heat production or to a selective increase in blood flow from some other site at an even higher temperature. Possible sites at a higher temperature are the liver and the heart. However, the temperature close to the liver surface was always lower than the deep colonic temperature and it seems unlikely that heat produced by cardiac work should not be recorded by the deep colonic thermocouple.

A selective increase in blood flow to brown adipose tissue has in fact been demonstrated in hibernating animals during arousal (Johansen, 1961) and in the cold-adapted rat during infusion of noradrenaline (Hannon *et al.*, 1963). We have also seen flushing of exposed brown adipose tissue in lightly anaesthetized new-born rabbits exposed to cold. Such an increase in blood flow would be an advantage in heat exchange between a local site of thermogenesis and the rest of the animal.

The heat production by brown adipose tissue was clearly demonstrated by experiments in which a 10 min period of hypoxia interrupted the metabolic response to cold (Fig. 4). The inhibition of the metabolic response to cold by hypoxia in new-born and small adult animals has been well documented (Hill, 1959). During hypoxia the interscapular subcutaneous temperature fell rapidly and approached the lumbar subcutaneous temperature. This indicated that the insulation over the two sites was similar. After hypoxia the subcutaneous temperature over brown adipose tissue rose immediately whilst the lumbar subcutaneous and deep colonic temperatures *continued to fall.* The actual temperature recorded in any tissue at equilibrium is determined by the local tissue blood flow, local thermal conductivity and environmental temperature. We are unable to make any quantitative assessment of the contribution of brown adipose tissue to the total heat production of new-born rabbits. However, its mass relative to body weight, rich blood supply and higher *in vitro* oxygen consumption all indirectly suggest that it could play a significant part in non-shivering heat production in new-born rabbits.

The mediator of the metabolic response to cold

In new-born rabbits both exposure to cold and administration of noradrenaline caused a large increase in oxygen consumption and therefore heat production. It has been proposed that noradrenaline is the mediator of the response to cold (Moore & Underwood, 1963). Both cold and noradrenaline lead to lipolysis (p. 233) and heat production in brown adipose tissue. If noradrenaline is the mediator of the metabolic response to cold by virtue of its ability to stimulate an adipose tissue lipase (Rizack, 1961) it could reach the adipose tissue cell either through the blood stream or by local liberation at sympathetic nerve endings. Since pronethalol blocks the increase in oxygen consumption following intravenous infusion of noradrenaline in new-born rabbits without affecting the metabolic response to cold (Hull, 1963), circulating noradrenaline is probably not involved in the metabolic response to cold. Local release of noradrenaline from sympathetic nerve-endings is a possible mechanism which is supported by the observation that denervation of brown adipose tissue in adult rats prevented mobilization of fat on exposure to cold (Sidman &

Fawcett, 1954). In this context the high noradrenaline content of brown adipose tissue (Sidman *et al.*, 1962) and the many nonmyelinated nerve fibres terminating in relation to brown adipose tissue cells (Napolitano & Fawcett, 1958) are significant.

The chemical basis of heat production

The changes in blood glucose during exposure to cold and after intravenous injection of noradrenaline were small. Similar small changes in blood glucose and blood lactate during intravenous infusion of noradrenaline were also reported by Scopes & Tizard (1963). Infusion of glucose and sodium lactate has no effect on the oxygen consumption of new-born kittens (Moore, 1963) and new-born rabbits (Scopes & Tizard, 1963; D. Hull, unpublished). The metabolic responses to cold and noradrenaline are probably not related to oxidation of glucose or lactic acid in these species.

Oxidation of fat has long been thought to be important in the metabolism of the animal exposed to cold. Alexander (1962) has shown that most of the calories produced by the cold, starved, new-born lamb are derived from oxidation of fat. Catecholamines increase the level of plasma free fatty acids in adult animals (Dole, 1956) and the rate of release of free fatty acids from isolated slices of adipose tissue (White & Engel, 1958). Exposure to cold causes a marked increase in urinary catecholamines (LeBlanc & Nadeau, 1961) and a rise in plasma free fatty acids (Hannon, *et al.*, 1963). An increased capacity of adipose tissue to release free fatty acids has also been demonstrated in the cold-acclimatized rat (Hannon *et al.*, 1963). These authors also showed a fall in R.Q. when cold-acclimatized rats were exposed to cold or given an I.V. infusion of noradrenaline. They concluded that non-shivering thermogenesis and the calorigenic response to noradrenaline were largely supported by oxidation in the liver of plasma free fatty acids mobilized from adipose tissue by noradrenaline.

In new-born rabbits exposed to cold or given an I.V. injection of noradrenaline, the rise in plasma free fatty acids was small compared with the marked rise in plasma glycerol. Brown adipose tissue free fatty acids also showed only a small rise during exposure to cold. The rise in plasma glycerol indicated that hydrolysis of triglycerides was taking place in adipose tissue, both on exposure to cold and after injection of noradrenaline. The plasma half-lives of glycerol and free fatty acids in the rabbit are both about 2–3 min (Bierman, Schwartz & Dole, 1957; Hagen, 1963). It may be concluded that most of the free fatty acids liberated by hydrolysis of triglycerides in brown adipose tissue of new-born rabbits did not appear as plasma free fatty acids, but were metabolized within the cell. This conclusion was supported by the *in vitro* studies of glycerol and free fatty

acid liberation from slices of brown and white adipose tissue. The rate of release of glycerol was faster from brown adipose tissue than from white adipose tissue, indicating a more rapid hydrolysis of triglycerides in brown adipose tissue. However, the rate of release of free fatty acids from brown adipose tissue was slower than from white adipose tissue. A slower rate of release of free fatty acids from brown adipose tissue compared with

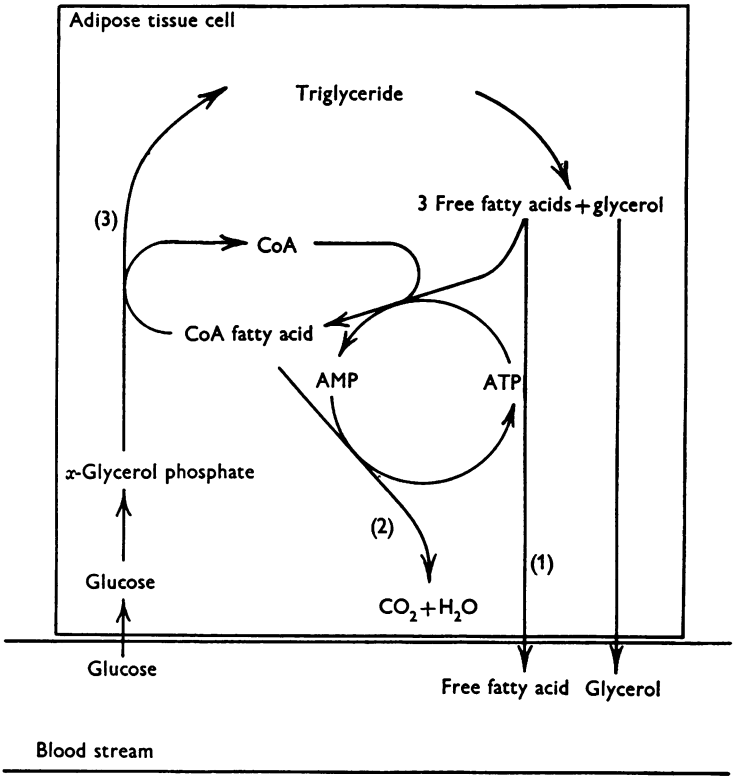

Fig. 9. Metabolism of fatty acids in adipose tissue. ATP = adenosinetriphosphate, AMP = adenosinemonophosphate, CoA = Coenzyme A.

white adipose tissue has also been shown in adult rats (Wertheimer, Hamosh & Shafrir, 1960). A further possible indication of a rapid rate of hydrolysis of triglycerides in brown adipose tissue was the presence of monoglycerides, since monoglycerides can be detected in white adipose tissue of adult rabbits only after administration of catecholamines (Wadstrom, 1957).

The fact that hormones which increase the release of free fatty acids from adipose tissue also increase the rate of oxygen consumption of adipose tissue was first stressed by Ball & Jungas (1961). They pointed out that free fatty acids have three alternative routes in the adipose tissue cell

(Fig. 9). They may be released from the cell into the blood stream (pathway 1), oxidised to carbon dioxide and water (pathway 2) or re-esterified to triglyceride (pathway 3). Ball & Jungas (1961) also drew attention to the fact that the apparently purposeless hydrolysis and re-synthesis of triglycerides in adipose tissue was potentially a highly exothermic process, since there is utilization of adenosine triphosphate in the formation of the coenzyme A–fatty acid complex which is an intermediate in the reaction. Resynthesis of adenosinetriphosphate from adenosinemonophosphate would be accomplished by oxidative phosphorylation during the oxidation of fatty acids or glucose and all oxidative processes are accompanied by heat production. Re-esterification of the coenzyme A–fatty acid complex requires a supply of α-glycerol phosphate derived from glucose since adipose tissue is unable to phosphorylate the glycerol liberated from triglyceride (Margolis & Vaughan, 1962).

The metabolism of brown adipose tissue appears to differ only quantitatively from that of white adipose tissue in respect of the reactions shown in Fig. 9. The suggestion of Ball & Jungas (1961) that hydrolysis and re-synthesis of triglycerides might contribute to heat production is even more applicable to brown adipose tissue than white adipose tissue. In brown adipose tissue from new-born rabbits the over-all rate of hydrolysis of triglycerides (indicated by release of glycerol) was considerably higher (Fig. 8) and the proportion of fatty acids released from the cell considerably less than in white adipose tissue from adult rabbits. The very much higher rate of oxidative metabolism of brown adipose tissue would be of advantage in the rapid oxidation of intracellular free fatty acids. On the assumption that all the oxygen consumption of the slices of brown adipose tissue is derived from the oxidation of free fatty acids, it can be calculated that, *in vitro*, approximately 60 % of the free fatty acids are re-esterified to triglyceride, 30 % are oxidized and less than 10 % released into the medium. Similar calculations for interscapular white adipose tissue from adult rabbits are, release into medium 40 %, re-esterification 50 % and oxidation less than 10 %.

The stimulatory effect of noradrenaline and insulin on the oxygen consumption of slices of brown adipose tissue of newborn rabbits has also been found with brown adipose tissue from adult rats (Joel & Shackney, 1962). Noradrenaline has been shown to activate an adipose tissue lipase (Rizack, 1961) and insulin would facilitate the supply of α-glycerol phosphate necessary for re-esterification. Since insulin alone did not affect the oxygen consumption it would appear that the supply of α-glycerol phosphate only became rate-limiting when lipolysis in the isolated tissue was increased by noradrenaline. The mechanism by which noradrenaline stimulates lipolysis by activation of a lipase is unknown. It does not seem

to involve the formation of cyclic 3′-5′-adenosinemonophosphate (Vaughan, 1960) which has been shown to participate in the activation of phosphorylase by catecholamines (Sutherland & Rall, 1960).

CONCLUSIONS

In the intact new-born rabbit exposed to cold, local heat production in brown adipose tissue coincided with a large rise in plasma glycerol. Since the rise in plasma and adipose tissue free fatty acids was small, most of the free fatty acids liberated by hydrolysis of triglycerides must have been metabolized within the cell. This conclusion was supported by *in vitro* studies of slices of brown adipose tissue in which it was found that more than 90% of free fatty acids liberated within the cell were metabolized within the cell. The major metabolic paths open to intracellular free fatty acids are oxidation or re-esterification to triglycerides. Both processes are exothermic and could well account for the demonstrated local heat production in brown adipose tissue.

SUMMARY

1. Brown adipose tissue between the scapulae and around the neck represents 5% of the body weight of new-born rabbits.

2. Exposure to cold or I.V. infusion of noradrenaline increased the oxygen consumption of new-born rabbits and was accompanied by local heat production in brown adipose tissue.

3. Local heat production in brown adipose tissue during exposure to cold was abolished by hypoxia.

4. Local heat production during infusion of noradrenaline was abolished by pronethalol.

5. Exposure to cold or infusion of noradrenaline caused a small rise in plasma free fatty acids but a large rise in plasma glycerol. Adipose tissue levels of free fatty acids during cold exposure indicated that most of the free fatty acids liberated by hyrolysis were metabolised within brown adipose tissue.

6. *In vitro* studies of interscapular adipose tissue showed that the rate of lipolysis was three times greater in the new-born than in the adult, but the rate of release of free fatty acids was one half that of the adult.

7. Glycerol kinase activity was absent from brown adipose tissue but was present in the liver of new-born rabbits.

8. Brown adipose tissue had exceptionally high *in vitro* oxygen consumption, in slices and homogenates.

9. These observations indirectly suggest that brown adipose tissue makes a significant contribution to the metabolic response of new-born rabbits to cold and noradrenaline.

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