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FAT METABOLISM AND HEAT PRODUCTION IN YOUNG RABBITS

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SUMMARY

1. The rates of oxygen consumption were measured in 6–8-day-old rabbits at 34 and 15° C after varying periods of starvation and cold exposure. At the start of the experiment the rabbits had been fasted for 24 hr. Eight rabbits were studied immediately, six after 24 and six after 48 hr in a cold environment (20° C), and twelve after a further 48 hr in a warm environment (34° C). All the animals had a similar increase in oxygen consumption during the final hour of cold exposure (15° C).

2. The rabbits kept at 20° C lost 83 % of the fat stored in their brown adipose tissue within 24 hr and a further 11 % in the next 24 hr. The fat content of white adipose tissue had fallen by 75 % at 48 hr. In contrast rabbits kept unfed at 34° C had lost 47 % of the fat in brown adipose tissue and 44 % of the fat in white adipose tissue after 48 hr.

3. In six rabbits subcutaneous thermocouples demonstrated that local heat production continued in brown adipose tissue after 48 hr cold exposure.

4. In the rabbits kept at 34° C the final cold exposure caused a large increase in the serum free fatty acid and glycerol concentrations. Much lower concentrations were found in rabbits kept at 20° C.

5. The results show that the fat stored in the brown adipose tissue of young rabbits exposed to cold is preferentially used for heat production. When this store of fat is exhausted, brown adipose tissue still produces heat presumably by oxidizing fat and glucose taken from the circulation.

INTRODUCTION

The energy metabolism of a week-old rabbit differs from that of the new-born in a number of important ways. Within a week of birth the rabbit has already doubled its body weight, it has established oral feeding, and its metabolism is adjusted to fat as well as glucose as a source of cellular energy. Rabbit's milk contains 15 % of fat, and each day the

young rabbit receives a milk feed weighing between 10 and 20% of its body weight (Davies, Widdowson & McCance, 1964), so that in the first week it receives orally five times more fat than is present in its stores at birth. It was interesting therefore to compare the metabolic responses of week-old rapidly growing rabbits to cold exposure and starvation with those of new-born rabbits (Hardman, Hey & Hull, 1969).

METHODS

The rabbits used in this investigation had been reared in a thermoneutral environment (34–36° C) since birth and they had been fed once each day by the does (Bernard & Hull, 1964). Thirty-one rabbits 6–8 days old weighing 85–150 g were separated from the does immediately after a feed and kept in a thermoneutral environment for 24 hr. Eight rabbits were then challenged with cold exposure (15° C) for 1 hr (Group I). Twelve rabbits were placed in individual chambers at an environmental temperature of 20° C for 24 hr (Group II) or 48 hr (Group III), before their rates of oxygen consumption were measured at 34 and 15 °C respectively for 1 hr. A further twelve rabbits were kept unfed for 48 hr at 34° C, six were killed immediately (Group IV*a*) and the remaining rabbits were challenged by exposure to cold (15° C) for 1 hr (Group IV*b*). Colonic temperatures were monitored continuously. At the end of each experiment the animal was killed, blood collected and the cervical and interscapular brown adipose tissue, the perirenal and inguinal white adipose tissue, and the liver were dissected free and weighed.

Oxygen consumption, body temperature, tissue glyceride content, blood glucose, serum free fatty acids and serum glycerol were measured as described in the previous paper (Hardman, Hey & Hull, 1969).

In order to demonstrate heat production in fat depleted brown adipose tissue, a further six rabbits were treated as Group III but before the final cold exposure, copper-constantan thermocouples were sited subcutaneously over the cervical brown adipose tissue and over the dorsal lumbar muscles.

Heat production was calculated on the assumption that the consumption of 1 l. oxygen produced 4·8 kcal of heat, and that the surface area (A) in m^2 can be estimated from the animal's body weight (W) in kg from the equation $A = 0{\cdot}1\ W^{2/3}$.

Seven rabbits intended for study in Group III became hypothermic (colonic temperature below 28° C) within 18 hr of cold exposure; these animals were excluded from this study.

Statistics. Where the mean of a series of observations is reported the standard error of the mean has been supplied.

RESULTS

The metabolic response to cold exposure

The colonic temperatures and rates of oxygen consumption of rabbits in the different groups are given in Table 1. The ambient temperature of 34° C is within the thermoneutral range and therefore the rabbit's rate of oxygen consumption is at a minimum; the ambient temperature of 15° C is a severe cold stress and stimulates maximal oxygen consumption (Hull, 1965). With starvation the rate of oxygen consumption at 34° C fell,

but the rabbits in all four groups responded to cold exposure with a similar increase in their rate of oxygen consumption. Values for specific thermal insulation can be calculated from these measurements of equilibrium colonic temperature and oxygen consumption. The mean specific thermal insulation in a warm environment (34° C) was $0{\cdot}141 \pm 0{\cdot}005$° C.m²/hr/kcal and this rose to $0{\cdot}297 \pm 0{\cdot}013$° C.m² hr/kcal in an environment of 15° C.

TABLE 1. The final rates of oxygen consumption of four groups of 1-week-old rabbits measured at environmental temperatures of 34 and 15° C for 1 hr following varying periods of starvation and cold exposure. Groups I and IV had been kept unfed at an ambient temperature of 34° C, whilst Groups II and III had been kept unfed at an ambient temperature of 20° C

	34° C		15° C	
	Minimal rate of oxygen consumption (ml./kg.min)	Colonic temp. (° C)	Maximal rate of oxygen consumption (ml./kg.min)	Colonic temp. (° C)
Group I Studied at zero time (See text methods)	$18{\cdot}7 \pm 0{\cdot}6$	$37{\cdot}0 \pm 0{\cdot}2$	$37{\cdot}0 \pm 0{\cdot}6$	$29{\cdot}5 \pm 1{\cdot}6$
Group II unfed for 24 hr at 20° C	$18{\cdot}3 \pm 0{\cdot}9$	$37{\cdot}5 \pm 0{\cdot}2$	$37{\cdot}7 \pm 2{\cdot}9$	$30{\cdot}6 \pm 0{\cdot}6$
Group III unfed for 48 hr at 20° C	$16{\cdot}2 \pm 0{\cdot}7$	$37{\cdot}2 \pm 0{\cdot}2$	$31{\cdot}7 \pm 1{\cdot}1$	$28{\cdot}7 \pm 0{\cdot}2$
Group IV unfed for 48 hr at 34° C	$14{\cdot}2 \pm 0{\cdot}7$	$36{\cdot}7 \pm 0{\cdot}05$	$31{\cdot}5 \pm 0{\cdot}5$	$28{\cdot}3 \pm 0{\cdot}2$

Changes in the circulating concentration of glucose, free fatty acids and glycerol

Glucose. The resting concentration of blood glucose in 6–8-day-old rabbits was 135 ± 8 mg/100 ml. which is approximately double the concentration found in rabbits at birth. In control rabbits (Group I), cold exposure for 1 hr at 15° C stimulated a small but not significant rise to 142 ± 6 mg/100 ml. The blood glucose concentrations in the rabbits kept for 24 or 48 hr at 20° C (Groups II and III), after they had been rewarmed at 34° C to measure their rate of oxygen consumption and then re-exposed to cold (15° C) for 1 hr were 103 ± 6 mg/100 ml. and 74 ± 10 mg/100 ml. respectively. After 48 hr unfed at an ambient temperature of 34° C (Group IV *a* and *b*), the resting blood glucose concentration had fallen to 78 ± 10 mg/100 ml., but cold exposure caused a small rise to 100 ± 8 mg/100 ml. which was just significant statistically ($P = 0{\cdot}1$).

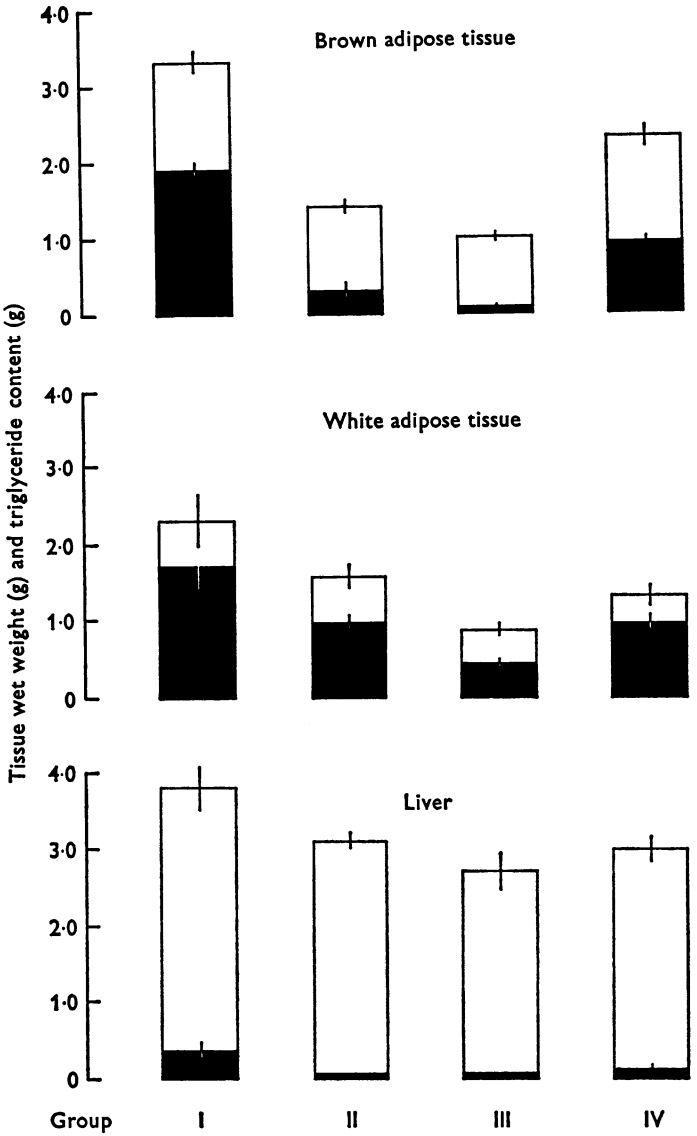
Free fatty acids. The circulating concentration of free fatty acids in the control rabbits was $0{\cdot}50 \pm 0{\cdot}06$ m-equiv/l. which was similar to the concentration found in new-born rabbits. Cold exposure stimulated a rise to

1·25 ± 0·08 m-equiv/l. In rabbits in Groups II and III the concentrations were 0·95 ± 0·15 m-equiv/l. and 0·72 ± 0·06 m-equiv/l. respectively after the final cold stress at 15° C. These are significantly lower than the concentrations in the rabbits in Group I ($P < 0·1$, $P < 0·001$ respectively). In contrast the rabbits kept unfed but warm for 48 hr (Group IV) had a resting concentration of 0·59 ± 0·06 m-equiv/l.; in similar rabbits exposed to cold for 1 hr it was 1·54 ± 0·04 m-equiv/l. The highest concentrations of free fatty acids in response to cold stress for 1 hr were found in rabbits exposed to cold after 48 hr starvation in a warm environment; the lowest concentrations were found in rabbits kept unfed in the cold for 48 hr.

Glycerol. The resting concentration of serum glycerol in Group I was 0·10 ± 0·01 mM/l. which is similar to that found in rabbits at birth. With cold exposure for 1 hr it rose to 0·36 ± 0·03 mM/l. In Groups II and III it was progressively less at 0·31 ± 0·04 mM/l. and 0·15 ± 0·02 mM/l. respectively, after 1 hr cold exposure. On the other hand the resting concentration in rabbits in Group IV*a* was 0·07 ± 0·01 mM/l. and after 1 hr cold exposure it had risen to 0·56 ± 0·05 mM/l. Thus the highest concentrations of glycerol were found in rabbits exposed to cold after they had been kept warm and starved for 48 hr and the lowest concentrations were found in rabbits kept cold and starved before the final cold exposure.

Fat stores of young rabbits

The wet weights of the liver, cervical and interscapular brown adipose tissue and perirenal and inguinal white adipose tissue lobes with their glyceride contents for the four groups of rabbits are given in Text-fig. 1. The calculated weight of glyceride (g/kg body weight) for the adipose tissues are given in Table 2. After 24 hr in a cold environment, the mean glyceride content of the liver had fallen from 9 to 2·6 % and it did not fall further over the next 24 hr. Similarly the glyceride content of the liver of rabbits kept in a warm environment for 48 hr had fallen to a mean of 3·5 %. With cold exposure the weight and the glyceride content of brown adipose tissue fell sharply, within 24 hr its weight had halved and its glyceride content was reduced to a sixth (Group II), and it fell a little more over the next 24 hr (Group III). The weight and glyceride content of brown adipose tissue from rabbits starved for 48 hr but kept warm was significantly less than that of Group I, but was considerably higher than that of either Group II or III. White adipose tissue steadily decreased in weight and glyceride content with prolonged cold exposure and starvation (Groups II and III) and to a lesser extent with starvation alone (Group IV).



Text-fig. 1. The mean wet weights and triglyceride contents of the cervical and interscapular brown adipose tissue, inguinal and perirenal white adipose tissue and the liver of four groups of young rabbits after the final cold exposure. All the rabbits were fasted for 24 hr before the experiment. Rabbits in Group I were studied immediately and those in Group IV remained unfed for a further 48 hr in a warm environment (34° C). The rabbits in Groups II and III had been kept unfed in a cold environment (20° C) for 24 and 48 hr before the final cold exposure. The vertical lines are the S.E. of mean of at least six experiments.

Heat production in fat depleted brown adipose tissue

The rabbits in Group III had a good response to cold exposure but their brown adipose tissue was largely depleted of glyceride. To establish whether these rabbits were still producing heat in brown adipose tissue, thermocouples were placed under the skin over brown adipose tissue and the temperature of the tissue was compared with the colonic temperature before and during cold exposure. In all the rabbits the temperature over brown adipose tissue rose above the colonic temperature during cold exposure, thus demonstrating heat production in brown adipose tissue.

TABLE 2. The glyceride content of the cervical and interscapular brown adipose tissue and inguinal and perirenal white adipose tissue (g/kg body weight) for the four groups of rabbits illustrated in Text-fig. 1 (mean and S.E. of mean of at least six animals in each group)

	Group	Brown adipose tissue	White adipose tissue
I	Killed at zero time (see text methods)	16·54 ± 1·50	14·31 ± 2·74
II	Killed after 24 hr unfed at 20° C	2·76 ± 0·64	9·81 ± 1·11
III	Killed after 48 hr unfed at 20° C	0·90 ± 0·10	3·80 ± 0·45
IV	Killed after 48 hr unfed at 34° C	7·75 ± 0·56	8·08 ± 0·69

In this situation the tissue was presumably using an external supp'y of energy for heat production and it was interesting therefore to examine the histological appearance of the tissue in this state under the electron microscope. Plate 1 shows an electron micrograph of fat depleted brown adipose tissue from a rabbit in Group III. The cytoplasm is filled with large complex mitochondria, but between the mitochondria are many small vacuoles or channels.

DISCUSSION

One-week-old rabbits achieve thermal stability over a much wider range of ambient temperatures than they do on the day of birth. This is mainly due to their larger size and increased insulation. The specific thermal insulation of a week-old rabbit in a thermoneutral environment was only half that achieved in the cold. Rabbits on day of birth also demonstrated some ability to adjust their specific thermal insulation but to a lesser extent (Hardman *et al.* 1969).

The extra heat produced by new-born rabbits (mean body weight 52 ± 2·2 g) on cold exposure (25° C) was about 11 kcal/day, whereas that produced by the week-old rabbits (mean body weight 111 ± 4·5 g) at 20° C was 14·2 kcal/day. This is equivalent to the oxidation of 1·17 and 1·51 g of triglyceride/day respectively. In new-born rabbits brown adipose tissue

weighed on average 2·2 g, of which 1·0 g was glyceride and in the week-old rabbits it weighed on average 3·3 g, of which 1·9 g was glyceride. Thus if brown adipose tissue is the major site of heat production (Hull & Segall, 1965) and if the glyceride it stores is preferentially used for heat production (Hull & Segall, 1966) then on the basis of these figures the brown adipose tissue of new-born rabbits would be depleted of glyceride in 24 hr and that of the 1-week-old rabbits within 36 hr of the onset of cold exposure. This proved to be so, but whereas new-born rabbits ceased to produce heat at a time when their brown adipose tissue was depleted of glyceride (Hardman *et al.* 1969) the week-old rabbits did not. It is possible that the week-old rabbits started to generate heat by muscular activity when their brown adipose tissue became fat depleted, in the same way as the guinea-pigs studied by Brück & Wünnenberg (1965) started to shiver when heat production in brown adipose tissue was blocked by drugs. However, the higher subcutaneous temperature found over the fat depleted brown adipose tissue of rabbits after 48 hr in the cold demonstrates that the tissue is still capable of heat production. It follows that this continued thermogenesis in brown adipose tissue after prolonged cold exposure must be largely maintained by an external source of energy.

The most likely explanation is that white adipose tissue releases fatty acids which are taken up by brown adipose tissue. The week-old rabbit has five times more glyceride in its white adipose tissue than it had at birth. Even after 48 hr intense cold exposure the white adipose tissue analysed still contained 0·5 g glyceride which is enough to support maximal thermogenesis in brown adipose tissue for a further 10 hr. This could be the main reason why young rabbits withstand prolonged cold exposure better than they do at birth, and it is interesting to note that they also maintain a satisfactory blood glucose concentration in the face of prolonged cold exposure and starvation.

If brown adipose tissue when depleted of fat but producing heat takes from the circulation sufficient fatty acids to meet its metabolic requirements then there will be considerable transport of fatty acids across the capillary membranes into the cells. In many tissues including the bowel (Palay & Karlin, 1959) and white adipose tissue (Williamson, 1964) increased transport of fatty acids and the formation of triglyceride has led to the increased prominence of the smooth reticular endothelium in the cytoplasm of the cells. Plate 1 shows that this is also true in brown adipose tissue. What is not clear at the moment is whether or not the fatty acids are re-esterified before they are transported within the reticular endothelium or whether they remain as fatty acids linked to a carrier substance. The complex network around the mitochondria emphasizes again the close proximity of the 'cellular furnace' to its fuel and oxygen supply.

In a previous investigation (Hardman & Hull, 1969) it was shown that the rate of oxygen consumption after cold exposure for 1 hr was directly related to the rise in serum glycerol in both new-born and young rabbits. In the present study the rate of oxygen consumption after cold exposure for 1 hr was the same in the four groups of rabbits but the changes in the serum glycerol concentration were not. After cold exposure and starvation the rise in serum glycerol concentration was smaller, while with starvation alone it was greater than that observed in normal rabbits (Group I). If the changes in the serum glycerol on cold exposure are largely due to changes in the rate of its release from brown adipose tissue (preliminary investigations on the glycerol content of the venous outflow from the tissue support this suggestion; M. J. Hardman & D. Hull, unpublished), then presumably it reflects the rate of hydrolysis of glyceride in the tissue. The lower concentration of serum glycerol found in young rabbits after prolonged cold exposure may in part be due to the fact that as the glyceride content of brown adipose tissue falls, less glyceride is available for hydrolysis and more free fatty acids are being used directly from the circulation. However, this does not explain the greater rise in the serum glycerol concentration in rabbits exposed to cold after starvation in a warm environment. In general the changes in the serum glycerol concentration were accompanied by similar variations in the concentration of serum free fatty acids, and thus the large rise in glycerol may be a consequence of an increased rate of glyceride hydrolysis for release of fatty acids into the circulation.

The response of brown adipose tissue to starvation changed over the first week of life. In new-born rabbits there was no significant fall in the glyceride content of brown adipose tissue during starvation for 48 hr in a warm environment, whereas in the week-old rabbits half the glyceride was used in this time, presumably for metabolism elsewhere. Further study of these changes may well enlarge our understanding of the mechanisms which control adipose tissue metabolism.

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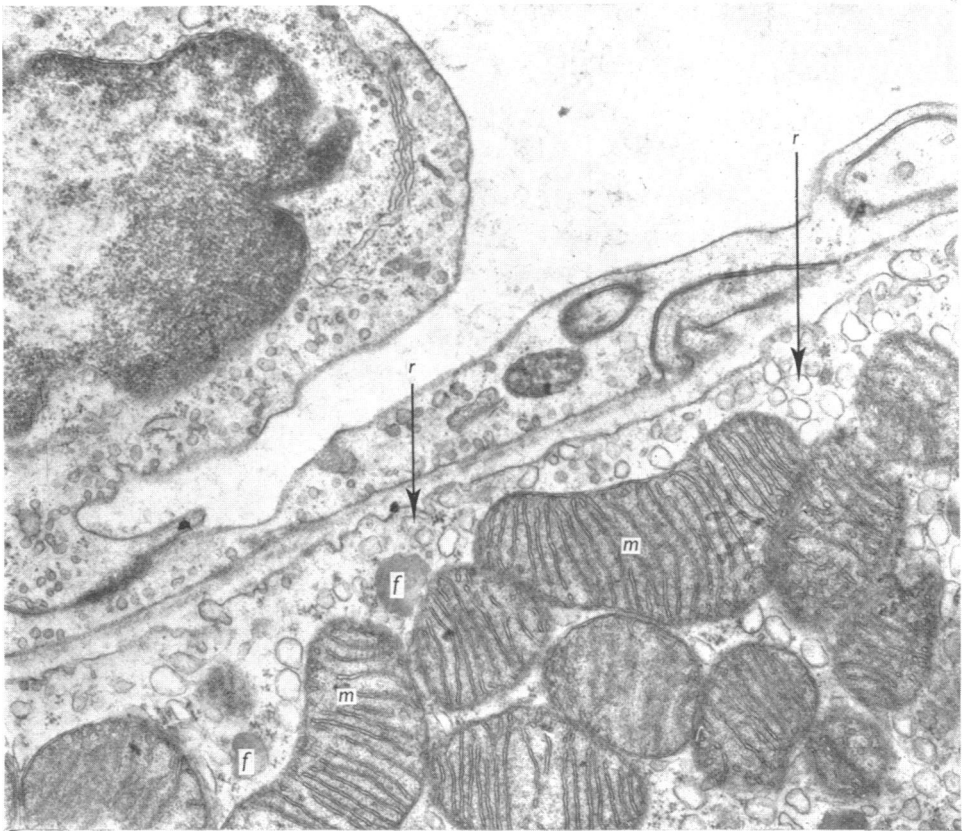
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EXPLANATION OF PLATE

An electron micrograph (magnification × 18,000) of the cervical brown adipose tissue of a week-old rabbit from Group III, which had been kept unfed for 48 hr at an ambient temperature of 20° C. The animal was killed after the final cold exposure (15° C) and a piece of brown adipose tissue was immediately fixed in buffered gluteraldehyde followed by osmic acid. The sections were stained with uranyl acetate and Reynold's lead acetate. The cytoplasm of the fat cell contains many large mitochondria (*m*) and a few discrete fat droplets (*f*) and the smooth endoplasmic reticulum (*r*) is prominent.