J. Physiol. (1965), **181**, *pp.* 468–477
With 8 *text-figures*
Printed in Great Britain

HEAT PRODUCTION IN THE NEW-BORN RABBIT AND THE FAT CONTENT OF THE BROWN ADIPOSE TISSUE

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(*Received* 23 *March* 1965)

In the new-born rabbit heat production in response to cold exposure occurs principally in brown adipose tissue (Hull & Segall, 1965*a*). There is evidence that the energy for heat production is provided by the oxidation of fat stored in the brown adipose cell (Dawkins & Hull, 1964). During prolonged exposure of the new-born rabbit to cold, the fat vacuoles within brown adipose tissue gradually decrease in size and finally disappear. The fat content of the tissue falls at a rate which is dependent on the environmental temperature (D. Hull, H. J. Shelley & M. Young, unpublished). The present experiments were designed to investigate the relation between the maximal ability of the new-born rabbit to consume oxygen (and hence to produce heat) and changes in its brown adipose tissue. The results show that this ability is related to the amount of fat in the brown adipose tissue.

METHODS

Forty-seven new-born rabbits weighing from 45 to 79 g at birth were used. Twenty-seven were born normally and these animals fed soon after delivery. Twenty were delivered by Caesarean section at term and were not fed on the day of birth. The results from the two groups were similar and are reported together. Rates of O_2 consumption of individual rabbits were measured by a closed circuit method and are expressed as dry gas at s.t.p. The minimal rate of O_2 consumption (ambient temperature 35° C) and the maximal rate on cold exposure (ambient temperature 20° C) were measured in unanaesthetized rabbits. The rate of O_2 consumption during intravenous infusion of noradrenaline was measured in thirty-three rabbits shortly before they were killed. These rabbits were anaesthetized with ether and a polyethylene catheter was inserted into an external jugular vein. After recovery from the anaesthetic, a fresh solution of noradrenaline bitartrate (Winthrop Laboratories) was given at a rate of 0·02 ml./min by means of a motor driven syringe. Ten-minute infusions of noradrenaline were used (ambient temperature 35° C) and the mean rate of O_2 consumption during the last 6 min of the infusion was calculated. Doses of noradrenaline are expressed in terms of the base. The bulk of the brown adipose tissue in the new-born rabbit lies around the neck and between the scapulae. This tissue was dissected out after death and weighed. The total lipid content of the tissue was measured by the method of Bloor (1928). Phospholipid and cholesterol account for only 4 % of the total lipid content of brown adipose tissue in the new-born rabbit (Dawkins & Hull, 1964) and were not specifically measured.

On the day of birth (day 0) O_2 consumption was measured in all forty-seven rabbits. Twelve were then killed and eleven subjected to fat analysis. The surviving thirty-five rabbits were placed in an incubator for 24 hr at a cool ambient temperature (30° C). Oxygen consumption was measured in these rabbits on the second day of life (day 1). Twelve were then killed and eleven subjected to fat analysis. Of the surviving twenty-three rabbits, thirteen were allowed to feed from the suckling doe, each taking 2–7 g of milk. All the twenty-three rabbits were then kept for a further 24 hr at 30° C. Finally, the O_2 consumption was measured again in these rabbits on the third day of life (day 2). All were then killed and subjected to fat analysis. The O_2 consumption during noradrenaline infusion was measured in eight rabbits on day 0, nine rabbits on day 1, and twelve fed and four unfed rabbits on day 2.

RESULTS

Maximal rate of O_2 consumption on cold exposure

Figure 1 shows the mean minimal rates of O_2 consumption and the mean maximal rates on cold exposure on the first 3 days of life. The maximal rate on day 1 was only slightly lower than on day 0. In the fed animals on day 2 the maximal rate had again dropped only slightly, but in the unfed rabbits on day 2 the maximal rate had dropped very greatly. In these rabbits there was virtually no response to cold exposure.

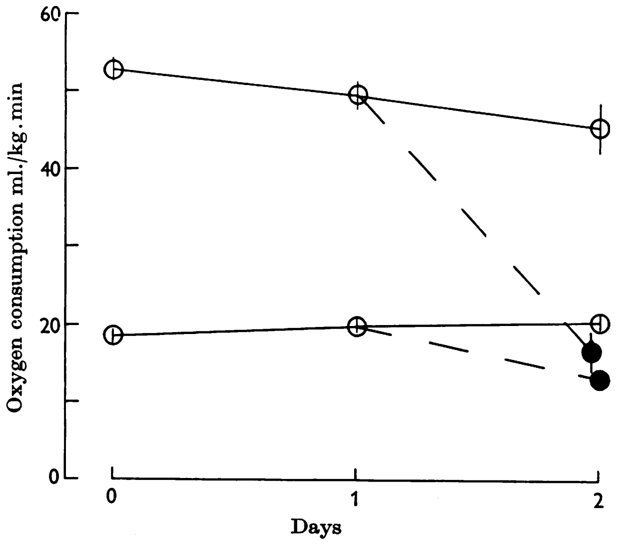

Fig. 1. The minimal rates of O_2 consumption (ambient temp. 35° C) and maximal rates on cold exposure (ambient temp. 20° C) of new-born rabbits on the first 3 days of life. Each symbol represents the mean ± S.E. of mean of the results from at least ten rabbits. The filled symbols (●) indicate the results from the unfed rabbits on day 2.

Maximal rate of O_2 consumption during noradrenaline infusion

Three infusions of noradrenaline were given at 30 min intervals in each rabbit. Doses of 2 µg/kg.min and 4 µg/kg.min were used. (A dose of 6 µg/kg.min had previously been found to produce a less than maximal response in some rabbits.) In alternate animals infusions of 2, 4 and 2 μg/kg.min or of 4, 2 and 4 μg/kg.min were given. In just over half the rabbits the response to a dose of 4 μg/kg.min was greater than that to 2 μg/kg.min, sometimes considerably so. In many, however, the response to 4 μg/kg.min was the same as that to 2 μg/kg.min and in one instance was less. The mean rate of O_2 consumption during 2 μg/kg.min infusions on day 0 was $50{\cdot}7 \pm 2{\cdot}6$ (S.E. of mean) ml./kg.min and during 4 μg/kg.min was $58{\cdot}9 \pm 4{\cdot}1$ ml./kg.min. The difference between these means is not significant ($P \simeq 0{\cdot}10$). On day 1 the mean rates were respectively $46{\cdot}6 \pm 3{\cdot}4$ ml./kg.min and $47{\cdot}3 \pm 4{\cdot}0$ ml./kg.min. Thus the mean rate during 4 μg/kg.min infusions was not significantly higher than during

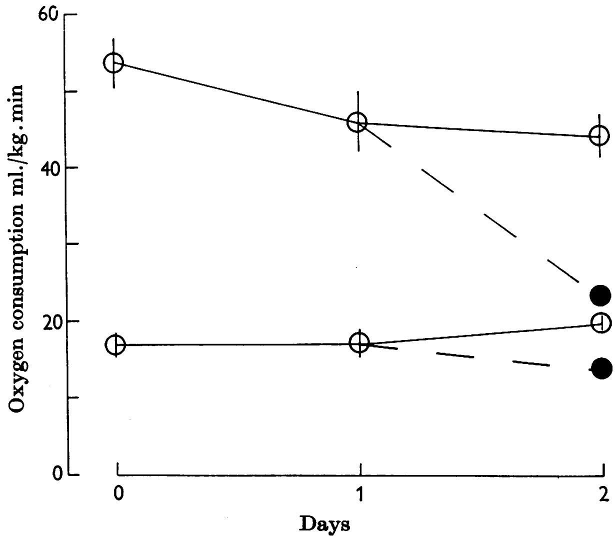

Fig. 2. The minimal rates of O_2 consumption and maximal rates during infusion of noradrenaline (ambient temp. 35° C) of new-born rabbits on the first 3 days of life. Each empty symbol (○) represents the mean ± S.E. of mean of the results from at least six rabbits. The filled symbols (●) indicate the mean results from four unfed rabbits on day 2.

2 μg/kg.min, and so the results obtained with these two doses of noradrenaline were combined to estimate the mean maximal rate of O_2 consumption during noradrenaline infusion.

Figure 2 shows the mean minimal rates of O_2 consumption and the mean maximal rates during noradrenaline infusion on the first 3 days of life. The results are similar to those shown in Fig. 1. There was only a small fall in the maximal rate from day 0 to day 1. In the fed animals on day 2 the maximal rate was maintained, whereas by day 2 in the unfed animals it had fallen considerably. In these rabbits there was still a definite, though reduced, response to noradrenaline.

Changes in the brown adipose tissue

Figure 3 shows the mean wet weight of the brown adipose tissue on the first 3 days of life. A large fall in weight of the tissue occurred from day 0 to day 1. There was then only a small further fall by day 2 in both the fed and unfed rabbits. The difference between the mean weight of tissue in the two groups on day 2 was not statistically significant.

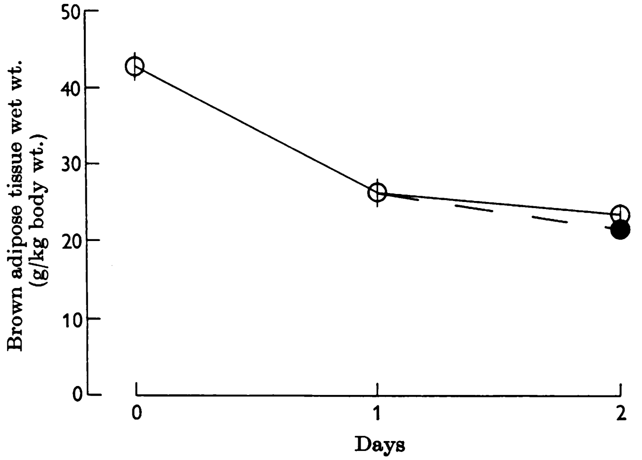

Fig. 3. The wet weight of brown adipose tissue of new-born rabbits on the first 3 days of life. Symbols as for Fig. 1.

Figure 4 shows the mean fat content of the brown adipose tissue on the first 3 days of life. The fall in fat content from day 0 to day 1 was in the same proportion as the fall in the tissue's wet weight between these days. In the fed animals on day 2 there was a small further fall in fat content, whereas in the unfed animals on day 2 the fall was significantly greater ($P < 0{\cdot}02$).

Figure 5 shows the mean concentration of fat in the brown adipose tissue on the first 3 days of life. The concentration was constant on days 0 and 1. In the fed animals on day 2 there was a small fall in the concentration of fat, whereas in the unfed animals on day 2 there was a considerable fall. The difference between the mean fat concentration in the fed and unfed animals on day 2 was significant ($P < 0{\cdot}05$).

Maximal rates on cold exposure and noradrenaline related to the state of the brown adipose tissue

Figures 6 and 7 include additional results from a previous series of experiments (Hull & Segall, 1965*a*). Figure 6 shows the maximal rates of O_2 consumption of individual rabbits on cold exposure plotted against the wet weights of their brown adipose tissue. The maximal rate was independent of the brown adipose tissue weight until the latter fell to 20–30 g/kg body weight. At this tissue weight some animals still had good responses whilst others had almost none. The relation between the maximal

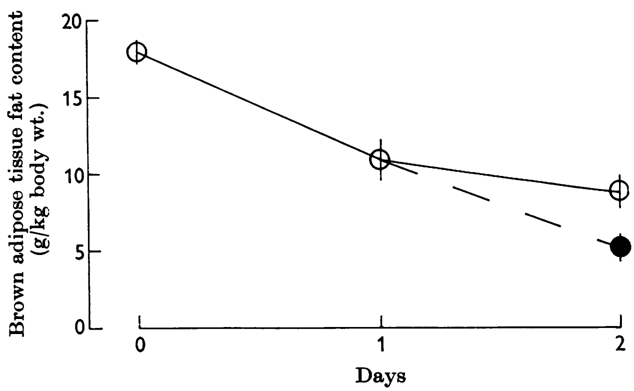

Fig. 4. The fat content of brown adipose tissue of new-born rabbits on the first 3 days of life. Symbols as for Fig. 1.

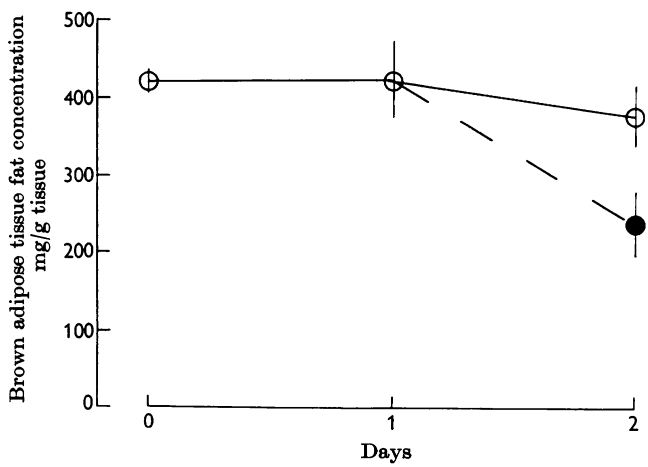

Fig. 5. The fat concentration in brown adipose tissue of new-born rabbits on the first 3 days of life. Symbols as for Fig. 1.

rate during noradrenaline infusion and the weight of the brown adipose tissue was similar (Fig. 7). The rabbits with the poorest responses to cold exposure had in general the lowest brown adipose tissue fat contents (Fig. 8). These were mostly the unfed animals on day 2. Since the weights of their brown adipose tissue were not lower than those of rabbits with good responses, those with poor responses to cold were characterized by having

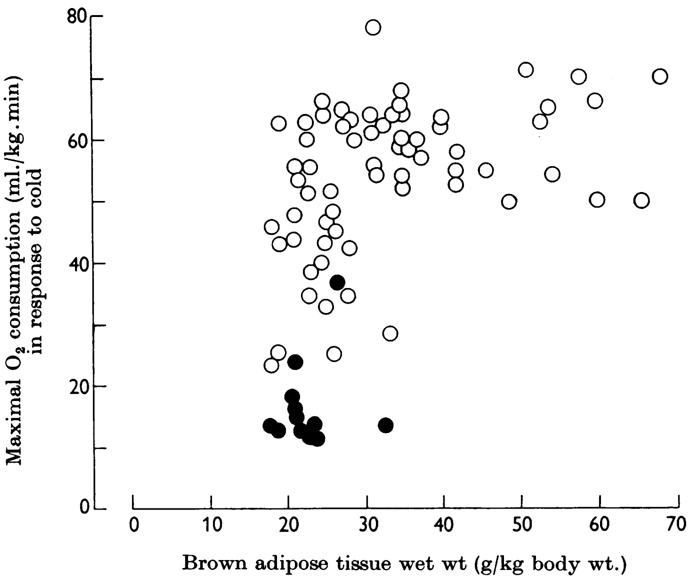

Fig. 6. The maximal rate of O_2 consumption on cold exposure plotted against the wet weight of brown adipose tissue. The filled symbols (●) represent the results from the unfed rabbits on day 2. This scattergram includes additional results from a previous series of experiments.

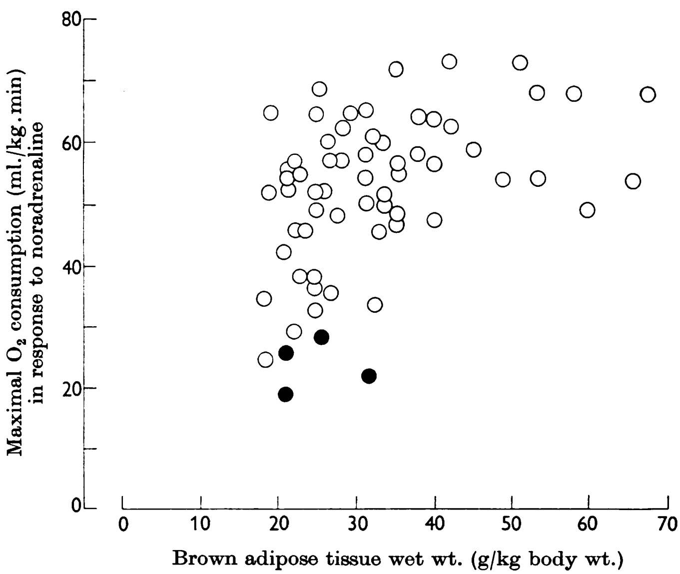

Fig. 7. The maximal rate of O_2 consumption during infusion of noradrenaline (2–4 μg/kg. min) plotted against the wet weight of brown adipose tissue. Symbols as for Fig. 6. This scattergram includes additional results from a previous series of experiments.

both lower contents and lower concentrations of fat in their brown adipose tissue. The maximal response to noradrenaline showed a similar relationship to the fat content of the tissue.

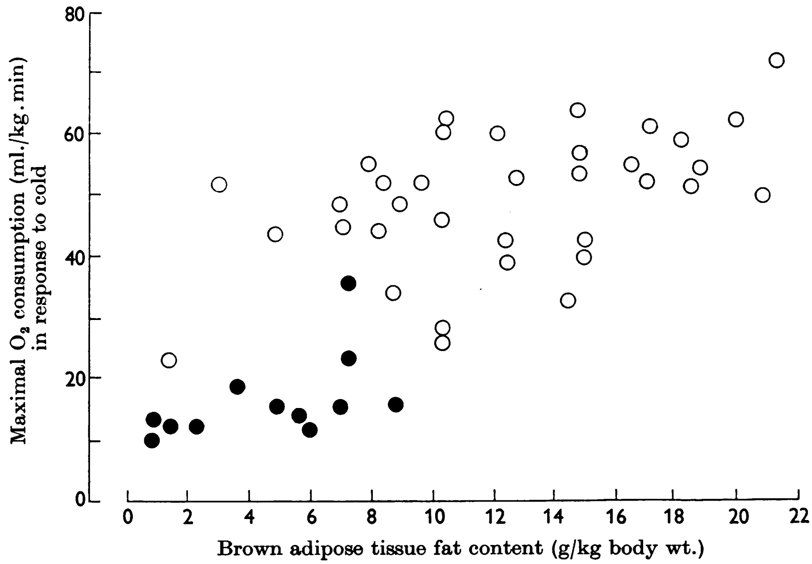

Fig. 8. The maximal rate of O_2 consumption on cold exposure plotted against the fat content of brown adipose tissue. Symbols as for Fig. 6.

DISCUSSION

The maximal responses to cold exposure and to noradrenaline were both highest on the day of birth, when the brown adipose tissue constituted 4·3 % and its fat content 1·8 % of the body weight. The tissue contained 42 % by weight of fat. On day 1 the brown adipose tissue constituted 2·6 % and the fat content 1·1 % of the body weight, but there were still good responses to cold and noradrenaline. The concentration of fat in the brown adipose tissue was still 42 % despite the decline in its fat content, so that the fall in wet weight of the tissue must have been due equally to a loss of lipid and non-lipid constituents. In the fed animals on day 2 a similar phenomenon was seen. The wet weight of tissue had declined slightly further to 2·4 % and the fat content to 0·9 % of the body weight, but the rabbits still had moderately good responses. The brown adipose tissue still contained 38 % by weight of fat. In the unfed animals on day 2 the brown adipose tissue constituted 2·2 % of the body weight. This is about the lowest weight that can be obtained by depleting the tissue of fat. The brown adipose tissue of unfed rabbits kept for 48 hr at 30° C appears histologically to be largely depleted of fat vacuoles (Hull & Segall, 1965*b*) and presumably this weight represents the basic cellular constituents. In these animals the fat content of the tissue had declined greatly to 0·5 % of the body weight and the animals had poor responses to cold and noradrenaline. The loss of fat was not apparently accompanied by a corresponding loss of non-lipid substance, so that the tissue now contained only 24 % by weight of fat.

The results thus show that the responses of the new-born rabbit to cold exposure and infusion of noradrenaline are initially unaffected by a declining fat content of the brown adipose tissue. A threshold of fat content is then reached, below which the animals lose the ability to raise their O_2 consumption in response to cold or noradrenaline. This phenomenon is illustrated by the individual results shown in Fig. 8.

In the new-born rabbit heat production in response to cold exposure or noradrenaline occurs principally in the brown adipose tissue (Hull & Segall, 1965*a*). Heat is produced in the adipose tissue cell by the hydrolysis and subsequent re-synthesis of triglyceride in a process that involves oxidation (Ball & Jungas, 1961). The availability of fat for hydrolysis will depend on the total surface area which the fat vacuoles present to the lipolytic enzymes. Provided an adequate surface area of fat exists, a decline in fat content would not limit the oxidative capacity of the cell. As the fat in the cell is utilized the fat vacuoles decrease in size and so present a relatively large surface area for the amount of fat present. This would tend to preserve the metabolic ability of the cell despite the reduced content of fat. When, however, the fat content declines below a certain level the surface area of the vacuoles will decrease sufficiently to limit the availability of fat for metabolism. This is the probable explanation of the present findings and it is supported by the electron microscopic appearance of brown adipose tissue. In the cell which is replete with fat, mitochondria are seen closely applied to the surface of the vacuoles (Napolitano & Fawcett, 1958). In the cell which is depleted of fat, mitochondria may be seen lying free in the cytoplasm out of contact with the fat vacuoles (W. Aherne & D. Hull, unpublished)

The initially constant concentration of fat in the brown adipose tissue despite the declining fat content was an unexpected finding. Presumably water was the major constituent of the non-lipid substances lost from the tissue during fat depletion. Only in the animals with poor responses was the concentration of fat reduced. The significance of the concentration of fat, as distinct from the fat content, in the metabolism of the fat cell is not clear but it appears from these results to be related to the ability of the brown adipose tissue to produce heat.

The decrease in the metabolic responses to cold and noradrenaline observed in the unfed animals on day 2 might have been due to factors other than the fat content of the brown adipose tissue. For example, these animals were probably hypoglycaemic (D. Hull, H. J. Shelley and M. Young, unpublished). Apart from its effect on the central nervous system, hypoglycaemia might impair the ability of brown adipose tissue to produce heat by limiting the re-esterification of triglyceride (Ball & Jungas, 1961). Blood-sugar estimations were not made in the present experiments but occasional unfed animals on day 2 had convulsions which were relieved by the administration of glucose. However, in one animal intravenous glucose stopped the convulsions but did not improve the response to cold exposure. The present investigation examined only the association between the maximal ability of new-born rabbits to produce heat and the weight and fat content of their brown adipose tissue. The fat content of the brown adipose tissue appears to be a determining factor in this ability.

In these experiments only rabbits weighing 45 g or more at birth were used. It appears that new-born rabbits (or at least those above this birth weight) placed in a cool environment (30° C) are born in a state of nutrition sufficient to maintain their metabolic response to cold independently of being fed in the first 24 hr. After this the response depends upon whether the animals are fed or not. Rabbit's milk contains a high content of fat and maintains the fat content of the brown adipose tissue.

SUMMARY

1. New-born rabbits were kept in a cool environment (30° C) and the relation between their maximal ability to consume oxygen and the state of their brown adipose tissue was investigated over the next 48 hr.

2. The maximal increases in O_2 consumption in response to cold exposure and to intravenous infusion of noradrenaline were highest on the day of birth. The responses after 24 hr and of fed rabbits after 48 hr were only slightly lower. The responses of unfed rabbits after 48 hr were poor.

3. The wet weight of brown adipose tissue was highest on the day of birth. It fell considerably in the first 24 hr and slightly further in the second 24 hr in both fed and unfed rabbits.

4. The fat content of the brown adipose tissue was highest on the day of birth. It fell considerably in the first 24 hr and slightly further in the second 24 hr in fed rabbits. It fell to very low levels in the second 24 hr in unfed rabbits.

5. The concentration of fat in the brown adipose tissue was constant in the first 48 hr of life in fed rabbits. It fell in the second 24 hr in unfed rabbits.

6. It is concluded that the ability of the new-born rabbit to produce heat

in response to cold exposure depends on the fat content of the brown adipose tissue being higher than a threshold level.

We wish to thank Dr G. S. Dawes for his useful advice. We are grateful to the staff of the Nuffield Institute for their technical assistance and in particular to Mrs M. Young who performed the fat analyses. One of us (M.M.S.) held a Nuffield Foundation fellowship during this work.

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