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BROWN ADIPOSE TISSUE METABOLISM *IN VIVO* AND SERUM INSULIN CONCENTRATIONS IN RABBITS SOON AFTER BIRTH

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SUMMARY

1. In rabbits kept unfed for 4 or 24 or 48 hr after delivery by Caesarean section at term, noradrenaline infusion (I.V. for 30 min) caused a similar increase in oxygen consumption but the increase in serum free fatty acid concentration was greatest in rabbits kept unfed for 48 hr.

2. The brown adipose tissue of anaesthetized rabbits under 3 hr old took glucose from the circulation but did not release fatty acids. In similar rabbits noradrenaline infusion stimulated the tissue to generate heat, but there was no release of fatty acids even though the rate of triglyceride hydrolysis was high (as judged by the rate of glycerol release).

3. In rabbits kept unfed for 48 hr from birth in a warm environment, brown adipose tissue released small amounts of fatty acids but continued to take glucose from the circulation. Heat production in response to noradrenaline infusion was accompanied by an increased release of fatty acids. The fat content of the brown adipose tissue did not fall with starvation.

4. The mean serum insulin concentration of rabbits at birth was 54 μu./ml. compared to 23 μu./ml. in the mother. In new-born rabbits kept unfed for 48 hr the insulin concentration had fallen to 14 μu./ml.

5. It is concluded (i) that at birth brown adipose tissue has the capacity to generate heat but the tissue is slow to release its stores of fat in response to starvation, (ii) that brown adipose tissue has a high rate of glucose uptake even during starvation and (iii) that the high circulating concentration of insulin may be responsible for the tissue's slow adaptation to the demands of starvation.

INTRODUCTION

In utero the developing mammal enjoys continuous placental nutrition and warm ambient conditions. At delivery the foetus enters a relatively cool environment and may, for the first time, experience starvation.

Brown adipose tissue, in addition to being a store of fat, oxidizes fatty acids for heat production in response to cold exposure (Hull & Segall, 1965). Recent experiments on brown adipose tissue *in vivo*, in 1-week-old rabbits, have shown that the tissue also release considerable amounts of fatty acids in response to starvation (Hardman & Hull, 1970*a*).

The purposes of this investigation were, first, to measure the exchange of glucose, free fatty acids and glycerol by brown adipose tissue *in vivo* in rabbits soon after birth and, secondly, to see how the exchange of these metabolites was influenced by starvation or by stimulating the tissue to generate heat. Insulin has important effects on adipose tissue metabolism (Rieser, 1967) and it was therefore of added interest to measure the serum insulin concentrations in rabbits during the first 2 days of life. A preliminary report of some of this work has been given (Hardman & Hull, 1970*b*).

METHODS

All the rabbits studied were delivered by Caesarean section. The mothers were killed by a blow on the neck, maternal blood was taken from the heart and the litter were delivered immediately and were either killed instantly and blood collected, or dried and placed into individual beakers which were submerged to their rim in a water-bath set at a temperature of 36° C. An hour was allowed for the rabbits to adjust to extra-uterine life.

Oxygen consumption. The rates of oxygen consumption and colonic temperatures of six rabbits under 4 hr old, ten rabbits 24–30 hr old and six rabbits 48 hr old were measured at a thermoneutral temperature (36° C) before and during a 30 min infusion of L-noradrenaline bitartrate (Winthrop Laboratory). The infusion was given at a dose of 4 μg base/kg.min via a catheter in the jugular vein which had been previously inserted under light ether anaesthesia. The animals were allowed to recover before their rate of oxygen consumption was measured with a closed circuit method (Hardman, Hey & Hull, 1969). At the end of the experiments the rabbits were killed and blood samples taken for analysis.

Metabolism of brown adipose tissue in vivo. For these experiments the rabbits were anaesthetized with urethane (1 g/kg body weight I.P.). One catheter was placed in the left jugular vein to collect the venous outflow from the lateral vein of cervical brown adipose and, in those given noradrenaline infusions, a second catheter was placed into a branch of the right jugular vein. Arterial samples were obtained by cutting the carotid artery. After one flow measurement (for details, see Hardman & Hull, 1970*a*), venous and arterial samples were taken and no further measurements made because the blood volume taken, approximate total 1 ml., is a significant fraction of the total blood volume of new-born rabbits (Mott, 1965).

Twenty rabbits under 3 hr old were studied; in seven, measurements were made without stimulation; in thirteen litter-mates, noradrenaline was infused for 10 min before measurements were made. A further fourteen rabbits were studied after they had been kept at a thermoneutral ambient temperature for 48 hr. In seven of these rabbits measurements were made after noradrenaline infusion. These rabbits were given water and phenidione B.P. (3 mg/kg) on the day before the experiment to minimize clotting in the flow catheter.

In all rabbits the lateral lobe and upper half of the posterior lobe (the tissue drained

by the lateral vein) was dissected free and weighed. The total wet weight of the cervical and interscapular brown adipose tissue was also noted. The tissue glyceride content was measured with a modification of the glycerol method (Hardman *et al.* 1969).

Serum insulin. For these measurements blood was taken from rabbits delivered by Caesarean section and killed immediately or 2 or 48 hr later. The serum insulin concentrations were estimated by a radio-immunoassay method (Grant, 1968) which is a modification of the double antibody method of Morgan & Lazarow (1963). As rabbit insulin standard was not available human insulin standard was used. The validity of this substitution was checked by extracting insulin from adult rabbit pancreatic tissue using the acid-alcohol method of Scott & Fisher (1938) and comparing the dilution curves of this extract with that of a human insulin standard. The curves obtained were parallel. As this did not eliminate the possibility of interference in the assay by a non-insulin factor in rabbit serum, recovery experiments were carried out in which 0·1 ml. aliquots of rabbit serum were added to one of two identical serial dilutions of rabbit insulin. The immunoreactive insulin content was increased by the same amount (5 μu./ml.) throughout. Assay of serial dilutions of new-born rabbit serum with high insulin content produced results consistent with the dilution. The results are expressed in terms of the human insulin and not in absolute terms. The standard deviation of the mean of replicate estimations of insulin in the same serum with mean concentration of 36 μu./ml. was $\pm$ 2·5 μu./ml.

In all experiments the blood glucose was estimated by the method of Huggett & Nixon (1957), the plasma free fatty acid concentration by the method of Novák (1965) and the plasma glycerol by the micro-method of Boehringer (Biochemical Test Combination). The S.D. of the mean of replicate estimates of glucose was +1·0 mg/100 ml., of free fatty acids was +0·035 m-equiv/l. and of glycerol was $\pm$ 0·003 m-mole/l.

RESULTS

Metabolic response to noradrenaline infusion. Noradrenaline is a powerful stimulant of thermogenesis in brown adipose tissue (Hardman & Hull, 1970*a*). The effect of noradrenaline infusion, 4 μg/kg min on the rates of oxygen consumption and colonic temperatures of three groups of unfed rabbits aged 2–4 hr, 24–30 and 48 hr is shown in Table 1. Although the minimal rate of oxygen consumption fell with age the increase with noradrenaline infusion was very similar in the three groups.

The concentrations of blood glucose, serum free fatty acids and glycerol at the end of the infusions are shown in Table 2. In previous experiments infusion of noradrenaline was found to cause a rise in circulating concentrations of all three metabolites in rabbits 2 hr after birth (Hardman & Hull, 1970*a*). From Fig. 1 it can be seen that over the first 48 hr the blood sugar of unfed rabbits gradually falls whilst the concentrations of fatty acid and glycerol remain unchanged. On the basis of this information the results shown in Table 2 suggest that noradrenaline stimulated a greater rise in fatty acid mobilization with increasing starvation.

Brown adipose tissue metabolism in vivo. The arterial concentrations of glucose, free fatty acids and glycerol in the anaesthetized new-born

rabbits were similar to those of unanaesthetized rabbits of similar age. The net exchange of glucose and fatty acids are given in Table 3*a*. Under these conditions the venous sample was not large enough to permit measurement of glycerol concentration. The blood flow through the tissue was only a little less than that found in 7-day-old rabbits. The rate of glucose uptake was high and there was little or no release of fatty acids.

TABLE 1. The rate of oxygen consumption and colonic temperature (mean ± S.E. of mean) before and during noradrenaline infusion (4 μg/kg.min for 30 min) into rabbits previously kept unfed at an environmental temperature of 36° C for varying periods after birth. The environmental temperature during the experiments was maintained at 36° C

		Oxygen consumption (ml./kg.min)		Colonic temp. (°C)	
	Body wt. (g)	Initial	Noradrenaline infusion	Initial	After 30 min noradrenaline infusion
Rabbits aged 2–4 hr (n = 6)	52·3 ± 2·1	19·0 ± 0·9	43·8 ± 3·5	37·9 ± 0·1	39·9 ± 0·2
Rabbits aged 24–36 hr (n = 10)	52·6 ± 1·9	18·7 ± 1·5	42·2 ± 3·6	37·7 ± 0·1	39·5 ± 0·3
Rabbits aged 48 hr (n = 6)	48·5 ± 2·1	11·6 ± 1·3	38·5 ± 3·8	36·3 ± 0·2	38·0 ± 0·5

TABLE 2. The final concentrations of blood glucose, plasma free fatty acids and glycerol after noradrenaline infusion (4 μg/kg.min for 30 min) for the three groups of unfed new-born rabbits shown in Table 1. The figures shown are the mean ± S.E. of mean

	Blood glucose (mg/100 ml.)	Serum free fatty acids (m-equiv/l.)	Serum glycerol (m-mole/l.)
Rabbits age 2–4 hr (n = 6)	104 ± 16·0	0·78 ± 0·08	0·21 ± 0·04
Rabbits aged 24–36 hr (n = 10)	82 ± 9·0	1·04 ± 0·08	0·20 ± 0·02
Rabbits aged 48 hr (n = 6)	78 ± 12·7	1·14 ± 0·14	0·23 ± 0·04

Noradrenaline infusion for 10 min in rabbits which were litter-mates of the previous group caused a rise in the temperature over brown adipose tissue of 1·4 ± 0·14° C (mean ± S.E. of mean). The blood flow and net exchange of glucose, free fatty acids and glycerol at the end of the infusion is shown in Table 3*b*. The arterial concentration of all three metabolites was higher and the venous outflow considerably greater than that

in the animals not given noradrenaline. Although the arteriovenous difference of glucose was less, the net uptake had increased due to the increase in blood flow. Again there was little or no release of fatty acids although there was a high rate of glycerol release.

Forty-eight hours after birth the arterial concentrations of metabolites in anaesthetized rabbits were a little higher with respect to free fatty acids and glycerol in comparison with those found in unanaesthetized rabbits.

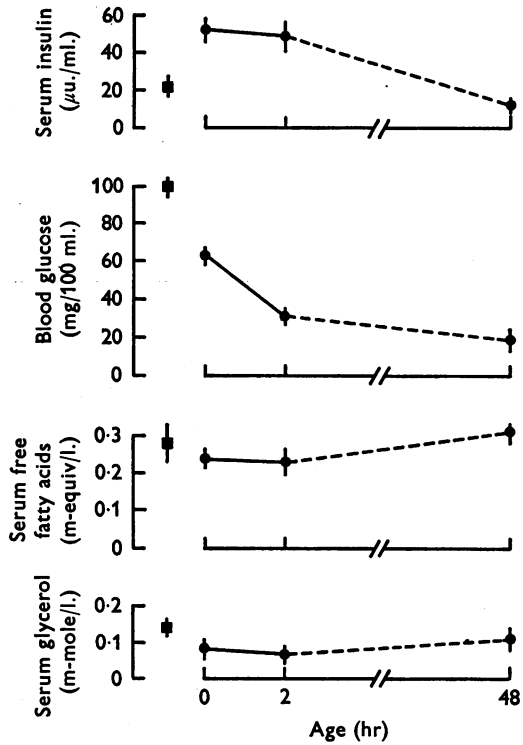

Fig. 1. The mean concentrations of serum insulin, blood glucose, free fatty acids and glycerol in the doe are compared to those in new-born rabbits kept unfed in a thermoneutral environment (36° C) for varying periods after delivery by Caesarean section at term. The vertical line is the S.E. of mean of at least nine experiments at each point.

At this time (Table 4*a*) the tissue was taking up glucose at a rate not far short of that found at birth and was now releasing fatty acids. However, the fat content of brown adipose tissue from these rabbits was similar to that found in the rabbits killed soon after birth, that is 24·6 ± 1·2 g/kg body weight compared to 24·6 ± 1·6 g/kg body weight at birth (mean ± S.E. of mean). In a further seven rabbits given noradrenaline infusions, the arterial concentrations of all three metabolites were higher. During the

infusion the temperature over the tissue rose 0·9 ± 0·07° C and the end the blood flow was three times greater than in the rabbits not infused (Table 4*b*). During noradrenaline infusion the net release of fatty acids was considerable.

TABLE 3(*a*). The arterial concentrations and the arteriovenous differences of glucose, free fatty acids and glycerol of seven unfed rabbits 1–3 hr old (body weight 53·1 ± 4·2 g). The weight of the lateral lobe and the upper half of the posterior lobe was 0·49 ± 0·03 g and the total weight of brown adipose tissue was 2·40 ± 0·25 g. The rate of venous outflow from the lateral vein of the cervical brown adipose tissue is given. The figures shown are the mean ± S.E. of mean

	Arterial concentration	Arteriovenous difference
Blood glucose (ml./100 ml.)	69 ± 4·2	−7·8 ± 1·7
Plasma free fatty acids (m-equiv/l.)	0·21 ± 0·03	+0·04 ± 0·03
Plasma glycerol (m-mole/l.)	0·12 ± 0·01	—
Blood flow (ml./min)	0·34 ± 0·03	

(*b*) The arterial concentrations and the arteriovenous differences of glucose, free fatty acids and glycerol at the 10th min of noradrenaline infusion in fourteen unfed rabbits 1–3 hr old. Body weight 57·1 ± 2·2 g. The weight of the lateral lobe and the upper half of the posterior lobe was 0·60 ± 0·03 g and the total weight of brown adipose tissue was 2·75 ± 0·15 g. The rate of venous outflow from the lateral vein of the cervical brown adipose tissue is given. The figures shown are the mean ± S.E. of mean

	Arterial concentration	Arteriovenous difference
Blood glucose (mg./100 ml.)	102 ± 7·3	−4·1 ± 1·4
Plasma free fatty acids (m-equiv/l.)	0·60 ± 0·05	+0·03 ± 0·02
Plasma glycerol (m-mole/l.)	0·37 ± 0·04	+0·09 ± 0·02
Blood flow (ml./min)	1·0 ± 0·13	

Maternal, foetal and new-born serum insulin concentrations. The foetal insulin concentration was surprisingly high, much higher at term than the maternal level. In the starved new-born rabbit kept in a thermoneutral environment the serum insulin concentration fell over the first 48 hr of life (Fig. 1).

DISCUSSION

In rabbits delivered by Caesarean section the circulating concentration of fatty acids and glycerol at birth were similar to those found 2 hr later. On the other hand the glucose concentration had already begun to fall. The present experiments confirm a previous finding (Milner, 1969) that

the plasma insulin concentration in the foetal rabbit at term is three times that in the mother. It remained around this level over the first 2 hr of life. Experiments on adipose tissue *in vitro* have shown that the action of insulin is to increase glucose uptake and reduce fatty acid release (Fain, Reed & Saperstein, 1967). The present experiments on brown adipose tissue *in vivo* showed that in the first hours after birth, whilst the level of

TABLE 4(*a*). The arterial concentrations and the arteriovenous differences of glucose, free fatty acids and glycerol of eight unfed rabbits 48 hr old. Body weight 48·6 ± 1·5 g. The weight of the lateral lobe and the upper half of the posterior lobe was 0·40 ± 0·02 g and the total weight of brown adipose tissue was 2·08 ± 0·13 g. The rate of venous outflow from the lateral vein of the cervical brown adipose tissue is given. The figures shown are the mean ± S.E. of mean

	Arterial concentration	Arteriovenous difference
Blood glucose (mg/100 ml.)	49 ± 6·9	−4·9 ± 1·1
Plasma-free fatty acids (m-equiv/l.)	0·58 ± 0·08	+0·22 ± 0·05
Plasma glycerol (m-mole/l.)	0·20 ± 0·02	+0·07 ± 0·02
Blood flow (ml./min)	0·40 ± 0·11	

(*b*) The arterial concentrations and the arteriovenous differences of glucose, free fatty acids and glycerol at the 10th min of noradrenaline infusion in rabbits 48 hr old. Body weight 52·0 ± 2·4 g. The weight of the lateral lobe and the upper half of the posterior lobe was 0·46 ± 0·04 g and the total weight of brown adipose tissue was 2·35 ± 0·21 g. The rate of venous outflow from the lateral vein of the cervical brown adipose tissue is given. The figures shown are the mean ± S.E. of mean

	Arterial concentration	Arteriovenous difference
Blood glucose (mg/100 ml.)	56 ± 9·5	−0·5 ± 1·4
Plasma free fatty acids (m-equiv/l.)	1·26 ± 0·06	+0·33 ± 0·07
Plasma glycerol (m-mole/l.)	0·44 ± 0·02	+0·08 ± 0·03
Blood flow (ml./min)	1·34 ± 0·29	

insulin is still high, the tissue took up glucose and did not release fatty acids. It seems probable that in the rabbit, at least, brown adipose tissue at birth is under the influence of insulin. The persisting effect of insulin may be one reason why the blood glucose in the new-born falls after birth despite considerable stores of fat in adipose tissue.

Within 2 hr of birth, exposure to cold or infusion of noradrenaline stimulates a large increase in the rabbit's rate of oxygen consumption and thermogenesis in its brown adipose tissue. These stimuli also cause a rise in the circulating concentrations of glucose, free fatty acids and

glycerol (Hardman & Hull, 1970*a*). The present experiments demonstrate at this age, as in older rabbits, thermogenesis is accompanied by an increase in blood flow through the brown adipose tissue. The high rate of glycerol release indicates rapid hydrolysis of triglyceride, and yet *in vivo*, as was previously shown *in vitro* (Dawkins & Hull, 1964), noradrenaline does not stimulate fatty acid release from brown adipose tissue. Presumably the tissue itself uses all the fatty acids liberated by triglyceride hydrolysis. Thus brown adipose tissue is not responsible for the rise in circulating free fatty acids which accompanies thermogenesis. This could be due to increased mobilization from white adipose tissue, which does not have such large intracellular needs; on the other hand it could be a consequence of decreased utilization secondary to the rise in blood glucose. The high rate of glycerol release from brown adipose tissue during thermogenesis explains why the circulating glycerol concentration rises in response to cold exposure after birth (Hardman & Hull, 1969).

After 48 hr starvation the blood glucose concentration had fallen whilst the concentration of free fatty acids and glycerol remained unchanged. In the anaesthetized rabbits the concentration of metabolites was a little higher than in the unanaesthetized animals. Even after this prolonged period of starvation and in the face of a low arterial concentration, brown adipose tissue took up glucose from the circulation but it had begun to release fatty acids. However, this rate of release is only half that found in young rabbits after a similar period of starvation (Hardman & Hull, 1970*a*). Furthermore, the fat content of their brown adipose tissue is essentially similar to that found in the rabbits killed soon after birth; this confirms a previous finding (Hardman *et al.* 1969). It appears that immediately after birth the tissue is slow to release its stored triglyceride for metabolism elsewhere. These experiments give no evidence why this should be so, but it is interesting to note that the circulating level of insulin is slow to fall after birth and even after 48 hr is similar to that found in the mothers at the time of delivery.

Cold exposure (Hardman *et al.* 1969) and noradrenaline infusion after starvation for 48 hr causes a large rise in the circulating concentration of free fatty acids and glucose. At this time the rate of fatty acid release from brown adipose tissue was considerable. Rabbits kept unfed for 2 days after birth in a warm environment have a poor and variable thermogenic response to their first exposure to cold (Hardman *et al.* 1969), whilst the rabbits in the present experiments had a good thermogenic response to noradrenaline infusion after a similar period of starvation. Thus the poor response to cold is not due to limited thermogenic function in brown adipose tissue.

In conclusion it has been shown that at birth brown adipose tissue has

the capacity to generate heat, but the tissue is slow to release, in response to starvation, its considerable stores of fat for metabolism elsewhere. The circulating insulin concentration is high in new-born rabbits and is slow to fall after birth. Insulin may be one factor involved in the sluggish adjustment of fat and glucose metabolism to the demands of starvation after birth. From a practical point of view the present experiments emphasize that any study of adipose tissue metabolism in the new-born must take into consideration the age and nutrition of the animal.

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