review

Development of brown and white adipose tissue

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In this review, it is assumed that the reader is familiar with the basic properties of white and brown adipose tissue. The role and metabolic characteristics of both tissues in adult mammals have been adequately and repeatedly reviewed (1–3) and will be mentioned here only insofar as they impinge upon developmental aspects. Macroscopically brown fat is found in particular sites of the body, usually under the skin between the shoulder blades, in the axilla, and surrounding the aorta and the kidneys. It is assumed to have a brown appearance because of its high cytochrome *c* content. A typical brown adipose tissue cell contains many densely packed mitochondria and several smaller fat vacuoles. A white fat cell during fetal and perinatal development may appear to be very similar under the microscope, and even today it has not been settled definitely whether or not brown fat changes to white fat during ontogeny or whether they are separate tissues.

The development (ontogenic) of white adipose tissue is fairly well known with regard to structure, cell size, and some microscopic changes. However, metabolic development has been inadequately described and studied. This is particularly true for mammals other than man. In man, it is now possible to describe in some detail early postnatal changes and to compare them with adult parameters. The opposite is true of brown adipose tissue. This tissue has been examined fairly thoroughly in rats, guinea pigs, and rabbits. However, ontogenetic changes in man are unknown. This is due to the fact that it is relatively easy to obtain samples of white fat from normal newborns but almost impossible to sample brown fat from the same source.

For reasons that will become obvious later, we shall first discuss the development of brown fat. This has been reviewed several times (4, 5). Data contained in these reviews will only be touched upon.

Abbreviations: BAT, brown adipose tissue; WAT, white adipose tissue; NE, norepinephrine.

BROWN ADIPOSE TISSUE

The tissue was first described in hibernators (for review see Ref. 2) and was then found to play a role in nonshivering thermogenesis. The production of heat not due to shivering is particularly pronounced in newborn mammals of many species, including man. The stimulus at the cellular level appears to be norepinephrine (NE) (for reviews see Refs. 1, 6, and 7). Removal of 80% of the BAT in newborn rabbits reduces the rise in oxygen consumption in the response to cold by 70% (7), and the same is true for suckling rats (8). The relative role of nonshivering thermogenesis is usually gauged by injecting NE and determining the rise in oxygen consumption at neutral temperature.[1] Calculations show that, in the sheep, nonshivering thermogenesis produces 33% of the extra heat in the newborn and 3% in the adult (9). In the rat, the figures are 70% and 3%, respectively (6); they are about the same in the rabbit. In newborn infants, using thermocouples (7, 10) or thermography (11, 12), the area between the shoulder blades has the highest temperature when the body is cooled (21°C). The same is true for guinea pigs (6) and rabbits (7), indicating that BAT is a site where heat is produced.

Histologically, BAT from newborn mammals differs considerably from WAT; in particular, the number and size of mitochondria are much greater in the former. The appearance of mitochondria also differs, the cristae being more tightly packed and more numerous. Detailed descriptions of developmental changes in BAT ultrastructure have been published (7, 13–17). In the rat just before birth, dense granules are found in the mitochondria. These disappear after birth, the rate of disappearance depending on the degree of cold exposure. In vitro addition

[1] Neutral temperature is that ambient temperature at which an animal's oxygen consumption at rest is lowest. In most mammals, the neutral temperature decreases between birth and weaning.

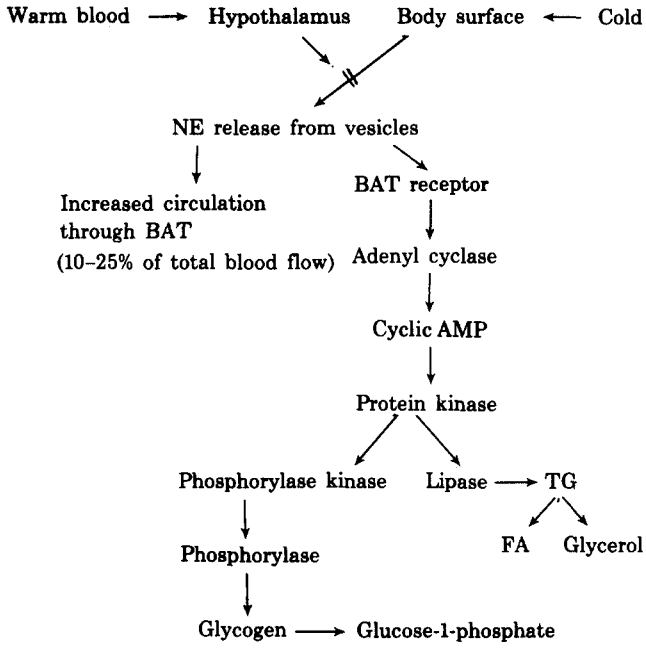

Fig. 1.

of NE or dibutyryl cyclic AMP accelerates their disappearance (14, 15). It has been suggested that these granules are precursors of the inner mitochondrial membrane (13). However, no direct proof for this hypothesis has been forthcoming. It has also been reported that BAT contains sympathetic nerve fibers going to the cells, whereas in WAT only blood vessels are sympathetically innervated (18).

There are considerable differences among species in the time and rate of development of BAT. These differences seem to depend on the degree of maturity of the young at birth. In rats, changes are most pronounced at the time of birth (4); in guinea pigs, they occur about halfway through gestation; and in the hamster, they are seen 15 days after birth. In lambs, all adipose tissue of the newborn has the appearance of BAT and gradually changes to WAT (16).

Composition of BAT

BAT represents from 0 to 6% of the body weight in newborn mammals. With the exception of the hamster, this figure decreases with age and, in fact, adult humans and rabbits do not seem to have any BAT at all. Some species never have any BAT (e.g., the pig). The gross composition of the tissue is given in Table 8 of Ref. 5. In newborn rabbits starved in the cold (25°C), fat gradually disappears from BAT but not from WAT, whereas starvation at 37°C causes no changes in BAT but depletes WAT of fat; the same is true for newborn infants (7). However, rearing rabbits at 25°C from birth for 1 month leads to a final lower weight and a lower fat and nitrogen content of BAT than in rabbits reared at higher temperatures (19–21).

Metabolic patterns

Because there is no doubt that BAT in neonatal mammals and cold-adapted rodents plays a role in heat production, and because BAT is particularly prominent in newborn and cold-adapted mammals, a study of developmental changes and changes during cold acclimation might throw some light on the mechanisms involved in heat production. This kind of study, however, has not been particularly successful. There is no doubt that the mitochondria are involved, and it seems fairly well established that fatty acid oxidation in these mitochondria plays a role. The earlier suggestion that heat is produced by a cycle of triglyceride synthesis and breakdown, which does appear to play a role in WAT, has been ruled out (3, 22, 23), and today the consensus of opinion is that the extra heat produced on cold exposure or after NE injection is due, to some extent at least, to uncoupling of oxidative phosphorylation, which, in the guinea pig at least, is greater at birth than 30 days later (24) (see below).

Let us now follow the chain of events leading from NE release in brown adipose tissue to increased heat production in that tissue (**Fig. 1**).

According to Brück (25), cold stimulates cutaneous thermoreceptors that activate the sympathetic nervous system and lead to NE release in BAT. A center in the hypothalamus keeps this activation in check and is itself regulated by the core body temperature. In newborn guinea pigs, destruction of this center causes a continuous rise in body temperature, which can be checked by injection of hexamethonium. In adult animals, destruction of the same center is without effect because no BAT is present and such animals do not produce heat by nonshivering thermogenesis. The rate of turnover of NE is greater in BAT of cold-adapted than of normal rats (26). At birth, there is a rapid decrease in NE content of BAT and a subsequent rise to prenatal levels within 2 days (26). In vitro labeled NE is bound to BAT to the greatest extent 10 days after birth (18, 27), when most parameters of BAT function have reached a peak. The next step is the activation of adenyl cyclase. This enzyme has been demonstrated in fetal rat BAT (28, 29). Its basal activity increases after birth. The response to NE in vitro in very young fetuses is absent and is highest on about the 10th day (29, 30). The cyclic AMP content of BAT increases very rapidly after birth (when NE content decreases) and then falls, but it never again reaches the low fetal values. Protein kinase is present at all ages. It is always activated by cyclic AMP, but this substance is most effective between days 10 and

20 (29). Because addition of cyclic AMP (or NE) to isolated BAT leads to increased glycerol release in late fetal and suckling rats (31), it is obvious that the whole sequence of events from NE to lipolysis is already developed in late fetal rats and that the one link in the chain that appears later than the others is the sensitivity of adenyl cyclase to NE.

In contrast to the rising fat content of BAT during development is the high glycogen content in fetal tissue, which declines precipitously at birth (29). This is accompanied by a large rise in phosphorylase *a* and *b* activities (29). However, phosphorylase kinase activity, although showing a peak at birth and found primarily in the activated form at the time, increases in total activity up to day 20. Thus, the chain of events leading to glucose-1-phosphate formation from glycogen is also present at birth, and it may be assumed that both glycogen and triglyceride breakdown at birth are initiated by activation of the adenyl cyclase, a rise in the cyclic AMP content, and activation of protein kinase, phosphorylase kinase, phosphorylase, and lipase. In this connection, it should be mentioned that hormone-sensitive lipase activity in BAT and WAT is relatively low in the suckling period (5). An interesting finding is the presence of an inhibitor of protein kinase in BAT. More of this substance is found in fetal than in adult tissue (32). It is tempting to speculate that it has a regulatory role in neonatal lipolysis and glycogenolysis and perhaps also enzyme synthesis. Finally, 3′,5′-cyclic nucleotide phosphodiesterase activity was found to decrease with age in both BAT and WAT (33), although it must be pointed out that the specificity of the method used is now in doubt. If activity is really higher in the suckling period, this might explain the low activity of hormone-sensitive lipase (5).

It is obvious from the above that the immediate effects of NE on BAT are (*a*) an increase in blood flow, (*b*) an increase in cyclic AMP content, (*c*) a decrease in glycogen content, (*d*) activation of phosphorylase, and (*e*) activation of hormone-sensitive lipase. In the rat, at least, all these mechanisms are normally triggered by exit of the fetus from the uterus. However, very little is known about the possibilities of evoking such a chain of events in the fetus. Experiments with injections of NE directly into the rat fetus 1–2 days prenatally did not show any unequivocal changes in BAT, although its development was somewhat accelerated if NE or cyclic AMP was injected prenatally (34). In contrast, the effects of repeated injections of NE into adult rats mimic the effects of chronic cold exposure (35).

Newborn rabbits born into a warm environment apparently do not react by NE release (7), suggesting that it is the cold exposure that initiates postnatal changes in BAT. Similar data are available for newborn infants (7), in whom blood levels of glycerol do not rise if they are kept warm.

Lipolysis itself is not responsible for the increased heat production seen in the newborn. Hence, because heat production is evidently started by NE, metabolites of glucose-1-phosphate, glycerol, or fatty acids must be involved. First, we shall examine the further fate of glucose-1-phosphate.

Glycolysis

The rate of glycolysis in rat BAT probably increases after birth and reaches a peak between days 10 and 20, as indicated by the rise in phosphofructokinase and pyruvate kinase activities (36). In contrast, glucose-6-phosphate dehydrogenase activity decreases considerably after birth. Whether this indicates a decreasing rate of fatty acid synthesis or, more probably, a decrease in the rate of reductive synthesis of other metabolites has not been determined. Malic enzyme, also involved in supplying reducing equivalents for fatty acid synthesis, does not rise in activity until after weaning (36). Since cytoplasmic (NADH-dependent) α-glycerophosphate dehydrogenase activity shows the same trend (5), it may be assumed that the rate of triglyceride synthesis also decreases postnatally. However, this is in contrast to the postnatal accumulation of fat in BAT (for discussion see Ref. 5) and might be related to the large amount of fat in the diet (milk).

Glyceroneogenesis

Even though both BAT and WAT contain a glycerol kinase (5, 22, 37), α-glycerophosphate synthesis from pyruvate also occurs (5, 38). One of the rate-limiting enzymes of this synthesis is phosphoenolpyruvate carboxykinase. The activity of this enzyme in rat BAT rises already prenatally and decreases only after weaning. Synthesis of glucose-6-phosphate from gluconeogenic precursors does not seem possible because adipose tissue lacks a fructose diphosphatase, although Stave (39) has reported the presence of this enzyme in neonatal but not adult BAT of the rabbit.

Fatty acid synthesis

The enzymes that have been examined during development are acetyl CoA synthetase, acetyl CoA carboxylase, and citrate cleavage enzyme (5). All three show a decrease in activity in BAT after birth and a rise after weaning. The rate of incorporation of [^{14}C]acetate or [^{14}C]citrate into triglycerides of BAT homogenates is also lower in the suckling period than after weaning (5). Again, this is very probably due to the high fat content of the milk.

Fatty acid oxidation

There is considerable direct and indirect evidence that fatty acid oxidation is related to the increase in heat pro-

TABLE 1. Retention of radioactive carnitine in brown adipose tissue, heart, and muscle after subcutaneous injection into rats

Age of Rats	BAT	Heart	Muscle	BAT/ Heart	BAT/ Muscle
days	*dpm/mg wet wt*				
5[a]	12,000	4,000	1,000	3	12
45[a]	7,000	6,800	3,000	1	2.5
	dpm/mg tissue: dpm/ml blood				
33[b]	2.5	0.8	1.0	3	2.5
33[b]	0.1	0.1	0.5	1	0.2

[a] Measurements were made 24 hr after subcutaneous administration of L-[^{3}H]carnitine (60 μCi and 0.9 mg/100 g body wt).
[b] Measurements were made 2 hr after subcutaneous administration of DL-[^{14}C]carnitine (0.5 μCi and 0.9 mg/100 g body wt).

TABLE 2. Effect of glucagon or insulin on phosphoenolpyruvate kinase and pyruvate kinase activity in culture of fetal rat brown adipose tissue[a]

	Fresh Tissue	After 24 hr	Plus Glucagon[b]	Plus Insulin[b]
PEPK[c]	56	21.3	27.8	34.2
% of fresh tissue	100	38	50	61
PK[d]	1.0	0.56	0.56	0.5
% of fresh tissue	100	56	56	50

[a] BAT was cultured for 24 hr in Eagle's minimal essential medium under 95% O_2 and 5% CO_2.
[b] 10^{-4} M final concentration during the entire incubation period.
[c] Phosphoenolpyruvate kinase activity, nmoles·mg of protein^{-1}·min^{-1}.
[d] Pyruvate kinase activity, μmoles·mg protein^{-1}·min^{-1}.

duction seen during nonshivering thermogenesis (5, 40). There is no doubt that this occurs on the numerous densely packed mitochondria found in BAT (5). Cold exposure of the newborn infant raises the blood glycerol content to a much greater degree than the FFA content, suggesting utilization of fatty acid in situ (41). Similar changes are seen in the newborn rabbit, in which the rise in both glycerol and FFA content in the blood can be prevented by delivery into a warm environment (7).

As in other tissues, mitochondrial fatty acid oxidation in BAT is carnitine-dependent (5). The greatest increase in oxygen consumption in isolated BAT mitochondria is found after addition of palmitoylcarnitine or palmitate, ATP, malate CoA, and carnitine. In isolated BAT cells, oleate also causes a large rise in oxygen consumption (40). Because the mitochondria are obviously involved, developmental changes in these organelles have been examined (4). All indicators of mitochondrial function increase to a peak 10–20 days after birth in the rat and then decline again. Thus, a BAT cell from a 10-day-old rat contains more mitochondria than one from a newborn or adult. Each mitochondrion contains more α-glycerophosphate dehydrogenase, succinic dehydrogenase, cytochrome oxidase, monoamine oxidase, and cytochromes (4, 42), and also carnitine acetyltransferase and palmitoyltransferase (43). In addition, the cell also contains more carnitine (43). Thus, BAT from suckling rats seems well equipped for maximum oxidation of substrates. If fatty acid oxidation in BAT is an essential part of the response to cold via NE, then any interference with this process should eliminate nonshivering thermogenesis. We have already mentioned that removal of interscapular BAT impairs the response to cold or NE in the newborn rat (8) and rabbit (7). Injection of deoxycarnitine or D-carnitine, which competes with L-carnitine for carnitine acetyltransferase and carnitine palmitoyltransferase, also has the same effect (8), and this can also be shown in in vitro experiments (see also WAT below). An interesting point is the very high activity of carnitine acetyltransferase in BAT from suckling rats, and also from late fetal and newborn monkey BAT (see later WAT). Perhaps acetylcarnitine is used as a "sink" in BAT (43). However, only 10% of the carnitine present in BAT is in the form of acetylcarnitine, much less than, e.g., in the heart (43). We have suggested that the high carnitine acetyltransferase activity in BAT might be related to a high rate of ketone utilization (43).

In the suckling period, BAT not only has the highest carnitine content of any tissue examined, but also takes up labeled carnitine to a greater extent (**Table 1**). There are some suggestions that a binding protein is involved.

BAT from newborn infants has only rarely been examined. It is not easily accessible in vivo, and the one report on needle biopsies (12) does not convince us that the tissue obtained was really BAT. Samples collected at autopsy by us (6–24 hr after death) did not show the high carnitine acetyltransferase activity found in newborn monkeys. This may have been due to autolysis. Alternatively, BAT might not play such an important role in man as it does in other species, particularly since the maximum amount reported to be present in a newborn infant is only 35 g (i.e., about 10 g/kg) as against 330 g of WAT (see also the discussion below on newborn WAT).

Effects of hormones

The adenyl cyclase of BAT seems particularly responsive to NE and epinephrine (22, 29). Increased lipolysis is evoked by glucagon and ACTH in vitro in adult BAT (3, 31). In newborn rat BAT, glucagon and ACTH induce lipolysis but neither is effective in 10-day-old rats, whereas after weaning the effect of both hormones is again apparent (31). This agrees well with the effect on adenyl cyclase, which is activated slightly by both hormones in BAT from newborn and adult rats but not from suckling animals (29). However, the effect of NE is always much greater. This may be related to the low adrenal steroid levels in the blood of suckling rats (see WAT).

Cortisone injections to infant rats lead to accumulation of fat in BAT and to alterations in the tissue mitochondria (27, 44). In fact, the changes produced resemble those seen normally after day 17 postnatally, and it is perhaps worth speculating that on that day the many metabolic changes normally observed in many tissues (45) might be related to increasing blood levels of adrenal steroids (46).

Finally, it should be pointed out that BAT in young rats is also responsive to the composition of the diet. A high fat diet induces phosphoenolpyruvate kinase activity in BAT from 21-day-old rats (47) just as it does in WAT, and, in addition, the inhibitory effect of a high carbohydrate diet can be counteracted by injections of NE or glucagon (48). Recent experiments with cultures of fetal rat BAT indicate that glucagon and also insulin partly prevent the decrease in phosphoenolpyruvate kinase activity normally seen during 24 hr of culturing (**Table 2**). How these data are related to the special role of BAT in nonshivering thermogenesis is difficult to assess.

In summary, BAT in neonatal mammals plays an important role in nonshivering heat production. Fatty acid oxidation is of prime importance. Nevertheless, glycolysis and Krebs cycle intermediates are necessary for normal BAT function. Partial uncoupling of mitochondria seems to be induced by released fatty acid. An interesting point is the very high activity of phosphoenolpyruvate carboxykinase. This suggests the possibility of a cycle between this enzyme and pyruvate kinase and might account for the dissipation of energy resulting from a decrease in the P/O ratio induced by increased cellular fatty acid content, while substrate-level phosphorylation, which apparently is functional in BAT mitochondria, remains unaffected.

Fig. 2 shows how this cycle might be working. The high activity of pyruvate kinase ensures a large supply of pyruvate through the mitochondria. This pyruvate is probably directed to a large extent via pyruvate carboxylase to oxaloacetate since the acetyl CoA is supplied by fatty acid oxidation. The oxaloacetate formed is then transported out of the mitochondria, probably in the form of malate or aspartate, and in the cytoplasm it is changed to oxaloacetate, which then gives rise to phosphoenolpyruvate and GDP. The GDP is then reutilized in the mitochondria for substrate-level phosphorylation, and the GTP formed is again used for phosphoenolpyruvate formation. This would leave the ATP formed in the pyruvate kinase reaction, which might be utilized for fatty acid activation and for glucose phosphorylation. The greater the supply of fatty acid, the more mitochondria are uncoupled, the more ATP is formed in the pyruvate kinase reaction, and the more ATP is required for fatty acid activation and glucose phosphorylation. In addition, it has been shown that the ouabain-sensitive sodium pump probably participates in BAT thermogenesis (49) and would thus be another site of ATP utilization.

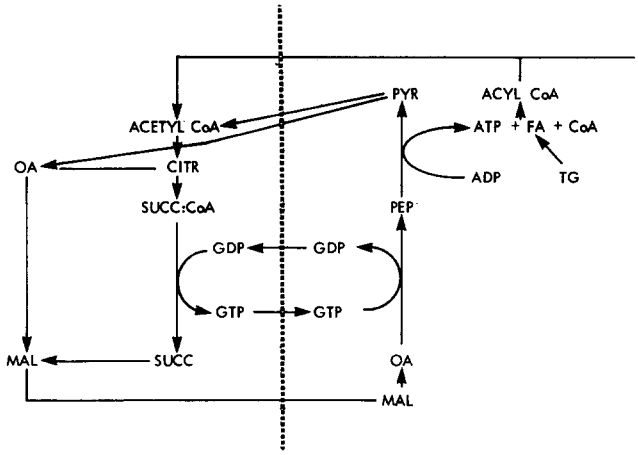

Fig. 2. Diagram illustrating possible role of phosphoenolpyruvate kinase (PEPK) in BAT. Pyruvate is carboxylated to form oxaloacetate (OA) in the mitochondria. This reaction is activated by acetyl CoA, the rate of formation of which is accelerated when more fatty acids (FA) are oxidized. Malate (MAL) itself or aspartate (aspartate transaminases are present in BAT according to our unpublished observations) is transported into the cytoplasm and via OA is decarboxylated by PEPK to phosphoenolpyruvate (PEP). GTP supplies the high-energy phosphate bond. The GDP is returned to the mitochondria to be utilized in substrate phosphorylation while PEP gives rise to pyruvate (PYR) and ATP. The latter is used for (*a*) FA activation, (*b*) cyclic AMP formation, (*c*) protein kinase activation of phosphorylase *b* kinase and lipase, (*d*) glucose phosphorylation, etc. The assumption of this scheme is that GTP and GDP are shuttled across the mitochondrial membranes. For simplicity, the role of carnitine is not shown, nor are all the reactions of the Krebs cycle indicated.

There is some interesting evidence that indirectly supports this scheme. In the first place, the activity of phosphoenolpyruvate carboxykinase is about 10 times higher in brown than in white fat, as expressed per milligram protein of the 100,000 *g* supernate. In other words, an equal amount of cytoplasm in a brown fat cell contains 10 times more enzyme than a white fat cell. Nevertheless, the rate of incorporation of [1-^{14}C]pyruvate into triglycerides is four times greater in WAT than in BAT (**Table 3**), although in both tissues half the label goes into the glycerol moiety. Since these experiments were done with tissue slices, the actual amount of WAT cytoplasm per unit weight was at least half that of BAT cytoplasm so that the total phosphoenolpyruvate carboxykinase available in WAT was at least 20 times less than in BAT. Nevertheless, this amount of enzyme is sufficient to support new α-glycerophosphate synthesis in WAT at a rate that is three to four times that of BAT. Hence, it may be assumed that the extra phosphoenolpyruvate carboxykinase is required for other reactions.

Secondly, the rate of fatty acid synthesis is higher in fetal BAT than in the same tissue from suckling rats (5), yet phosphoenolpyruvate carboxykinase activity increases before birth.[2] This may, of course, be related to the in-

[2] Frohlich, J., and P. Hahn. Unpublished data.

TABLE 3. Ratios of white adipose tissue to brown adipose tissue of different metabolic parameters in suckling and weaned rats

	Suckling Rats (2–10 days old)	Weaned Rats (30–40 days old)
Incorporation of ^{14}C into triglyceride[a]		
$^{14}CO_2$	3.5	0.6
[1-^{14}C]Pyruvate	4.0	3.0
[2-^{14}C]Pyruvate	3.0	2.0
[1-^{14}C]Glucose	1.5	3.0
[6-^{14}C]Glucose	3.0	1.8
[2-^{14}C]Acetate	1.6	0.2
[1,5-^{14}C]Citrate	0.9	0.3
Conversion of substrate to fatty acid[b]		
Citrate	1.0	0.4
Acetate	0.11	0.45
Enzyme activities[c]		
Malic enzyme	1.0	2.0
Glucose-6-phosphate dehydrogenase	0.3	0.5
α-Glycerophosphate dehydrogenase	0.3	0.3
Phosphofructokinase	0.14	0.14
Pyruvate kinase	0.10	0.10

[a] Whole tissue, see Ref. 61.
[b] 100,000 *g* supernate, see Ref. 61.
[c] 100,000 *g* supernate, see Ref. 36.

creasing utilization of exogenous fatty acids (from milk) for triglyceride synthesis, particularly since in weanling rats phosphoenolpyruvate carboxykinase activity can be raised by feeding a high fat diet (47) or by glucagon or norepinephrine injections (48). However, this activity is also increased in WAT, yet the 10:1 ratio for phosphoenolpyruvate carboxykinase in BAT and WAT remains the same. It is also noteworthy that pyruvate oxidation accounts for at least one-fourth of the CO_2 produced in both resting and NE-stimulated BAT from 1-day-old rabbits (37), i.e., NE more than doubles the oxidation of pyruvate. Because pyruvate dehydrogenase activity is high in rat BAT[3] and thus presumably also in rabbit tissue, and assuming that this enzyme is not affected by NE, then the increase in cycling of phosphoenolpyruvate to phosphoenolpyruvate could also account for the rise in CO_2 formation from pyruvate, because more of this labeled substrate would be returned to the pyruvate dehydrogenase reaction.

The actual mechanism of uncoupling, supposed to be due to the greater rate of fatty acid release, is still not quite clear. It has been known for some time that mitochondria in general are uncoupled by fatty acids. However, those from BAT appear to be much more sensitive (40), particularly in newborn mammals (24), where under the most stringent conditions the P/O ratio can never be raised to values found in liver mitochondria, for example. Nevertheless, there is evidence that addition of carnitine leads to some coupling, and it has been shown that only about one-tenth of the total fatty acids present is oxidized during this process (50). Hence, we are presented with the following question: If fatty acids uncouple phosphorylative oxidation in BAT mitochondria and if the oxidation of one-tenth the amount of fatty acids in the presence of carnitine leads to recoupling, is uncoupling the actual basis for the increased heat production? Opinions on this differ. It is possible that a special pool of fatty acids within the mitochondria plays a decisive role, as suggested by Pedersen, Christiansen, and Grav (24). It is also possible that increased fatty acid release leads to uncoupling followed by recoupling as more carnitine enters the mitochondria with the fatty acids. As fatty acid oxidation proceeds, more carnitine leaves the mitochondria and, if lipolysis continues, more fatty acids are released, uncouple the mitochondria, etc. Essentially, oscillations of uncoupling and coupling could be at work during heat production in BAT. Further studies in this field could be very rewarding.

[3] Bailey, K., and P. Hahn. Unpublished data.

WHITE ADIPOSE TISSUE

The pre- and postnatal development of WAT in laboratory animals has not been studied to any large extent. Considerable work has been done on morphological changes during ontogeny (51). Metabolic patterns in the perinatal period have been described in some detail only for man. In the rat, for reasons of convenience, the tissue usually studied is the epididymal fat pad, and this is supposed to represent WAT in general. However, even such global indicators as nitrogen and hydroxyproline content show that the composition of WAT from the region of the gonads, the groin, and the kidney differs in both male and female rats (52). There are hardly any data on the development of WAT from different sites, with the exception of two papers that show that enzyme development and the reaction to hormones are different in different types of WAT (31, 53).

Composition of WAT

In the rat at birth, it is usually impossible, macroscopically, to find any WAT. The fat content of a newborn rat varies between 1 and 2% of the body weight. Apparently, most of this fat is a constituent of body tissues, while some is accumulated in WAT (54). The first WAT visible to the naked eye is in the gluteal region and appears between the 1st and 2nd day of postnatal life. Occasionally, if the litter is small and the newborns very large, some fat can be found here already at birth. Epididymal or ovarian fat

pads appear much later and can be discerned between the 14th and 20th postnatal days (but see Refs. 51 and 54). Intraabdominal fat is also completely absent at birth and usually is still very scanty 20 days later.

The rabbit has about 50 g of fat/kg of body weight at birth, half in brown fat, a quarter in the liver, and 1.25% in inguinal, axillary, and perirenal WAT (55, 56). The guinea pig is born with large fat reserves and in fact loses fat during the 1st wk of postnatal life (45).

In man, histological development of WAT has been described (reviews, Refs. 51 and 57). The content of lipid, water, nitrogen, and DNA in subcutaneous and perirenal fat has been analyzed by Baker (58). At all ages, the latter tissue contains more cells per gram wet weight than the former. Baker also carried out some interesting calculations showing that if we accept a figure of 26% body fat in a 4-month-old infant, adipose tissue accounts for 50% of the body weight (100 g of body contains 26 g of fat and adipose tissue contains just over 50% fat; hence, 1 g of fat is equivalent to 1.9 g of adipose tissue and 26 g of fat to 50 g of adipose tissue). In the newborn, we calculate a figure of 33% (45% fat in adipose tissue, 15% fat in body). Both these figures indicate how great a metabolic role WAT might play in the newborn period.

An even more dramatic illustration of how neonatal adipose tissue is relatively more important than adult adipose tissue comes from the work of Stave.[4] In the newborn rabbit, 39.0% of the total β-hydroxyacyl CoA dehydrogenase activity in the body is found in adipose tissue. In the adult, this figure has dropped to 2.6%. The figures for citrate synthase are 70.6% against 4.5%. It must be emphasized, however, that BAT was the newborn and WAT the adult tissue. Nevertheless, even total enzyme activity per gram of body weight decreased by 50% between the fetus and the adult for β-hydroxyacyl CoA dehydrogenase and increased by more than 10 times for phosphofructokinase. In other words, the ratio of fatty acid oxidation to glycolysis decreased considerably with age. This is in good agreement with data showing that the main energy source of many suckling mammals (rat, rabbit, man, guinea pig) is fat (45).

Metabolic patterns

Histochemically, adipose tissue cells are first recognized by the appearance of glycogen and α-naphthylacetate esterase (lipase?) (57). Glycogen can also be demonstrated chemically. Its content is very high at birth in human WAT (59) and decreases very rapidly postnatally. There is a negative correlation between the length of labor and the glycogen content in WAT, indicating that the highest values (ca. 200 mg %) are found prenatally.

[4] Stave, U. Personal communication.

Considering the two accepted functions of WAT, lipogenesis and lipolysis, what is known of their development and of the development of glycogenolysis, glycolysis, and gluconeogenesis?

First, let us reiterate some of the changes that occur at birth in vivo. In the human neonate, there is a rise in free fatty acid and glycerol content of the blood within 3 hr after birth (45), indicating increased lipolysis (45, 60). Similar changes are found in most newborn mammals (rat [5], rabbit [7], lamb [9]). Depending on the species, this rise may also be due to the ingestion of the high fat diet (milk), particularly in the rat, whose body fat stores are very small (for reviews of these changes, see Refs. 5 and 61), or to lipolysis in adipose tissue, initiated soon after birth before milk is consumed.

If we compare the in vivo with the in vitro changes that occur postnatally in man, we obtain an interesting picture. At a time when the spontaneous in vitro release of glycerol from incubated WAT decreases, the blood glycerol content reaches its peak. The explanation for this might lie in the fact that glycerol utilization by the baby increases more slowly after birth (41) than its release from WAT and that some of the glycerol released by lipolysis may be reesterified in WAT itself (5). In addition, the diglyceride content of newborn WAT is greater than later in life, suggesting some partial hydrolysis of triglycerides in the neonatal period (45).

A surprising finding is the much greater rate of oxygen consumption by isolated cells or mitochondria in adult white fat compared with the newborn (for method see Ref. 62); particularly, isolated mitochondria from newborn WAT supplied with malate, ATP, and CoA respire much more slowly than mitochondria obtained from adult tissue (63, 64). We have, as yet, no explanation for this. However, the lower rate of respiration per gram of tissue of adult as against newborn WAT (65) is presumably due not only to the fact that adult cells are much bigger but also to the fact that they contain a much smaller number of mitochondria than newborn cells. Perhaps these mitochondrial differences are related to their ability to utilize different substrates at different ages. Addition of L-carnitine to mitochondria and isolated cells (66) from newborn WAT enhances the rate of oxygen consumption, but no such effect is seen in the adult.

Comparison of WAT and BAT

Electron microscopic data (67) show that WAT from newborn babies contains at least two types of cells. One type is large and has one large fat vacuole and small mitochondria, and the other is small and has several fat vacuoles and contains large and numerous mitochondria. The two types of cells can be separated by flotation and are found to react differently to carnitine (68). The small cells

increase their respiratory rate when L-carnitine is added, and the effect of norepinephrine is inhibited by deoxycarnitine. These changes are not seen in large cells nor are they observed in adult cells.

It is interesting to note here that the histology of brown adipose tissue from newborn babies is very similar (12, 17) to that of white fat. Considering all these data, it would appear that newborn WAT (at least the small cells contained in it) is similar to BAT and might play a role in nonshivering thermogenesis of the newborn. There are also other similarities: L-Carnitine enhances the rate of lipolysis in newborn but not in adult WAT in vitro (68), and it has the same effect on BAT from newborn rabbits.[5] However, it would be premature to draw this conclusion, because in other respects human BAT and WAT do differ histologically and no biochemical data are available on human newborn BAT (69). The carnitine acetyltransferase shows the same activity in mitochondria from newborn and adult human WAT, whereas carnitine palmitoyltransferase activity is lower in the adult (66). Because the number of mitochondria per cell decreases considerably with age and carnitine palmitoyltransferase activity is also lower in the adult, the ability of adult cells to oxidize fatty acids is presumably much smaller. This is also borne out by direct measurements (67).

If we compare these data with the much greater respiratory rate of adult mitochondria, we must again conclude that some qualitative age change has occurred. On the other hand, the carnitine content of newborn WAT is low (66), much lower than in other human fetal organs (70), in contrast to the rat, where BAT contains more carnitine than most other tissues (43) and is known to play an important role in heat production.

The most serious objection to comparing WAT from newborn babies with BAT in other species is our lack of data on metabolic patterns in human BAT. In the three babies (all premature) that died within 2 wk after delivery and from whom tissues were collected by us within 24 hr after death, mitochondrial carnitine acetyltransferase activity was between 135 and 269 nmoles/mg of mitochondrial protein, i.e., within the range found for newborn WAT. No carnitine palmitoyltransferase could be detected in isolated mitochondria from this tissue, but in the 3000 *g* supernate, activity was 30 nmoles/mg of protein/min as against 26 in the liver of the same infant. In suckling rats the ratio of palmitoyltransferase activity in BAT against liver was 3:1 (47). In fetal (133 days of gestation) monkey, on the other hand, carnitine acetyltransferase activity in BAT was 549 nmoles/mg of mitochondrial protein/min vs. 142 nmoles in liver, and the corresponding values for carnitine palmitoyltransferase were 71 and 32.[6] Thus, it is possible that the data for human BAT were affected by the 12–24-hr postmortem period.

[5] Novak, M., and U. Stave. Personal communication.

[6] Beatty, M., M. Bocek, and P. Hahn. Unpublished data.

In contrast to the equivocal results obtained with human WAT and BAT in the newborn period, the metabolic development of rat WAT and BAT is clearly different and is obviously related to the much greater metabolic load placed on the latter. Table 3 compares some enzyme activities of BAT and WAT in the rat during development. It is apparent from the table that glycolytic enzyme activities are much higher in brown than in white fat, and the same is true for phosphoenolpyruvate carboxykinase, i.e., new α-glycerophosphate formation (but see discussion on this enzyme under BAT). The rate of fatty acid oxidation is also much higher in BAT. The one function in which differences between the two tissues are slight and in which, in fact, WAT develops more rapidly than BAT is fatty acid synthesis.

However, some caution must be observed here, as discussed by us some time ago (5) and again pointed out by Mersman and Brown (71) for the development of pig WAT. WAT cells increase in number and size during development. In the rat, only size continues to increase after the 9th postnatal wk, whereas in the guinea pig cell number increases throughout life as well (72). If we limit our discussion to the rat, then we find that per unit tissue weight, the rate of fatty acid synthesis is greater in suckling than in adult animals. This is in contrast to the *decrease* in the activities of enzymes of fatty acid synthesis calculated per milligram of supernatant protein seen after birth and the subsequent increase after weaning. The reason for this is the increasing cell size, so that although enzyme activities for fatty acid synthesis are low (the diet of suckling mammals is milk, a high fat diet), a unit volume of fat cells from a suckling rat contains more cells and hence more protein than the same volume in older animals (for discussion see Ref. 5).

Let us now consider the sequence of events that probably occurs during and after delivery of the human fetus as it pertains to WAT.

The stress of delivery itself induces changes in WAT that are presumably initiated by catecholamine release, either into the fetal blood or locally directly into adipose tissue. This activates and perhaps even induces an adenyl cyclase in WAT because there is a positive correlation between the duration of labor and the activity of WAT fluoride-stimulated adenyl cyclase (73). The cyclic AMP formed activates a protein kinase(s) and this eventually leads (via phosphorylase kinase) to activation of phosphorylase (73) and breakdown of glycogen. This probable sequence is supported by the fact that WAT glycogen content is negatively correlated with the length of labor and that phosphorylase activity is highest immediately after birth (73).

We still do not know, however, whether the rise in blood levels of glycerol and fatty acids is due to triglyceride breakdown in WAT, BAT, or both. (Is cold the only stimulus? See Ref. 7.) Further, the rate of incorporation of

fatty acids into WAT triglycerides in vitro declines much more slowly (45) after birth than the glycogen or ATP content. However, other data indicate increased lipolysis in WAT immediately at birth or very soon after birth. The rates of glycerol (45, 73) and fatty acid release from isolated WAT or cells are highest within hours after birth, when there is a decline in ATP and glycogen content of the tissue. In fact, a good case can be made for glycogen breakdown being required for ATP synthesis and ATP being essential for lipolysis. We have put forward the hypothesis that L-carnitine enhances lipolysis in isolated WAT from newborns because it recouples WAT mitochondria (as it does in BAT, see Ref. 50) and thus ensures a sufficient supply of ATP for further fatty acid activation. Other data bear this out. Any interference with glycolysis and/or mitochondrial electron transfer suppresses lipolysis (74). However, there is no direct proof for this hypothesis.

Lipolysis

If, as suggested, the stimulus for lipolysis at birth is catecholamine release, it is not surprising to find only a slight effect of NE on lipolysis in vitro in WAT from newborn infants. Glycerol release (without hormone) is very high at that time (45, 73, 74) and it may be assumed that NE is already acting on WAT before it is sampled. The rate of spontaneous glycerol release from WAT in vitro correlates with the length of labor, as does the ATP content.[7] This is also borne out by the fact that the rate of release of glycerol can be enhanced by α-adrenergic blockers (phentolamine) without addition of catecholamine.[7] In adult WAT, α-adrenergic blockers enhance the effect of norepinephrine but are ineffective without it (75). Lipolysis is high in infants from diabetic mothers, and in such infants the glycogen content of WAT is very high and decreases more slowly after birth (73, 76). Because the serum insulin levels are high prenatally and fall postnatally (77), even in normal infants, it may be assumed that the initiation of lipolysis after birth depends not only on catecholamine release but also on the level and rate of decrease of serum insulin and the level of glycogen in WAT. Supportive evidence for this comes from work with BAT from newborn rabbits, where insulin blood levels are found to decrease before BAT in vivo begins to release glycerol in response to cold and, to some extent, starvation (78, 79).

Another hormone that enhances the rate of lipolysis in adult rat epididymal WAT is glucagon. Blood levels of this hormone are elevated in both rats (80) and man (81, 82) immediately after birth. In subcutaneous WAT from suckling rats, glucagon has no effect on lipolysis (31) nor is it effective in human newborn WAT.[7] Its effect on epididymal and ovarian fat increases after the 20th postnatal day (31). Hence, it appears unlikely that this hormone has any direct effect on WAT in the neonatal period. However, it is essential for hepatic glycogen breakdown and gluconeogenesis and, perhaps by raising the blood level of glucose, enhances neonatal lipolysis in WAT. In newborn WAT, addition of glucose or pyruvate in vitro enhances lipolysis (59), presumably by supplying energy for ATP synthesis.

Another lipolytic hormone is ACTH. Burns and Langley (75) did not find any lipolytic effect of ACTH in adult human WAT. In the neonatal rat it is very effective, but its lipolytic effect decreases after the 4th day. This may be related to the decrease in blood levels of adrenal steroids (46) because the spontaneous rate of release of glycerol in WAT from 10-day-old rats treated with cortisone for 2 days is twice that of controls. This is somewhat analogous to the situation described by Braun and Hechter (83) for adult WAT, in which the effect of ACTH on adenyl cyclase is decreased after adrenalectomy and is restored after dexamethazone injections.

In summary, the development of white adipose tissue in both man and rat must be considered in relation to the increase in fat content, and thus cell size, with age. This change can explain the decreases in many enzyme activities expressed per unit of wet weight observed during postnatal development (84–88). In addition, however, some enzyme activities per unit of cytoplasmic or mitochondrial protein are also found to be changed with age and also appear to be affected by the diet (e.g., fatty acid synthesis during the suckling period when a high fat diet is fed). Such developmental changes seem much more pronounced in man than in the rat. Experimental data indicate that, in man, WAT mitochondrial fatty acid oxidation is more carnitine-dependent in newborns than in adults. No data on the effect of carnitine on WAT are available for the rat, but indirect evidence (e.g., removal of BAT) suggests that, in this animal, WAT in the suckling period serves mainly as a lipid store. In the human newborn, we incline to the view that at least some of the WAT cells are more directly involved in heat production than WAT in the suckling rat. In both BAT from newborn rats and WAT from newborn humans, the pathways leading from adenyl cyclase activation to fatty acid release are functional. They are probably also functional in newborn rat WAT, but most of them have not been examined.

Finally, it should be mentioned that, in the rat, the final number of fat cells in the body is determined by the amount of food consumed in the early postnatal period (89, 90) whereas the size of individual cells depends on the momentary food intake. In man, a similar ontogenetic control of the final number of fat cells is probably also at work (91, 92), and it may be suggested with some confidence that in addition to genetic factors the amount and type of diet fed in early infancy and perhaps to the pregnant woman will have an effect on the final amount of WAT in adulthood. From these considerations, we may speculate that the early feeding of excess carbohydrates

[7] Novak, M., and D. Penn-Walker. Unpublished data.

(i.e., sufficient protein and calories and excess sugar), so common in our culture, accentuates lipogenesis directly and, through insulin release, leads to the formation of more WAT cells containing more fat and later perhaps also to exhaustion of the insulin-producing apparatus. Insulin may increase the number of fat cells if given to young rats but does not do so if injected later in life (93, 94), but this has been denied recently (95). In fact, it has long been known that infants born to diabetic mothers are larger and have more fat in their bodies. The same is found in newborn rats injected with insulin just before term (96). The important point here is that excess food intake must occur early in postnatal life, because at that time the final number of fat cells in the body is determined. An excess number of cells thus formed may later in life place an excessive load on the endocrine pancreas and may hence lead to diabetes.

The complex regulations involved are illustrated by the fact that in mice whose mothers were fed a high fat diet, by the 14th postnatal day (when the infants consume milk only) the adipose cell volume is already sevenfold larger than in mice whose mothers consumed the normal laboratory diet (97). Cell numbers, however, are the same in both groups and continue to be the same until the 18th wk of life. Only after that do the experimental animals also show a larger number of cells so that by the 52nd wk mice on the high fat diet have three times the number of cells found in control animals of the same age. This work also shows that WAT at different sites reacts differently. For instance, in male mice the perirenal fat pad shows this difference whereas subcutaneous WAT does not. This renders somewhat doubtful calculations of the total number of fat cells based on counting a few cells from one site only.

In both obese men (98) and mice (99), insulin blood levels are elevated and there is a positive correlation between these levels and WAT cell size.

The mechanisms by which early overfeeding can lead to a larger final number of fat cells have not been examined. It has been accepted for decades that tissue can respond to stimuli either by hyperplasia (increase in cell number) or hypertrophy (increase in cell size). Apparently, during a certain stage of development hyperplasia can readily occur, but when growth has ceased, hypertrophy is the more common response. Thus, the heart of the very young rat reacts with hyperplasia to chronic anoxia. In the adult, cardiac hypertrophy is the response. A similar response is found in rat WAT. Since in both suckling and adult animals the initial stimulus is the same (overeating), the difference must lie in a difference in either the hormonal or the cellular response. We would incline to a primary difference in the latter because we found some time ago that drop cultures of both WAT and BAT grew well if the tissues were taken from young suckling rats but never grew if they were collected from animals older than about 15 days.[8] This is in agreement with the suggestion that the late increase in WAT cell number found in animals overfed when very young is due to the accumulation of fat in cells preformed in the suckling period (100).

Undoubtedly, recent reports make it clear that in our society, overeating, practically from the moment of birth, is now the rule rather than the exception (101, 102) and that as a consequence 6-wk-old babies already tend to be obese. It is thus a distinct possibility that the increased incidence of obesity, diabetes, and perhaps even atherosclerosis in our society is based on the excess food consumption established very early in postnatal life and perhaps even prenatally in the case of undiagnosed prediabetic pregnant women.

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