Morphological and metabolic alterations have been observed in brown adipose tissue (BAT) of animals subjected to a variety of environmental stresses (Smith & Horwitz, 1969). One of these alterations, marked hypertrophy of the tissue, has been reported in hibernating species, and in rats exposed to cold (Cameron & Smith, 1964), to high altitude (Blatteis & Lutherer, 1965), or to strenuous physical exercise (Selye & Timiras, 1949). During cold exposure, BAT growth is associated with enhanced respiratory and lipolytic activities, as well as by elevated tissue levels of nitrogen and norepinephrine (Roberts & Smith, 1967; Chaffee & Smith, 1963; Chaffee et al., 1966). Cold-induced enlargement of BAT is also associated with enhanced glucose oxidation and glucose conversion to fatty acids and to glyceride-glycerol (Steiner & Cahill, 1964). In contrast to cold stress, BAT from heat-stressed hamsters and monkeys is paler, weighs less, and has a lower palmitic acid level and to increases in oleic and linoleic acid levels (Chalvardjian, 1964). Fasting also leads to a decrease in glycogen level (Lachance, 1953). Fasting also leads to a decrease in fatty acid synthesis by white adipose tissue was depressed by cold exposure and increased compared to controls during subsequent refeeding.

INTRODUCTION

The effects of fasting, and fasting followed by refeeding, on glucose utilization and lipogenesis of BAT. These effects were compared to those occurring in epididymal white adipose tissue (WAT) and to those occurring under conditions of cold, heat, and altitude exposure.

MATERIAL AND METHODS

Male Holtzman rats ranging in weight from 300 to 400 g were used in all experiments. They were individually housed at 20°C in galvanized wire cages and fed ad libitum a semi-purified diet for 10 days before experimentation. The diet contained by weight the following components: 20% casein, 73% sucrose, 3% corn oil, 4% mineral mix and a complete vitamin supplement. After this dietary adjustment period the following studies were conducted.

In vivo

One group of rats was placed in a cold room at 5°C, a second group was placed in an environmental chamber at 35°C and relative humidity of 69%. Both groups were maintained at 1600 m altitude. A third group was transported to the summit of Pikes Peak, Colorado (4300 m) and housed in a laboratory facility at 20°C; a control group remained in the laboratory in Denver, Colorado (1600 m) at 20°C. Each group of rats was exposed to its environment for 7 days. During this period diet and water were freely available. There were 6–8 rats in each treatment group.

At the end of the exposure periods, the rats were injected intraperitoneally with 5 μCi/100 g body weight of uniformly labeled D-glucose-14C dissolved in saline. 2 hr after the injection, they were sacrificed by decapitation and epididymal WAT pads and intrascapular BAT pads were excised, weighed and chilled in ice-cold saline. Total lipids and fatty acids were extracted and isolated from the two tissues and radioactivity was determined according to procedures reported previously (Klain et al. 1970).

In vitro

Rats, maintained in the laboratory in Denver, Colorado, at 20°C, were divided into 3 treatment groups of 8 animals each. The first group was on the ad libitum feeding schedule for an additional 4 days while the second
group was subjected to a 4-day fast. The third group was subjected to 4 days of fasting followed by 4 days of ad libitum refeeding. At the end of these treatments the animals were decapitated, after which epididymal WAT pads and intrascapular BAT pads were quickly removed and chilled. Both tissues were cut into 10–15 mg fragments. Approximately 100 mg of fragmented BAT and 50 mg of WAT were placed in incubation flasks. The latter contained 3.0 ml of Krebs-Ringer bicarbonate solution (pH 7.4), 30 μmoles of glucose, and 0.25 μCi glucose-U-14C. They were gassed for 1 min with 95% O2-5% CO2, then sealed with a rubber stopper and were incubated in a Dubnoff shaking apparatus, at 37°C and 100 strokes/min, for 3 hr. At the end of the incubation period, glucose incorporation into lipid components and 14CO2 was measured according to methods described previously (Klain & Burlington, 1964; Klain et al., 1970).

Tissue nitrogen was determined by micro-Kjeldahl digestion followed by nesslerization (Johnson, 1941). All data were evaluated with analyses of variance and significant (P ≤ 0.05) differences between means were identified by the Newman-Keuls procedure (Winer, 1971).

RESULTS

Cold-exposed rats exhibited a marked increase in size of BAT when it was compared to the size of BAT in the control rats (Table 1). Furthermore, BAT from the cold-stressed rats converted about 2.5 times more glucose into fatty acids than control tissue. In contrast, cold exposure completely abolished fatty acid synthesis and led to a slight loss of total lipids from WAT. An increase in size of BAT pads also was observed in the rats exposed to high altitude. However, the increase was significantly less pronounced than that observed in cold-exposed animals. A comparative effect was not observed in WAT. Heat exposure had no effect on fatty acid synthesis or the size of BAT.

Fasting decreased the weight of BAT and WAT but increased the nitrogen concentration of both tissues (Table 1). In BAT, fasting was associated with a 3-fold increase in glucose oxidation and a 45-fold increase in glucose incorporation into glycogen. Fasting led to 4-fold increases in fatty acid and glyceride-glycerol synthesis and to a 150-fold increase in cholesterol synthesis. Refeeding restored the weight and nitrogen level in BAT pads to control levels. However, when compared to the control values, refeeding reduced BAT glucose oxidation and glucose incorporation into glycogen, fatty acids, and glyceride-glycerol. Cholesterol synthesis in the refeed animals was returned to control levels.

In contrast to the BAT results, fasting significantly reduced glucose oxidation and glucose incorporation into glycogen in WAT. Fatty acid and cholesterol syntheses, furthermore, were markedly reduced in fasting rats relative to control rats. Refeeding enhanced glucose oxidation and the syntheses of fatty acids and glyceride-glycerol. Glycogen and cholesterol syntheses were not affected in refeed animals.

DISCUSSION

Significant and opposite metabolic responses of BAT and WAT to cold, high altitude, and in particular to fasting and refeeding stress, have been observed in this study. Reports in the literature indicate reduced lipogenesis in WAT during fasting and enhanced lipid synthesis during subsequent refeeding.
(Moore, 1963; Klain & Burlington, 1964). The present data confirm these reports. However, we would not have expected an elevated rate of glucose oxidation and glucose incorporation into lipids in BAT during the period of food deprivation, as well as a depressed lipogenesis associated with refeeding. The enhanced incorporation of glucose into BAT fatty acids in cold-stressed rats observed in this study confirms the reports of others (Steiner & Cahill, 1964; Himm-Hagen, 1965); it suggests an increased lipid turnover in BAT. The present data also suggest that high altitude exposure and fasting, in addition to cold-exposure, stimulate lipid turnover in BAT.

It has been proposed that the main function of BAT is to generate heat in stressful situations, and to provide heat to the organism when other thermoregulatory mechanisms are not fully developed, or do not function adequately, as in newborn animals or hibernators (Smith & Horwitz, 1969). In such situations, energy released by substrate oxidation is transformed into heat. Enhanced thermogenesis can be accomplished via a fatty acid synthesis-oxidation cycle (Masoro, 1963) or a triglyceride hydrolysis-reesterification cycle (Ball & Jungas, 1961). The former cycle is based on a continual breakdown of triglycerides to fatty acids and to acetyl CoA and subsequent resynthesis of fatty acids from acetyl CoA. The latter involves a continual hydrolysis of triglycerides to glycerol and fatty acids followed by subsequent reesterification. In any event, the distribution of BAT around vital organs and the special arrangement of vascular network which allows warmed blood to be returned to the thorax via its venous drainage aid to regulate the body temperature (Smith & Roberts, 1964).

The present data strongly suggest the concept that BAT may play an equally important role in the maintenance of body temperature during periods of food deprivation when substrate availability is limited. Also, it appears that upon refeeding, when substrates are excessive, the heat-generating mechanism(s) in BAT is markedly limited. Metabolic activity in WAT is stimulated by refeeding but the available substrates, rather than being oxidized, are deposited as triglycerides. Since exogenous fatty acids inhibit lipogenesis in BAT preparations (Steiner & Cahill, 1966), BAT lipogenesis in refed rats may have been inhibited by fatty acids that had been synthesized in WAT and transported to BAT. Indeed, acetyl-CoA carboxylase, the rate-limiting enzyme in the synthetic pathway, is subject to inhibition by long-chain acyl CoA derivatives (Bortz & Lynen, 1963) or free fatty acids (Levy, 1963).

Increased turnover of lipids in BAT appears to be, at least in part, hormone-mediated. In this respect, the role of norepinephrine in stimulating BAT lipolysis in cold-exposed rats has been well documented (Himms-Hagen, 1967). Enhanced activity of adrenal enzymes participating in the synthesis of catecholamines in high altitude-exposed rats has been reported (Klain, 1972). Acute exposure to high altitude, like cold-exposure, produces a decrease in body temperature, especially in small animals such as rats (Roberts et al., 1969). Consequently, additive effects of cold and hypoxia on fatty acid metabolism in BAT of altitude-exposed rats cannot be excluded.

REFERENCES


