CELLULAR MECHANISM OF ADAPTATION TO HIGH ALTITUDE

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A critical review of the literature on acclimatization to high altitude with special emphasis on certain problems which are of interest to the armed forces stationed at high altitude is presented in this paper.

It is a well known fact that the physiological mechanisms in the body are not very rigid and that they respond to conditions external and internal which necessitate changes to adapt the body to the new situation. Such physiological changes can, ultimately, be traced to the cellular level because the cells are the biological units of function and in them take place biochemical processes which produce, conserve and utilize energy for the various functions of the body. The cell itself has a highly complicated structure and organization which differs from tissue to tissue depending upon the specific function it performs. All these cells need energy for the performance of their function and the energy producing reactions take place mostly in the mitochondria by oxidative processes. The mitochondrion is a highly organized component of the cell which contains the complicated array of enzymes and co-factors which catalyze the chain of biochemical reactions leading to the production, conservation and utilization of energy. If an animal is exposed to an environment which calls for a greater expenditure of energy, the physiological changes that take place in the body are those leading to the more efficient production and more economic utilization of energy.

Such an environment, for instance, is countered at high altitude due to extremely low temperature and decreased oxygen tension when specific physiological responses may be elicited in an individual to counteract the effects of cold and decreased oxygen tension. If such responses are adequate to counteract these factors the individual can be said to be acclimatized to the new environment. An understanding of the mechanisms involved in these adaptive responses and the various other factors influencing them, may help us a great deal in inducing and perhaps facilitating the adaptive changes effectively in a shorter time and thus enhancing the process of acclimatization and increasing the physiological efficiency of the individual.

LOW OXYGEN TENSION

At high altitudes these factors exist simultaneously and are complementary to each other. However, let us leave the effect of cold and consider only the effect of gradually decreasing pressure generally encountered by mountaineers. The effect of low oxygen tension will mainly be on the respiratory system. As a result, the primary response of the individual will be to ensure sufficient amount of air reaching the lungs. Increase in the pulmonary ventilation is, therefore, the immediate response to this situation. Smith *et. al*[1] have shown that increased haematopoiesis occurs on exposure to high altitude. A larger amount of erythrocytes will hold a larger amount of oxygen. Other tissues also develop mechanism to store as much of oxygen as possible. Thus, the myoglobin content of the muscle is increased [2-6]. Excretion of highly alkaline urine with low ammonia

content is the result of hyperventilation, which removes a lot of carbondioxide from the blood. The mountain sickness which sets in the beginning is got rid of, once adaptations set in. Because the physical activities of the individual are maintained by the muscle, a major portion of the oxygen utilized by the individual may be expected to be expended in the muscle. The increase in the myoglobin content of the muscle is certainly a mechanism to ensure enough of oxygen to the muscle to maintain its activity and may favour the kinetics of oxygen utilization during adaptation to high altitude. Once these adaptations have fully set in, the individual should be able to maintain his normal activities, if other factors do not interfere. But there are other interfering factors, the most important of which is cold.

COLD

In a non-hibernating homoeothermic animal, exposure to cold causes excessive loss of heat from the body and the cold stimuli evoke shivering which keeps up the temperature of the body. In a normal animal at sea level the body temperature is maintained by the heat generated during catabolic processes. The catabolic processes are mediated by a number of enzymes and co-factors which are present in the tissues in adequate quantities for the normal needs of the body. In animals exposed to cold, the normal rate of catabolic processes is inadequate to meet the increased caloric needs of the body. As a result, there is a tendency to increase the heat output by an increased metabolic rate as shown by several workers. A 25-30% elevation in metabolism was observed in men exposed to cold for periods upto two weeks [7-9]. In small animals, the metabolic rate may be doubled for many months[10]. According to Hart [10], the raised heat production at a given temperature does not appreciably enhance survival in the cold, but is a symptom of cold acclimation which is associated with increased metabolism of tissues measured *in vitro*. Then comes a stage when the increased metabolic rate also becomes inadequate to meet the energy requirements of the body. This probably results in shivering.

Apart from the elevation in heat production, other methods of cold resistance may develop in animals. This is by increasing body insulation with fall in environmental temperature as observed in the case of small birds[11,12]. One of the principal effects of acclimatization of white rats to winter is the reduction of heat loss in the cold[13]. These are associated in part with increased insulation of fur or feather, lowered peripheral temperature as in rats and ability of tissues to function at lower temperature as in the feet of herring gull[14]. In studies on man two opposing adjustments (*i*) peripheral heating as seen in Eskimos, Indians and cold acclimatized Caucasians[15,16] and (*ii*) toleration of greater peripheral cooling as seen in Australian Aborigins and Alaskan students [17-20] are also seen as natural climatic responses. Another important adjustment of man acclimatizing to cold is a relative increase in the temperature of his extremities[21-24]. The studies of Mackworth[25] showed a greater blood flow to the hand during winter. Metabolic alterations comparable with those in cold conditioned animals have never been observed in man because the extent and duration of exposure to cold have not been comparable. The presence of clothing and activity greatly retards natural acclimatization[26]. Field studies have suggested reduction in "Core" and greater heating of appendages [21], coupled with delayed metabolic response. These processes may be combined with the metabolic type of changes seen in cold conditioned animals. Much attention is, therefore, paid to the study of increased cold resistance through an increased capacity to produce heat as a result of metabolic alterations.

METABOLIC CHANGES

The existing knowledge on the metabolic alterations induced by cold has recently been reviewed by Depocas[27]. Acclimation to cold is a gradual process requiring 3-4 weeks in mice at 10°C, and approximately 2 weeks in white rats[28] at 6°C. Prolonged exposure of white rats to cold environment in which the animal survives results in a greater metabolic rate measured at any temperature and increased ability to maintain high rate of heat production in cold environment[29], and greater maximal thermogenic capacity[30]. Increased heat output in cold involves mobilization and oxidation of reserves and in the first days of exposure this usually results in loss of body weight[31-33] since food consumption does not immediately increase to a level sufficient to satisfy the increased caloric requirements. Increase in food consumption is gradual probably because this should be accompanied by increased production of enzymes and other factors which are needed for the metabolism of the food. This cannot be spontaneous and during the transition period shivering is probably the only means of additional heat production.

Since carbohydrate reserves are small and fat reserves are relatively abundant, it may be expected that the latter will participate more extensively as substrate for cold thermogenesis during exposure lasting more than few hours[27]. A fall in the R.Q. of animals exposed to moderate cold suggests a strict dependence of cold thermogenesis on fatty acid oxidation[27]. Page and Chenier[34] also noted a small decrease in R.Q. of fasted rats exposed to cold. It does not mean that carbohydrates do not participate as fuel for thermogenesis. But a greater contribution of non-carbohydrate reserves as fuel for cold thermogenesis is observed[27]. Direct measurement of the rate of oxidation of fatty acids as altered by exposure to cold has not yet been feasible. Fat accounted for 85-89% of the total calorico reserves lost by mice at —7°C, protein supplying most of the remainder[35]. Total body fat is much lower in cold acclimated mice and rats whether the animals are on a high carbohydrate or high fat diet[36,37]. There is some evidence to show that in cold environment[31,38,39] high fat diet prolongs survival of rats and lipid metabolism remains elevated[40]. Diets which normally induce fatty livers in rats at room temperature fail to do so in the cold [41,42]. Prolonged exposure to 1°C cures fatty livers previously developed in the warm[43]. Acclimation to cold prevents (*i*) ketosis normally observed in fasting rats at 25—27°C[44] and (*ii*) massive mobilization of lipids to the liver normally observed in warm acclimated rats fasted in the cold [45]. These results can be interpreted on the basis of greater ability to oxidize acetate and long chain fatty acids to carbon dioxide and water, thus preventing accumulation of fat and excessive ketogenesis.

Protein metabolism generally remains increased in the cold acclimated animals[46]. Klain and Vaughan[47] noted an increase in glutamic-oxalacetic-transaminase and glutamic-pyruvic-transaminase in the liver.

ENZYMIC AND OTHER BIOCHEMICAL CHANGES

Changes in the activity of various enzymes in tissues of rats exposed to cold are tabulated and presented by Depocas[27]. These studies indicate a relationship between length of time in the cold and changes in enzymic activity, and provide evidence for a slow elaboration of a "revised enzyme pattern" of the cold acclimated rat. It is significant to note that there is a tendency for reduction in the activity of some of the glycolytic enzymes and an increase in the oxidative enzymes in both liver and muscle. Homogenates from livers of cold acclimated rats were more active in oxidizing intermediates of the Krebs cycle but

not lactate[48]. Consequently, the activities of succinic and malic dehydrogenases were increased in the liver homogenates of cold acclimated rats, but lactic dehydrogenase remained unchanged[49].

The increase in the oxidative activity is associated with the increase in the oxygen consumption of rats acclimated to cold. The oxygen consumption of rats measured at 30°C increases during acclimation to cold[49,50]. A gradual increase in oxygen consumption could also be observed in various tissue slices of rat[48,51-55]. Hannon[49] interpreted these changes as manifestations of the increased capacity of the cold acclimated rat to produce heat by means other than shivering. However, at all temperatures cold acclimated rats have a higher oxygen consumption and non- shivering heat production is superimposed on the high resting oxygen consumption.

Increased concentration of cytochrome *C* in tissues of animals exposed to high altitude[56] may favour increased oxidative activity. Many authors have noted increase in the oxidative enzymes of animals exposed to high altitude[57,58]. It is not clear whether the greater capacity of certain oxidative enzymes of animals exposed to high altitude is the result of altitude acclimation, or acclimation to cold existing at high altitude. Tappan *et al.*[58] interpret these results as advantageous through a mass action effect in which enzyme activity compensates for limited amount of oxygen available.

From the foregoing observations it is possible to visualize the process of acclimatization to high altitude as taking place along two lines (*i*) compensating mechanism to meet oxygen deficiency as manifested by increased pulmonary ventilation, increase in the myoglobin content, haematocrit values, haemoglobin values etc. and (*ii*) adaptive changes in the cytochrome *C* content, increased enzymic activity, increased oxygen consumption etc. which lead to the development of non-shivering thermogenesis. Even in the absence of decreased oxygen tension associated with high altitude, these changes were noted in animals on chronic exposure to cold by a number of workers. It is, therefore, possible that whether in altitude acclimation or cold acclimation, the mechanisms are essentially the same but varying only in degree with regard to certain aspects. In both cases noradrenaline seems to play a very important role.

NON-SHIVERING THERMOGENESIS

Non-shivering thermogenesis is probably of wide occurrence in mammals. The development of non-shivering thermogenesis in rats acclimated to cold greatly extends its range for activity. This is because activity eliminates and reduces insulation in warm acclimated rats leading to hypothermia. Activity does not eliminate non-shivering thermogenesis in cold acclimated rats with the result that the total heat production is sufficient to offset the fall in insulation and the body temperature is maintained[10]. The increased thermogenic capacity of the cold acclimated rat manifested by its extended peak matabolic rate is understandable in terms of addition of heat resulting from non-shivering thermogenesis to the heat of shivering which can still be elicited by exposure to temperatures lower than that of acclimation[59]. Maintenance of animals continuously living in cold environment involves catabolic reactions associated with higher heat production. In a normal animal some of the chemical energy obtainable from the catabolic processes are trapped in the form of ATP, and is utilized for synthetic purposes or any other biological function. In cold, when the heat requirement of the body is more, the trapping of chemical energy may be

expected to be lower because of the diminished coupling of oxidation with phosphorylation. Reduction in P : O ratio in liver mitochondria and liver homogenates of cold acclimated rats has been demonstrated[60-62]. This would effect the electron transport system and involve NAD, NADP and the cytochrome systems. Lowered P : O ratio would also result in the maintenance of a higher cellular level of ADP. Slater and Hulsmann[63] consider this as one of the driving mechanisms for maintenance of a higher rate of oxygen consumption. As soon as the cold stimulus induces shivering through its nervous mechanism, the rate of ATP hydrolysis is increased due to increased muscular contraction cycle thus providing an increase in ADP availability to the coupled oxidative phosphorylation system. This results in an elevated rate of electron transport, substrate utilization, oxygen consumption and heat production from oxidation-reduction steps not involved in ATP synthesis as well as heat released during the increased hydrolysis of the terminal pyrophosphate bond of ATP[64]. It seems possible, therefore, that uncoupling is the principal mechanism of non-shivering thermogenesis. The increased activity of the thyroid at high altitude may further favour increased metabolic rate and uncoupling of oxidative phosphorylation because the thyroid hormone is known to be an uncoupling agent. Whether the heat produced is ultimately released directly or indirectly via ATP does not seem to be thermodynamically important in a steady state system of cold acclimated animal, in which the food consumed (about twice the normal caloric level) is mostly oxidized and does not enter into anabolic reactions, since a cold acclimated adult animal is severely catabolic. Decrease or extinction of shivering reflects important adaptive changes resulting from chronic exposure to cold[26] and would serve as an index of cold acclimatization in animals [59,65].

SITE OF NON-SHIVERING THERMOGENESIS

On the basis of studies on non-esterified fatty acid (NEFA) oxidation and the effect of noradrenaline and the measurement of temperature of the liver, Hannon *et al.* [66] contend that liver plays a strong calorigenic role in non-shivering thermogenesis. On the contrary, Depocas believes that muscle may be the tissue in which non-shivering thermogenesis takes place. His arguments are based on the fact that functional evisceration does not prevent the immediate rise in oxygen consumption seen in the anaesthetized cold acclimated rats transferred from 30°C to 6°C and curarization does not abolish this response once it is established[67]. Moreover, noradrenaline is as effective in increasing the oxygen consumption of the functionally eviscerated cold acclimated rat as that of the sham-operated animal[68]. Kawahata and Carlson[69] could not detect increased heat output from liver of curarized cold acclimated rats exposed to cold. These observations invalidate the presumed importance of the liver in thermoregulation in the cold acclimated rat. The demonstration of oxygen consumption by the muscle in the absence of muscular contraction[70] indicates that non-contracting muscle can produce heat and may be an important contributor of non-shivering thermogenesis in the cold. Davis [26] is also of the opinion that non-contracting muscle is an important site of non-shivering heat production.

EFFECT OF NORADRENALINE IN NON-SHIVERING THERMOGENESIS

The calorigenic action of noradrenaline in cold acclimated rats was first reported by Hsieh and Carlson[71]. This was confirmed and extended by Depocas[68] and later on by others[72—74]. The data on normal rats reported by Schotz & Page [75] would suggest that noradrenaline might exert its calorigenic action through the mobilization and subsequent

oxidation of NEFA. Other reports [72-74] appear to confirm this suggestion. By infusion of noradrenaline into a group of cold acclimated rats Hannon *et al.*[66] demonstrated a marked lowering of the R. Q. from an initial value of 0·88 ± 0·020 to a minimal value of 0·74 ± 0·016, strongly implying a noradrenaline stimulation of lipid oxidation. If NEFA were the supporting metabolites for the calorigenic action of noradrenaline, then we might expect that the greater effect in the cold acclimated animal might be due to at least in part to a greater tissue capacity for NEFA oxidation. It also seems possible that the greater calorigenic action of noradrenaline in the cold acclimated animal might include an increased capacity of NEFA mobilization and it was shown to be so by Hannon *et al*[66]. Further, if noradrenaline were the mediator we might expect an obligate sustained low R.Q. while the animal is in the cold. Kayser[76] and Page & Chenier[34] have presented some evidence to support this expectation. They have reported a lowering of the R.Q. during exposure of rats to cold and have concluded that such animals preferentially burn fat. Moore [77], studying the metabolism of kitten diaphragm found that the long chain non-esterified fatty acids were, apart from succinate, the only substance of biological origin which stimulates respiration *in vitro* in this tissue and concluded that NEFA is a component of non-shivering thermogenesis. Chatonnet *et al.*[78] observed that while in the case of dog extraneous injected adrenaline and noradrenaline are much less efficient in warm non-shivering conditions, they increase the maximum amount of heat which can be produced by the shivering animal in the cold. It is clear, therefore, that in small animals cold acclimation is largely a metabolic process with increased capacity to produce heat especially through non-shivering thermogenesis and is mediated by noradrenaline which improves the animal's capacity for mobilization and oxidation of NEFA.

SUBSTRATE

Let us now examine the status of carbohydrate, protein and lipid metabolism in the cold acclimated animal. There is very little evidence of enzyme changes that would favour an increased glycogen catabolism[79]. It is shown that prolonged exposure to cold induces an increase in the capacity of the liver to oxidize a wide variety of substrates ranging from the Krebs cycle from citrate to malate. It is also shown that cold acclimation leads to improved capacity of the oxidation of several substrates that feed into the cycle either directly or indirectly. These include amino acids and lipids. The role of lipids is highly significant because of the importance of NEFA in non-shivering thermogenesis. The increase in the capacity of electron transport system is also highly significant because this system constitutes the terminal stages of the oxidative process and it is here that hydrogen and electrons, removed from metabolic intermediates by dehydrogenases, are transported sequentially via flavoproteins and cytochromes to be combined with oxygen to form water. It is here also that most of the energy originally contained within the food molecules is released. Thus greater contribution of non-carbohydrate reserves as fuel for cold thermogenesis is evident.

The immediate effect of noradrenaline was not an increase of oxygen uptake but it was an increase of the plasma free fatty acid (FFA) level. As little as 0·05 μg/kg/min. had a clearly measurable effect up to 0·7 μg/kg/min. It was possible to get a good dose response curve in the dog. At this higher dose the plasma free fatty acid concentration increased five fold[80]. The greater heat output and oxygen consumption in the cold acclimated animal may, therefore, be considered as due to the higher tissue concentration of free fatty acid and they are known to stimulate oxygen consumption and uncouple oxidative phosphorylation[81]. It is also believed that the magnitude of cellular metabolism is determined at

least in part by substrate concentration and, therefore, a greater concentration of free fatty acid in the cell would lead to greater metabolism of the substrate. The data obtained on cold sensitive obese-hyperglycemic mice suggest that proper mobilization of fat is necessary for proper functioning of the non-shivering mechanism [26].

ADIPOSE TISSUE

Changes in the adipose tissue of cold acclimated animals is also very important in this context. Perirenal and subcutaneous fat from hamsters kept at 6°C exhibited a depression of melting point throughout the experimental period [82]. A decrease in melting point and increase in iodine number in fat was also reported by other authors [37, 40, 83, 84]. In the light of recent changes in our concept of the function of adipose tissue from that of an inert storage site to that of a very active system playing a central role in many phases of the intermediary metabolism, it would seem that changes in its composition may reflect yet another aspect of the metabolic changes associated with the process of cold acclimation. The principal changes in the adipose tissue of hamsters exposed to cold were a decrease in the mole fractions of the 16-carbon saturated palmitic acid and an increase in the mole fraction of the 18-carbon mono-unsaturated oleic acid. Significant differences were not found in the mole fractions of plamitoleic and stearic acids between control and cold exposed animals [82]. Judging from the changes in the fatty acid composition Kodama & Pace[82] believe that the softening of body fat during cold exposure may be the result of changes in the matabolism of adipose tissue as a whole rather than changes in metabolism of fatty acids *per se*. Alterations in the processes involved in the assimilation of fatty acids into fat cells, the esterification of fatty acids into glycerides, the lipolysis and lipogenesis of glycerides and the release of fatty acids, all might be expected to modify the fatty acid composition of adipose tissue. The new steady state mixture of fatty acids in the depot is more unsaturated and smaller in amount. Whether this is due to selective utilization of saturated or unsaturated fatty acids is not known. Smith [85] presents further evidence, in rats, for the participation of brown adipose tissue in thermoregulation.

OXIDATION OF FAT AT HIGH ALTITUDE

Let us examine how far fat would help in altitude acclimatization. All other conditions remaining the same, the limiting factor that might exist at high altitude on the oxidative activities of the tissues is the lower oxygen tension in the atmosphere. Whether this factor is so important as to exclude the possibility of fat being a major substrate for acclimatization to cold at high altitude by oxidative processes already discussed is a matter of great practical importance. Some elementary calculations will help to solve this problem. It cannot be denied that at high altitude, as on mere exposure to severe cold, energy production by oxidative process is of great survival value. This energy has to come either by the oxidation of carbohydrate or non-carbohydrate substrate or from both. The most favoured non-carbohydrate substrate is fat. Whether for the oxidation of carbohydrate or fat, large amount of oxygen is required. Thus the oxidation of one mole of glucose which produces 673 Calories of energy requires 6 moles of oxygen according to the following equation

$$C_6H_{12}O_6 + 6O_2 = 6\,CO_2 + 6\,H_2O + 673\ \text{Cal.}$$

In other words, 134·4 litres of oxygen is used up in this reaction to liberate 134·4 litres of carbon dioxide. So the R.Q. is (134·4 litres CO_2)/(134·4 litres O_2) =1. One litre of

the oxygen can, therefore, produce 5·01 Calories by the oxidation of 1·34 gm. of glucose. For 100 Calories, 26·6 gm. of glucose has to be oxidized and 20 litres of oxygen used up in the process.

Now, if we take a triglyceride containing 2 molecules of stearic and one molecule of palmitic acid, the oxidation of its 2 moles will produce 16353 Calories according to the following equation

$$2\ (C_{55}\ H_{106}\ O_6) + 157\ O_2 = 110\ CO_2 + 106\ H_2O + 16353\ Cal.$$

The R.Q. in this case is

$$(110\ CO_2)/(157\ O_2) \text{ or } (2464 \text{ litres } CO_2)/(3516{\cdot}8 \text{ litres } O_2) = 0{\cdot}701$$

1 litre of oxygen can produce 4·65 Calories by the oxidation of 0·49 gm. of the fat. For 100 Calories, therefore, 10·6 gm. of fat has to be oxidised at the expense of 21·5 litres of oxygen. The percentage increase in the consumption of oxygen for the oxidation of fat as compared to carbohydrate is only 7·5, whereas to produce the same amount of energy, the increase in quantity of carbohydrate that has to be oxidized compared to the amount of fat, is 150 %. If the energy is to be derived exclusively from carbohydrates, in a man requiring 5000 Calories per day, 1000 litres of oxygen will have to be used. If fat is the exclusive substrate, the amount of oxygen required is 1075 litres. On the average the amount of oxygen required per hour is 41·7 litres for carbohydrate and 44·8 litres for fat or 700 cc per minute for carbohydrate and 746 cc per minute for fat *i.e.*, only an additional 46 cc of oxygen is required per minute if fat is the exclusive substrate. But in an animal living on a mixed diet, it is almost impossible to think of carbohydrate or fat being the exclusive substrate for energy purposes although there is some evidence which shows that calories from fat can completely replace calories from carbohydrates in the diet without affecting the rate of growth or nitrogen retention [86]. The energy derived can be from carbohydrates, fats and also proteins. So in a cold acclimated person in the plains, the oxygen requirement will be more than 700 cc per minute because in such persons a good percentage of the energy comes from fat. Even in persons living at normal temperature in the plains, fat forms an important source of energy. In a cold acclimated person at high altitude also neither carbohydrate nor fat can be the exclusive substrate for energy production. Therefore, the requirement of oxygen will, in this case, be below 746 cc per minute. This cannot go to the lower limit of 700 cc per minute because fatty acid oxidation is an essential requirement for cold acclimation. It can be seen very clearly, therefore, that the oxygen requirement of cold acclimated animals in the plains, as well as at high altitude, will be the same and any attempt to economise on the utilization of oxygen by increased carbohydrate diet may not yield the required results because in carbohydrate fed cold acclimated animals there is increased lipogenesis and oxidation of fatty acids provides a major portion of the energy produced. Therefore, any argument against feeding man or animals on a high fat diet at high altitude on the basis of oxygen availability and utilization, may not be justified. In such cases adaptations, besides those required for cold acclimation, should come, not by changing the nature of the substrate used but by finding ways and means of ensuring adequate supply of oxygen to the tissue. If the working efficiency of a person suffers at high altitude it is not because of the inability to oxidize fatty acids but because of the lack of oxygen for oxidation, whatever may be the substrate. There exists, therefore, a strong case in support of the enrichment of the diet of people acclimatizing to high

altitude by increasing its fat content. Fat has certain other advantages also over carbohydrate. As mentioned earlier, the quantity of carbohydrate that has to be burnt up to produce a given amount of energy is $2\frac{1}{2}$ times that of fat. So fat is more advantageous from the point of view of bulk. Moreover, the metabolic production of water from fat is much greater than from carbohydrates, which again is advantageous for man or animals at high altitude.

OXIDATION OF FAT IN MAN

Contrary to the earlier concept that glycogen is the most readily available fuel for muscular activity, it has been shown that man and some other animals are capable of burning fat at rates adequate to support the basal needs as also intense and prolonged muscular activity. Muscle accounts for 35 – 40% of the total body oxygen uptake at rest, and following a 16 hour fast in man [84]. Muscle glucose uptake is relatively small and accounts for only 20% of the total body glucose uptake[84]. The oxidation of glucose would account for only 7% of oxygen uptake showing that glucose extracted from blood is only a minor fuel for skeletal muscle at the basal state. It is unlikely that glycogen in muscle is oxidized to an important extent in the basal state. Most of the oxygen uptake is probably spent on oxidation of non-carbohydrate material in the fore-arm muscle. The mean R.Q. of 0·80 suggested lipid to be a major substrate [84]. A study of the metabolism of 14C labelled acetate, octanoate and palmitate by isolated skeletal muscle showed an enhanced conversion of substrate to $14CO_2$[87]. Electrical stimulation of muscle under tension resulted in approximately 60% increase in oxygen comsumption and about 100% rise in fatty acid metabolism by muscle which suggested that fat degradation in isolated muscle may provide an energy source during activity. There is also strong evidence to suggest that fatty acids may well be the major source of substrate for the support of resting respiration of rat diaphragm *in vitro*[88]. The fall in total fatty acids observed by these workers could account for 100% of the oxygen consumption of diaphragms from fed rats. Neptune & Foreman[89] observed that glycogen that disappeared and could not be accounted for as lactate would support only 10% of the oxygen consumption of rat diaphragm. Bing[90] studying the metabolism of fat of the human heart by sampling the blood drawn from the coronary sinus by the catheter technique have shown that nearly 67% of the required energy is derived from fatty acids and only a portion of the remaining from carbohydrates. Gousios *et al.*[91] very recently emphasized the significant role of serun triglycerides and free fatty acids in the metabolism of the myocardium. George & Scaria[92] provided further evidence to show that lipids are the major source of energy for cardiac muscle.

IN OTHER ANIMALS

A large volume of literature is available to show the importance of fat as fuel of muscle metabolism. The subject has recently been reviewed extensively by Drummond & Black[93]. A few instances are worth mentioning here. Migratory insects and birds which indulge in sustained muscular activity need a lot of energy for their non-stop cross country flights. In these animals there is a heavy deposition of fat prior to migration and most of it is used up during the flight. In an exhaustive study of *Schistocerca gregaria*, the desert locust, Weiss-Fogh[94] established that fat is the principal source of energy during flight. The monarch butterfly *Daneus plexippus plexippus* which engages in continental migration similarly appears to use fat as fuel[95]. Zebe[96] concluded on the basis of R.Q. values that large number of lepidopteran species utilize fat during rest

and during flight even though they feed on nectar. The recent studies of George, Vallyathan & Scaria on the lipolytic activity of flight muscles of various insects suggested the utilization of fat for energy during flight.

Among vertebrates, birds present an interesting case of study. They usually indulge in sustained muscular activity and some of them undertake migratory flights over long distances. In migratory birds, seasonal changes occur in their fat content and the pre-migratory periods are especially marked by heavy fat deposition [97]. The humming birds which migrate a distance of 500 miles across the Gulf of Mexico, utilize mostly fat for metabolism. These birds prior to the migratory journey have an average fat content of 2·1 gm *i.e.*, 43% of the body weight. During summer they contain only 0·4 gm of fat[98]. George & Jyoti [99—102] have demonstrated a reduction in the free lipid content of the breast muscle of birds during prolonged flight or during continued stimulation of the muscle. It was shown that between 71 — 77% of the total energy is expended from fat. George & Scaria[103—106] and George, *et al.* [107,108] demonstrated the presence of lipolytic and oxidative enzymes that might favour the oxidation of fat in the narrow red muscle fibres of pigeon and bat. George & Scaria[92] showed that triolein is oxidized at a very rapid rate when incubated with pigeon breast muscle mitochondria. They also suggested that in muscle, which utilize fat as the chief fuel for energy, fatty acids are oxidized in preference to glycogen and glycolysis is resorted to only when oxygen is deficient. It can be seen, therefore, that in heart, diaphragm and skeletal muscle where continued supply of energy is needed, the energy is chiefly derived from the oxidation of lipids.

The skeletal muscles of vertebrates possess certain peculiarities the significance of which was not properly appreciated and documented until recently. It was known that there are two types of muscles—the red and white—and that even in the same muscle there exist two types of fibres—the narrow red and the broad white, along with types intermediate between the two. Even though their functional significance is not yet clear, their morphological and biochemical differences have been clearly pointed out by George & Scaria[104—106, 109], George & Naik[110—113] and Natchmias & Padycula[114]. The narrow red fibres have a small diameter, greater blood supply, higher myoglobin content, large number of mitochondria, a large amount of lipolytic and oxidative enzymes and are rich in lipids. The white fibres, rich in glycogen and devoid of lipids, have very little myoglobin; they have very few mitochondria, are poorly vascularised and have very little of the oxidative enzymes. Fatty acid oxidation can normally take place only in the narrow red fibres. In man also, the muscles which were formerly classed as pale and red, on closer examination, have turned out to be mixtures of red and white fibres[115]. The myoglobin content of the muscle fibre is associated with activity. The more myoglobin the muscle contains the greater is its capacity for respiratory metabolism[116-117]. Lawrie[118] has also shown that the myoglobin content of the muscle of rat and fowl is increased by continuous exercise. The increase in the myoglobin content and oxidative activity should naturally be accompanied by the increase in the enzyme and substrate, mitochondria, vascularization etc. because these factors are all interdependent. It is, therefore, logical to think that profound biochemical changes will take place in the skeletal muscle of humans and animals exposed to high altitude if muscle is of any importance in shivering and non-shivering thermogenesis.

CONCLUSION

High altitude presents a situation which calls for a higher rate of metabolism for its inhabitants because of the low ambient temperature and additional mechanisms to ensure

adequate supply of oxygen to the tissues where increased metabolism takes place. Acclimation to cold takes place by the development of non-shivering thermogenesis by increased metabolism and uncoupling of oxidative phosphorylation. It appears that fat plays a very important role in this process which may not become less important at high altitude. The availability of oxygen may not be a limiting factor, on the nature of the substrate metabolized at high altitude. Adaptive mechanisms take place primarily at the cellular level. Muscle seems to play a central role in this process and may undergo adaptive changes. The nature and degree of changes taking place in the muscle, the direct demonstration of the thermogenic capacity of the muscle in non-shivering thermogenesis, the elucidation of the mechanism of fat utilization, the nature of the fat utilized and its contribution to the process of acclimatization and all the related aspects need further experimentation and documentation.

The findings from these studies may be directly or indirectly utilized with advantage for the benefits of our troops in the border areas. For example, our knowledge regarding the importance of fat in cold acclimation, if extended further by some more experiments and field trials, may enable us to find out a suitable diet rich in calories and at the same time acceptable to our soldiers. Clothing and activity are said to retard natural acclimation to cold. For adaptive mechanisms to set in, the body must be allowed to cool. Once adaptations have set in, the amount of clothing required to protect the body when exposed to more severe stress may be less than what is required for an unacclimatized person. The available literature seems to suggest that persons can get properly acclimatized if they are stationed at places having moderately cold climate with minimum clothing and activity and fed a suitably adjusted diet. The exact conditions required can be stipulated only after further elucidation of the mechanism of acclimatization, which requires more objective experimentation along the lines already discussed. If the clothing can be reduced without sacrificing the comforts of the soldier it will certainly increase his mobility and efficiency and will be highly advantageous to the troops from the point of view of logistics.

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