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Human acclimatisation to cold in Antarctica, with special reference to the role of catecholamines

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ABSTRACT

The primary objective of this investigation was to see whether catecholamines are involved in the acclimatisation of humans to cold. The urinary excretions of noradrenaline and adrenaline over 24 hours were measured every month for all members of the expedition. To assess whether acclimatisation had occurred, a standard cold stress was given to half the expeditioners every 3 months. Changes in physiological responses to the cold stress were observed, as well as catecholamine and plasma cortisol reactions.

The principal findings were that the 24-hour noradrenaline excretion increased abruptly in Antarctica and then gradually subsided, remaining higher than in Australia. This closely follows the pattern observed in cold-exposed rats.

Adrenaline excretion showed a progressive rise in the second half of the year, when the weather improved. Adrenaline is involved in the responses to cold of both the acclimatised and non-acclimatised rat, but in an isolated group of humans, social stresses appear to over-ride climatic influences on the excretion of this hormone.

The basal diastolic blood pressure and basal pulse rate increased linearly over the time spent in Antarctica. These changes were probably associated with increased adrenaline excretion.

The standard cold stresses showed physiological changes that suggested an early form of cold-acclimatisation: increased peripheral cooling and a higher core temperature than in the unacclimatised subject. A late form of acclimatisation developed in the second half of the year. The core temperature was lower than in the unacclimatised or early acclimatised subject, and the rectal temperature was as well maintained as in the early form. Thus less energy was required to maintain core temperature. On the other hand, peripheral temperatures were better maintained in this late form of acclimatisation than in the early form or in the unacclimatised subject.

It is proposed that insulation may be either passive, due to subcutaneous fat, or active, due to altered vascular dynamics. In the latter improved vascular shunts may promote circulation in the limb, conserving heat by counter-current heat exchange between arteries and their venae comitantes.

Fat people rely on the passive insulation of subcutaneous fat. If they lose fat rapidly, the insulating properties of their skin are reduced more quickly than the vascular dynamics can be improved. Thus a formerly fat subject showed impaired insulation compared with those subjects who had always been lean. Nevertheless, there was a slight tendency overall, for subjects to become fatter during the year.

The altered responses in peripheral temperature to the standard cold stresses in Antarctica are considered to be due to cold acclimatisation rather than to the level of physical fitness, which did not alter during the year.

Plasma cortisol concentration will increase in response to any kind of stress. As it did not increase as much in the Antarctic cold stresses as in those done in Melbourne before and after the year in Antarctica, it appears that the body perceived the Antarctic cold stresses as less severe than the Melbourne stresses. This is interpreted as indicating that acclimatisation to cold had occurred.

Shivering occurred later in the cold stresses in the second half of the year in Antarctica than in either the earlier Antarctic series or in Melbourne; this change suggests non-shivering thermogenesis (NST) had developed.

In considering the present findings on humans in relation to animal experiments, it is suggested that NST is promoted by the re-development of brown adipose tissue (BAT). This tissue is known to provide the heat for NST in human infants, but retrogresses in the non-cold-stressed adult.

Noradrenaline promotes NST by activating an enzyme system, leading to increased lipolysis in BAT and thus providing free fatty acids as a substrate for heat-producing metabolism. The warmed blood leaving BAT warms the spinal cord shivering-centre, thus suppressing shivering. Hence it is suggested that noradrenaline is the key hormone in the cold-acclimatisation of adult humans.

A separate investigation was performed to study the effects, if any, of a cold environment on the structure of the skin. Skin from the covered lower abdomen and the exposed dorsum of the hand was biopsied in Melbourne and 10 months after arrival in Antarctica. The epidermis of the dorsum of the hand thickened and the dermis showed a reduction in elastica in Antarctica. The absence of changes in the covered abdominal skin indicated that skin responds to direct cold; there was no evidence of a systemic mechanism of cold acclimatisation affecting skin structure.

1. Introduction

1.1 THE ORIGINS AND AIMS OF THIS INVESTIGATION

Research stations in Antarctica provide unique opportunities for conducting observations on a closed population of humans living under fairly uniform conditions.

The most obvious environmental factor is the cold. Hence, the Antarctic provides a suitable milieu for investigating the effects of cold on temporary human populations. However, the winter temperatures of stations on the Antarctic littoral are comparable to those experienced in large cities in the Northern Hemisphere, although summer temperatures are much higher in the latter. A small, isolated, usually all-male group living in a cold climate is characteristic of an Antarctic station. In winter the sea is frozen and ships cannot reach the Antarctic continent. Even for countries using long-range aircraft, the physical isolation during the Antarctic winter is absolute.

Thus a suitable situation is provided for investigating a wide range of medical problems, not only those that are specific to polar regions, but also matters of general medical importance, such as blood coagulation and plasma lipids.

Efficient heating of the stations removes much of the cold stress. Nevertheless, research expeditions are necessarily exposed to conditions in which cold-climate indigenes would take shelter. Such conditions would be expected to induce cold acclimatisation, the effects of which could then be observed by changes in a number of biological variables.

Animal experiments indicate that catecholamines are involved in thermoregulation during whole-body cooling. Adrenaline promotes thermogenesis in warm- and cold-adapted rats, but noradrenaline is particularly important in maintaining body temperature without shivering in the cold-acclimatised rat. This has been demonstrated in two complementary lines of investigations: one investigation found oxygen consumption increased in response to infusion of noradrenaline (Hsieh and Carlson 1957b); the other measured the excretion of endogenous noradrenaline while animals were subjected to prolonged cold exposure (Le Duc 1961).

Infusion experiments with new-born infants (Karlberg et al. 1962) indicated that their heat-regulating system is similar to that of rats, and that cooling increases noradrenaline excretion (Stern et al. 1965).

Similar conclusions were reached in investigations of adult humans kept in a cold chamber (Joy et al. 1963) or living in Antarctica (Budd and Warhaft 1966b).

A review of the literature revealed no reports on the effects of prolonged cold exposure on the endogenous catecholamine excretion in adult humans, though Arnett and Watts (1960) have reported an increase in noradrenaline and adrenaline excretion in unacclimatised adults subjected to short-term cold stresses.

Acclimatisation may be defined as the physiological and biochemical adaptations that enable an organism to achieve a steady state with minimum energy output in a particular climate. The corollaries are that the organism has an enhanced capacity for survival in that climate and its comfort is also enhanced, an aspect of considerable importance to humans.

A comprehensive program was conceived to ascertain whether catecholamines were involved in the acclimatisation of adult humans to cold. Urine was collected

over a 24 hour period every month from twenty-four men at Australia's station, Casey. If there were no changes in the pattern of catecholamine excretion, it could mean either that catecholamines are not involved in the acclimatisation of adult humans to cold, or that cold acclimatisation had not taken place under the conditions prevailing at Casey. Therefore, tests of acclimatisation were also carried out. Measurements were made of a number of physiological and biochemical responses to a standard cold stress applied before, and periodically during the expedition, and at a follow-up of a maximum of twelve subjects. The physiological tests of acclimatisation closely followed previous Australian work to permit comparative evaluations.

The changes in rectal and skin temperatures in response to a standard cold stress, as well as the recovery rate, were measured, as these are important parameters in determining whether cold-acclimatisation has occurred. There are, however, differences of opinion as to what kind of pattern is associated with acclimatisation (Budd 1964, Wyndham et al. 1964c).

The skinfold thickness beneath the scapula and over the triceps was measured before each cold stress to determine the thickness of subcutaneous fat. This is thought to affect insulation and hence may be an important determinant of responses to whole-body cooling. The subjects were weighed, as responses may be affected by body mass as well as subcutaneous fat thickness. These measurements also enable the relationship between total body mass and subcutaneous fat thickness to be determined. The circumference of the arm was measured; together with skinfold thickness over triceps, it gives some idea of the relative variation of fat and other tissues, in particular muscle.

Observations on rats have shown that, with cold acclimatisation, noradrenaline is associated with non-shivering thermogenesis (NST). Shivering and oxygen consumption were therefore assessed. The former was evaluated by recording how long the subject remained in the cold chamber before starting to shiver. Oxygen consumption was measured before the subject entered the cold chamber and again near the end of the cold stress.

Urinary excretion rates of catecholamines were determined before and during each standard cold stress. The pre-cold stress values were obtained to give a base line for assessing how the cold stress values compared to those associated with general station activities. True basal values, however, could not be obtained because the subjects had to operate the station. The cold stress tests, including pre-test and post-test measurements, took over 3 hours.

Plasma cortisol concentration was determined on blood withdrawn immediately before and after each cold stress. This was done as a marker of cold acclimatisation, because it has been found that cold-adapted rats require fewer corticosteroids to survive a cold stress than do warm-adapted rats (Heroux and Hart 1954).

Blood pressure and pulse rates were determined in relation to the standard cold stresses to see if a consistent pattern emerged that could be related to altered pressor responses to catecholamines. Urine volumes were measured as part of the catecholamine assays; hence the rates of urine excretion before and during the cold stresses were readily determined.

Altered temperature responses of the extremities to cold have been attributed to improved physical fitness (Keatinge 1961, Heberling and Adams 1961). Therefore the Harvard Step Test was applied three times during the Antarctic year to

see whether there was a change in physical fitness that could account for any changes that might occur in the responses to the cold stress.

The responses to the standard cold stresses, although intended primarily as indicators of cold acclimatisation, also provided information on the response patterns and interactions of different acclimatisation mechanisms, their subtle interplay maximising survival capability.

Subjects were examined monthly according to the protocol laid down for Australian Antarctic stations: basal and casual blood pressure, pulse, oral temperature, weight, and measurement of skinfolds and arm circumference. As these measurements are made routinely on expeditions (Palmai 1962b, Hicks 1966, Lugg 1973), they can be compared to see whether subjects have responded to the environment in a typical manner.

As well as the physiological and biochemical changes that may be associated with cold-acclimatisation, changes possibly also occur in the skin, which is the interface between the subject and his environment. Ten subjects had skin biopsies from the dorsum of the hand and the abdomen, performed in Melbourne and in Antarctica.

The historical background to each part of this investigation will be given in some detail. Earlier discoveries will be discussed in relation to this investigation and, in turn, the present findings will be interpreted in the light of earlier observations made on experimental animals and human subjects.

1.2 THE ENVIRONMENT

1.2.1 Weather

The air temperatures and wind speeds measured at Casey during 1970 are illustrated in Figures 1 and 2, respectively. As the climate was of the 'dry-cold' kind, clothing did not become wet from water precipitation. Although minimum temperatures and overall mean temperature remained below freezing throughout the year, in the summer months from November the temperature rose above freezing point. The lowest temperature recorded was −33.3°C, in August; the highest was 5.6°, in November. In every month there were some high winds, on several occasions exceeding 100 knots, but there were also often periods of complete calm, so the mean windspeed for each month was less than 20 knots. Calm weather was often associated with clear sunny days, but on other occasions the sky was overcast. The maximum wind speed of 126 knots in May exceeded the anemometer maximum. The temperature range and wind speed for Melbourne at the time of the pre-Antarctic and follow-up series is shown in Table 1.

	Pre-Antarctic	Follow-up series	
	January	June	November
Temperature range (°C)	9.5–30.9	8.1–16.4	9.9–20.8
Windspeed range (kt)	0–12	0–12	8–10

Table 1. Temperatures and wind speeds in Melbourne

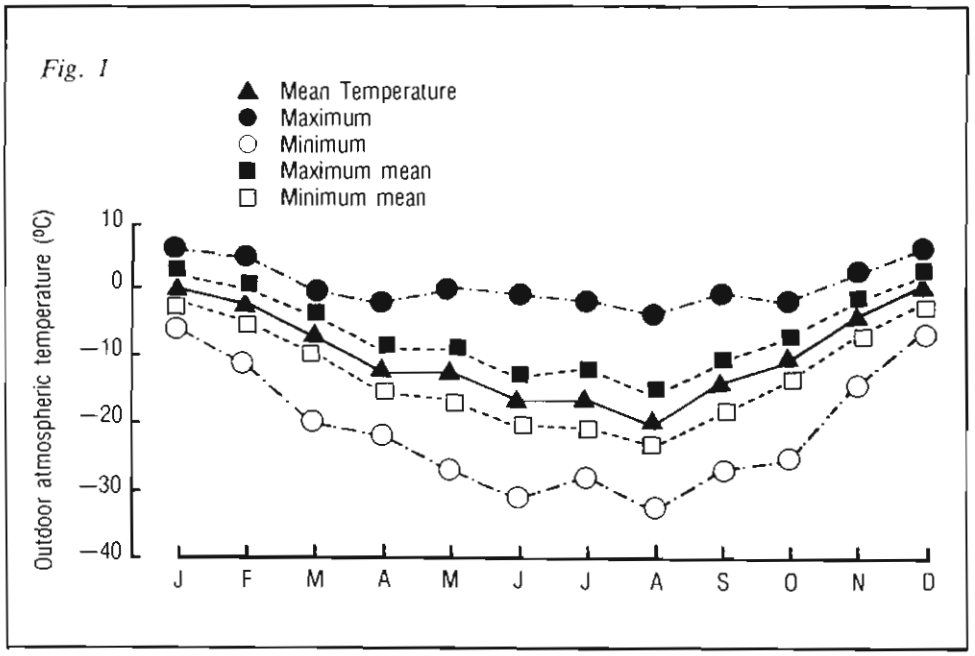


Figure 1. Air temperatures at Casey station, 1970

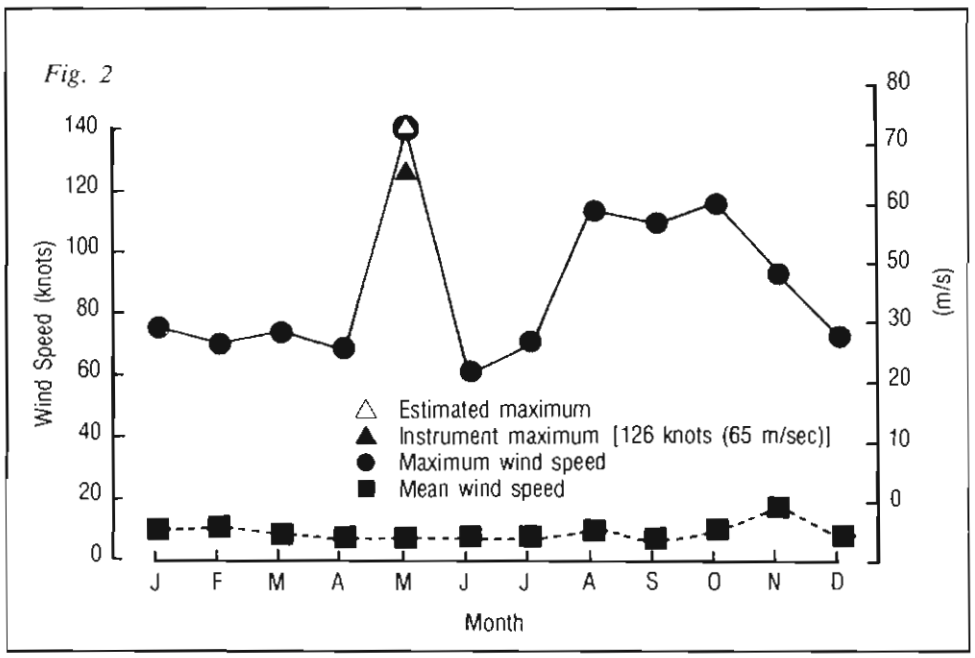


Figure 2. Wind speeds at Casey station, 1970

At a camp 24 km from the station, wind gusted to about 200 knots. In some areas of Antarctica the wind blows at 30 to 40 knots almost continuously but does not reach the very high speeds recorded at Casey. Though unpleasant, work can continue in those conditions, whereas at Casey outdoor work was impossible during strong winds. At 100 knots one cannot stand upright or keep a footing and a rope between huts is necessary.

Wind at any low temperature is effectively colder from a physiological standpoint than calm air at the same temperature. The faster the wind the lower the equivalent calm-air temperature. This concept, known as 'wind chill' was enunciated by Paul Siple (Burton and Edholm 1955). Windproof clothing abolishes wind chill except on exposed parts such as the face, which is normally partially protected by a tunnel-shaped parka hood. The hands, however, have to be exposed intermittently for fine work. The fairly long periods of calm weather at Casey enabled men to work outdoors mostly without maximum windproof clothing, the conditions being quite pleasant though cold. There was often a heavy fall of dry powder snow just before a blizzard, which then blew this snow away; such blizzards occurred about every ten days.

The station, being just above the Antarctic Circle, was not subjected to continuous darkness. The sun rose for about 2.5 hours on midwinter day, when it appeared to roll along the horizon, gradually setting. Twilight gave about 5 hours of light on that day. In summer, because of the long twilight, there was continuous bright daylight even for the few hours when the sun was below the horizon.

1.2.2 The station and facilities

Casey is located on Bailey Peninsula, Budd Coast at 66°17′S, 110°32′E.

The station consists of a number of huts of uniform size placed in line almost at right angles to the prevailing east wind. The buildings are raised on steel supports so that snow drift is blown under and over the buildings and does not build up against the station (Styles and Melbourne 1968).

The huts are made of wood sandwiched between zinc-coated steel sheets to provide strength and insulation. The interiors are heated by hot-water radiators. Additional heat is supplied by an oil-fired water heater if return water is too cool. If the water is too hot, as it is occasionally in summer, the power plant engine heat is dissipated through a radiator.

The entrances to the huts are joined by a corridor with a curved, corrugated galvanized iron wall to give an aerodynamic effect. The corridor is uninsulated and unheated, and snow drift sometimes penetrates very small gaps in the corridor wall during blizzards. The corridor temperature in winter is close to the ambient temperature, although on a few days in summer the corridor became much warmer than the insulated huts or the ambient temperature.

The interior of the huts was usually between 20 and 23°C, but on occasions in winter fell to 15°C. Occupants would walk in the huts quite lightly clad, some wearing shorts, shirt and thongs, or went bare-footed. Dressed thus, they would move from one hut to another along the corridor, and so were exposed to fairly severe cold pulses, temperatures −20°C or less being common.

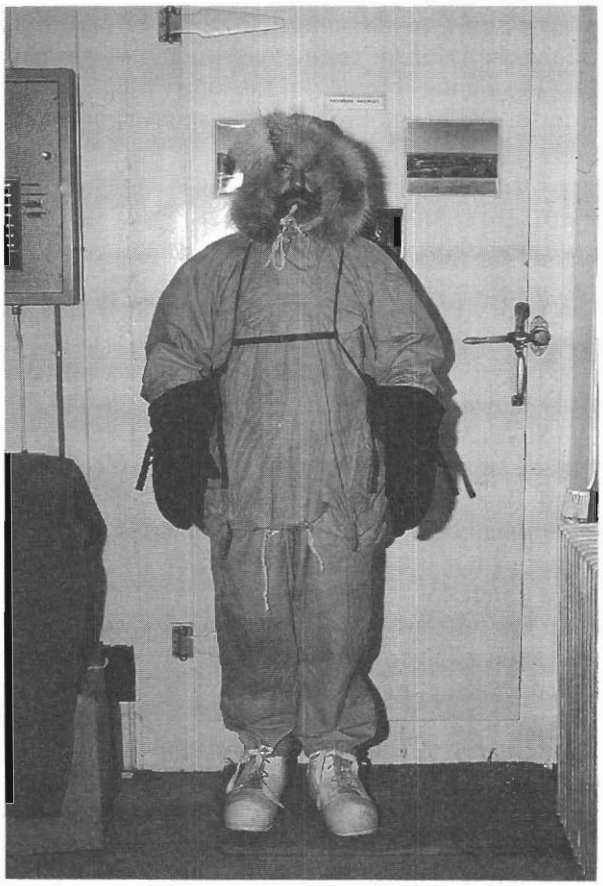


Figure 3. a) Clothing worn in the field
b) Ventile windproof outer garments

Two of the huts were divided into curtained cubicles for sleeping. The bunks were raised, with drawers, hanging space and a fixed desk underneath.

The medical block consisted of four areas: an operating theatre, a sick bay with a bed for a patient and one for the medical officer caring for patient, a consulting room and office partially partitioned off from the laboratory. Access to the medical block from the corridor is by means of a door in the consulting room. Another door opened from the sick bay onto the landing. The design of this building is discussed in Smith and Lugg (1968).

Fresh water was obtained from a large melt lake about 2 km inland. The surface froze and insulated the underlying water, so it remained in liquid form throughout winter.

There was an abundance of good-quality food, but only foods that can be stored indefinitely were available throughout the year. Apples and grapefruit lasted for 2 months; thereafter canned fruit was available. Canned and frozen meats, and dairy products, including frozen cream, powdered milk and cheeses, were provided. Vegetables were canned or dehydrated, though fresh potatoes and onions were stored throughout the year at 10°C. Bread was baked regularly and assorted sweets and pastries were prepared. Vitamin capsules were issued, but taking them was voluntary. Those who did not take them were just as healthy as those who did.

Most members of the expedition put on weight over most of the year (see 4.1) indicating that the diet was more than adequate. Even on field expeditions, personnel enjoyed such meals as roast poultry followed by plum pudding and cream. Fresh fruit was the main item missing from a very comprehensive cuisine.

The usual hot drinks, tea and coffee, were available at all meals. Other beverages were issued in standard amounts at regular intervals: two cans of soft drink each week, two cans of beer two nights per week, bottled home brew two nights per week, and table wines at dinner twice a week. Those so inclined could drink more. As this pattern was constant, it probably did not affect the yearly patterns of physiological measurements.

Indoor recreation was provided and included billiards, movies and a library.

1.2.3 Clothing

Personnel were equipped with string singlets for entrapment of warm air next to the skin. Over the underwear they wore heavy trousers of woollen material, a woollen shirt and pullovers (Figure 3a). To preserve clothing insulation, windproof but water-vapour permeable parka and overpants of ventile material were worn in the field (Figure 3b). The parka hood had a malleable wire sewn into the periphery, so it formed a tunnel to provide some protection from the wind. Wolverine fur, which tends to shed moisture, thereby minimising ice build-up from water vapour in exhaled air, was sewn to the periphery of the hood. Much lighter British Army windproofs were worn around the station.

The feet were usually protected by vapour-permeable mukluks, insulated with multiple layers of woollen material and felt innersoles over nylon mesh innersoles (Linton-Smith 1968). Alternatively, a few thermal boots made of an insulating layer enclosed in two vapour barriers were available.

The hands were covered by woollen mitts with outer mitts of leather and canvas.

Figure 4. Field medicine
a) Diesel mechanic preparing a syringe
b) Administering an intra-muscular antibiotic injection

1.2.4 Field expeditions

Expeditions were undertaken several times throughout the year. All were motorised. The geologist used a motor toboggan on which the rider sat astride as if on a motor cycle, which exposed him to the weather. These trips lasted one to two days. Glaciological traverses extended up to two months and were performed with large tracked vehicles with completely enclosed cabins.

Whilst traversing, the interior was kept warm by the engine; however, the driver had to get out every 30 m to place a cane marker in the snow. This enabled the traverse to run along a straight line by lining up the canes in mirrors. The driver of the following vehicle would then collect the canes, although he could grasp them by leaning out the open door. Thus the glaciological traverse personnel were mostly in warm conditions, but they were subjected to cold pulses. As the traverses were inland, ambient temperatures were somewhat lower than at the station, falling on occasions to −0°C.

The combined scientific and accommodation caravans were heated by oil-fired heaters. At night they were shut off and the interior of the vehicle would cool down. The occupants, however, remained warm in down-filled sleeping bags.

Personnel travelled between the station and field party in a small enclosed tracked vehicle, the interior of which was heated by the engine.

Each field party was equipped with a medical kit and trained in its use. The kit included a comprehensive array of antibiotics. Diagnoses were made by radio-consultations with the medical officer at the station. Personnel were then instructed as to what treatment should be given (Figure 4).

1.3 THE SUBJECTS

The characteristics of the subjects of this investigation are shown in Table 2.

The subjects, allof whom were Caucasian, covered a wide range of physical types although there were no great extremes.

The group comprised an officer-in-charge, medical officer, cosmic-ray physicist, upper-atmosphere physicist, glaciologist, geologist, electronics engineer, weather observer-in-charge, meteorological radar technician, weather observers (2), radio supervising technician, radio officer-in-charge, radio technician, radio officers (3),

	Age (yr)*	*Height (cm)*	*Weight (kg)*
Whole group (24 subjects)			
Mean	31.5	175.2	75.0
Range	22–49	164.5–187.0	62.3–103.6
Cold-stress subjects (12)			
Mean	28	178	80.3
Range	22–44	165–187	62.3–103.6

* at the beginning of the expedition.

Table 2. Characteristics of the subjects

plant inspector, senior diesel mechanics (3), electrician, cook and glaciological technician.

The glaciologist was responsible for studying features of Law Dome, the ice cap inland from the station, and as such was leader of the field traverses organised for this purpose. The diesel mechanics were responsible for the supply of electric power, the maintenance of vehicles as well as station repairs, such as plumbing.

The amount of cold exposure each man received depended to some extent on the nature of his work. Nevertheless, many whose main task was indoors participated in outdoor activity as recreation or as part of the station duties. All personnel were required to participate in a roster of duties for the running of the station. This ranged from indoor work of cleaning and setting tables in the mess to the exposure involved in carting water. Furthermore, moving from one hut to another along the unheated connecting corridor resulted in some degree of cold stress.

1.3.1 Thermal histories

Habitual exposure to cold in young children appears to induce cold acclimatisation of the peripheral vessels in the form of enhanced cold-induced vasodilatation of the finger vasculature on immersion in ice water. This could not be improved in young adults by repeated exposures (Yoshimura and Iida 1952). Thermal history during the formative years may therefore be of significance in cold-acclimatisation.

Most of the subjects had been reared in temperate or sub-tropical parts of Australia, but one New Zealander, two Germans and two Englishmen were reared in somewhat colder climates. It is unlikely, however, they received exceptional cold exposure during early childhood because of the protection offered by civilised communities to environmental stresses.

Before going to Antarctica many of the personnel engaged in recreations that would subject them to some degree of cold stress, e.g. swimming, skiing, yachting and bushwalking. In addition, one man served as a soldier during two Russian winters. These exposures, however, were probably too late in life to influence the vascular response pattern.

None of the subjects had previously been to the Antarctic.

1.3.2 Social and educational backgrounds

The personnel had a wide spectrum of educational backgrounds, ranging from primary schooling to postgraduate qualifications. Most had secondary or tertiary education. In socio-economic classification they ranged from low- to middle-income groups.

2. Catecholamines and cold

2.1 HISTORICAL SURVEY OF INVESTIGATIONS INTO ENDOCRINE CONTROL OF THERMOGENESIS

It is widely accepted by physiologists, biologists and anthropologists that man is a tropical animal. Archaeological work (particularly that of the Leakeys in Olduvai Gorge) tends to confirm this point of view. Worked stones and skeletal fragments of hominoid creatures suggest they used crude stone tools. Possibly the forebears of Homo sapiens evolved in the vicinity of this hot area, although South China has recently been suggested as the likely site of this evolutionary step.

Humans have an efficient system for dissipating excess heat: they can sweat over the entire body surface, which promotes cooling by evaporation. Unlike the primates, and indeed most other mammals, they lack an effective hair covering. Though the skin has many hair follicles, the short, fine hairs are of no practical significance except on the head. The only other sites where hair is conspicuous is in the pubic area and axillae, but its functional value in these sites is obscure; possibly it reduces frictional damage to opposing skin surfaces.

The result is that, by increasing blood flow in subcutaneous tissues and the dermis, the entire body surface can be used to dissipate heat, thereby enhancing efficiency during sustained high energy outputs in a hot climate. Morris (1967) believes this 'nakedness' of the human is supporting evidence for evolution in a tropical or hot climate.

However, the assumption that the temperate climate is neutral and causes minimum stress may be wrong. Man's mechanisms for heat conservation do not match the capacity of those for heat dissipation. His critical temperature (i.e. the ambient temperature at which the naked, inactive person neither gains nor loses heat and is quite comfortable) is 25 to 27°C. This is at the upper range for tropical animals. Hence a high nocturnal temperature is needed for undisturbed sleep.

Macpherson (1958) suggests, therefore, that in a temperate climate humans may be approaching the extreme range of adaptation to cooler conditions. Exposure to greater cold may only induce small changes. Nevertheless, some anthropologists believe that with the encroachment southward of the last Ice Age, some humans did not retreat and were trapped by the changing conditions. The survivors, cut off from interbreeding with other humans, differentiated into a characteristic type designated the Nordic race, a subtype of the Caucasian race. Nordic characteristics are generally genetically recessive compared with the alleles of other races (except the Australian Aborigine). This would further support the idea that they evolved more or less in isolation; in fact some degree of isolation is necessary for the differentiation of a characteristic type of any species.

By this time, man's survival in the cold was aided by artefacts of clothing and shelter, but those whose physiological mechanisms were more efficient at producing and conserving heat would eventually predominate. Some human beings may have developed enhanced cold-acclimatisation mechanisms as well as an efficient heat-dissipation system. If such enhancement occurred, subsequent dispersal of these people, with consequent interbreeding, would have made any such enhanced mechanisms less conspicuous. A cold ancestral abode enhances cold resistance (So

1975), but a low environmental temperature in early childhood appears to be more significant than genetic stock (Yoshimura and Iida 1952).

Nevertheless, mankind's chief form of adaptation to a cold environment is intellectual — the provision of adequate clothing and shelter. True physiological acclimatisation is effective only to just above freezing point. Indigenous peoples in cold climates have developed clothing that produces a subtropical microclimate beneath the clothing. In very severe conditions however, these people do not venture out. Western man, who goes into cold areas for some specific purpose, will tend to continue his activities in weather conditions in which indigenes would take shelter. Although the clothing developed by Western technology is not as thermally efficient as the Eskimos' furs, it is more suitable to wear while repairing vehicles and such like activities, as the grease would soon impair the insulating properties of furs. 'Comfort votes' taken at Australian Antarctic stations show that men working outdoors often had periods when they felt cold (Lugg 1965).

At Casey, the huts were joined by an uninsulated corridor, which had approximately the same temperature as that of the outside air. Some personnel would pass along the corridor clad only in shorts, light shirt and thongs. Thus man is still subjected to cold stress in Antarctica. If cold-acclimatisation greater than that required for life in the temperate zones is possible, it should be induced by dwelling in Antarctica.

Heat balance is maintained by reducing heat loss through increased insulation and increased heat production. Muscular activity produces heat. The muscle fibre, like any other heat engine, converts some of the energy of the fuel into mechanical energy, but also produces heat. The thermal balance at any given environmental temperature depends greatly on the level of muscular activity (Burton and Edholm 1955). In the days of dog-sledging, Antarctic explorers often became hot; their problem was to dissipate rather than conserve heat whilst sledging. On a sunny day in polar regions, insolation further contributes to a positive heat load.

The principal approaches to the investigation of human acclimatisation to a cold environment are, therefore, to elucidate the mechanisms of heat production and conservation in the resting state as near basal as possible, or alternatively to observe variables averaged over a 24 hour period in which the subjects are leading an ordinary life, with the usual daily activities and no unusual stresses.

2.1.1 Studies of thermoregulatory mechanisms

Shivering is a well recognised source of heat but it is both inefficient (see 3.2) and uncomfortable. Scientists have long been interested in the modalities of temperature regulation of mammals exposed to cold and in whether a mechanism of non-shivering thermogenesis (NST) exists.

Cannon et al. (1927) reviewed early literature on human cold response. In Germany in 1878, Voit studied the effects on clothed subjects kept for 6 hours at 30°C and 44°C. In 1881 he made observations in cold conditions and assessed the metabolic rate by measuring carbon dioxide output. The output increased in the cold without the subjects shivering. In 1897, Rubner and von Lewashaw studied subjects kept for 5 to 8 hours at 15°C and concluded that NST had occurred. Rubner in 1902 defined the chemical regulation of body temperature as the maintenance of normal temperature by an increase in heat production by the resting animal.

Carlson and Hsieh (1965) pointed out that Rubner had included shivering as a form of chemical regulation of heat production. This is of course literally true, but obscures the differentiation between shivering and NST. Rubner, after conducting further prolonged cold experiments in 1907, concluded that NST did exist. At about the same time, cold studies were being made in Sweden. In 1897, Johansson found that carbon dioxide output increased when shivering started. The subject was naked for 1 hour at 12.6° to 18°C. In 1913, Sjostrom found that in a subject exposed to cold for 2 hours, shivering occurred before the rectal temperature dropped. He concluded shivering was initiated by a reflex from cold skin. He also found that carbon dioxide output increased without shivering. Even so, the Swedish workers, unlike the Germans, considered there was insufficient evidence to support NST. Cannon et al. (1927) pointed out that the German experiments had a much longer duration than the Swedish; which may have been the basis for differing conclusions.

The role of adrenal secretion in the chemical control of body temperature was part of a broad study of the activity of endocrine glands (Cannon et al. 1927). Adrenaline subserved such responses to cold as erection of hair, ruffling of feathers, constriction of peripheral vessels and increased blood sugar.

When cold water was put into a cat's stomach, its heart and metabolic rates increased. In other experiments cats were immersed in cold water, with a stomach tube to eliminate the effects of agitation at being put in water. Shivering was found to coincide with the greatest adrenal medullary activity.

Human metabolism measured in response to cold was found to be sometimes depressed and sometimes raised initially, but later it always increased. This increase occurred without shivering. With cold 'hardened' subjects, there was less shivering. Cannon believed this was due to cold receptors in the skin becoming less sensitive to a lower temperature, and possibly a more rapid constriction of the blood vessels to conserve heat, though if skin temperature is the crucial factor in triggering shivering, quicker vasoconstriction ought to cause earlier onset of shivering. Cannon concluded that adrenaline is the first line of defence; if the temperature continues to drop, shivering is evoked.

2.1.2 Thyroid hormones

The thyroid gland has a profound effect on the basal metabolic rate and thus increases obligatory heat production. It is a common clinical observation that patients with hyperthyroidism can be quite comfortable while lightly clad in weather regarded as cold by normal people. Those with myxoedema show the reverse and feel cold when others are comfortable.

Attention has also been focussed on thyroxine as a hormone affecting thermoregulation (Ring 1942). The thermogenic response to adrenaline is potentiated by thyroid hormone. The maximum metabolic response to cold, as well as response to adrenaline, is potentiated by thyroid hormone. The thermoregulatory function of thyroxine may be in sensitising the response to adrenaline (Goetsch effect).

The antithyroid drug thiouracil interferes with the formation, but not the activity, of thyroxine. The amount of thyroxine required to inhibit thyroid hyperplasia in thiouracil-treated rats was greater in animals kept in the cold than in those kept in the warm, i.e. the cold animals had a higher utilisation rate of thyroxine (Dempsey

and Astwood 1943). Groups of rats were kept at 0 to 2°C, 4 to 6°C, 20 to 26.5°C, and 34 to 36°C. In animals not given thyroxine, but treated with thiouracil, thyroid gland hyperplasia as determined by increase in weight was more rapid, and reached the greater weight in the animals kept in the cold. Both aspects of their experiment indicate a greater thyroxine requirement in the cold.

Further work using intact and thyroidectomised rats adapted to 5°C and another group to 26°C, showed no difference in oxygen consumption of cold-adapted and warm-adapted rats receiving similar doses of thyroxine. In rats lacking thyroxine, adrenaline had no significant effect on oxygen consumption at 30°C, but it increased at 10°C. Untreated thyroidectomised rats could only maintain this raised oxygen consumption for 90 minutes. Exogenous adrenaline was well tolerated at 10°C in doses that were fatal at 30°C. Thyroxine, in cold-acclimatised rats, appears to potentiate the thermogenic effect of endogenous adrenaline (Swanson 1957).

At 30°C, oxygen consumption decreased twice as rapidly in thyroidectomised rats adapted to 5°C as in those adapted to 28°C. Thus cold-adapted rats depleted thyroxine stores at twice the rate of warm-adapted rats. On exposure to cold, cold-adapted, intact, curarised rats had a 20% higher oxygen consumption than intact, warm-adapted rats. In thyroidectomised rats, oxygen consumption fell progressively to a minimum, which was reached in 8 days by cold-adapted rats and 12 days by warm-adapted. During cooling, oxygen consumption increased in all rats, but this increase could be sustained only in the intact, cold-adapted animals. The initial rise was generally only for the first 50 minutes in the cold. After depletion of thyroxine stores, an initial increase in oxygen consumption in the cold could still be obtained; hence the metabolic response to cold is not directly dependent upon the amount of circulating thyroxine. After thyroidectomy the rectal temperature re-adjusted to a lower level in the warm; at 5°C the rectal temperature of thyroidectomised curarised rats decreased rapidly, the rate of decline in warm-adapted rats being greater than in the cold-adapted ones. Food consumption decreased after thyroidectomy. Lack of caloric intake may have contributed to the impaired temperature regulation. This feature of thyroidectomy complicates assessment of the role of thyroxine (Hsieh and Carlson 1957a).

Rats pre-treated with thyroxine are not more cold resistant than the controls. The raised basal metabolic rate of cold-adapted rats is not due to augmented thyroid secretion. Thyroid activity reaches a maximum in 3 weeks' exposure to cold, returning to near-normal after 6 to 10 weeks (Cottle 1960).

Hsieh et al. (1966) further confirmed that the regulation of NST is not associated with fluctuations in circulating thyroxine, but that animals in the cold have an increased thyroxine requirement.

Three groups of hamsters (intact, surgically thyroidectomised and thyroidectomised by ^{131}I) were exposed to temperatures between −6°C and −8°C. The first two groups survived more than 9 days, but all the animals of the third group died within 72 hours. The plasma protein-bound iodine of the surgically thyroidectomised animals was lower than in the intact animals, but higher than in the irradiated animals. This suggests the hamster has ectopic thyroid tissue. Oxygen consumption in the cold was highest in the intact animals, but increased significantly in all groups.

Thus thyroxine is not essential for a cold-induced increase in metabolic rate, although some thyroxine is necessary to permit continuation of metabolic processes. No single hormone represents the mediator of acclimatisation; rather, some

hormones play a part in some of the necessary mechanisms involved in the response. Carlson (1960) confirmed Ring's (1942) and Swanson's (1957) conclusions that the thermogenic effect of adrenaline is synergistic with thyroxine. It appears from the work cited above that thyroxine is a necessary component in thermogenesis. It seems to facilitate exothermic reactions to the extent required, but the actual drive for increased heat production is regulated by some other hormone, adrenaline appearing to be the likely one at that time.

2.1.3 Adrenal cortical hormones

Rats acclimatised for two months at 30°C and 6°C were found to require adrenal cortical hormones in greater amount on initial exposure to cold. The degree of acclimatisation was measured by survival time at −29°C. Once acclimatisation is established, it can be partly maintained by a constant and relatively low rate of administration of cortical hormones. After 17 days of cortical hormone treatment, adrenalectomised rats were more resistant to cold than normal or adrenalectomised warm-adapted rats, but less resistant than normal cold-adapted rats (Heroux and Hart 1954).

Adrenalectomised rats subjected to short daily exposures of 6°C for 39 to 42 days survived −18°C longer than did adrenalectomised and non-adrenalectomised warm-adapted rats. The adrenal glands are, therefore, not essential for cold acclimatisation. Untreated rats and rats treated with deoxycorticosterone acetate survived -18°C equally well (Heroux 1955).

Outdoor winter rats were compared with indoor rats maintained at 6°C. The outdoor rats showed no increase in basal metabolic rate (BMR), but the indoor cold-adapted rats had an increased BMR. The thyroid glands of the outdoor rats were regressing, but in the indoor rats they were active and hypertrophied. The adrenal glands of outdoor rats were hyperactive and of normal size, whilst indoor rats had less active but hypertrophied adrenals. These differences in BMR and the metabolic effects of thyroid and cortical hormones suggest there are different metabolic pathways in outdoor winter rats and indoor rats kept at 6°C (Heroux et al. 1959).

These observations may be of significance in studying the acclimatisation patterns of men dwelling in Antarctica, which may be different from those of men studied in a cold chamber in temperate climates. Antarcticans might be expected to be analogous to the outdoor winter rats.

Thyroxine and adrenal cortical hormones, though necessary for maintenance of heat production, do not appear to be regulating factors. Since Cannon's 1927 work, attention has focussed on adrenaline and later, after its discovery, on noradrenaline.

2.1.4 Non-shivering thermogenesis in rats

Evidence to support the existence of NST in the rat was elicited by comparing warm- and cold-acclimatised rats. At room temperature, the muscle activity of cold-acclimatised rats, as measured electromyographically, was less than that of non-acclimatised rats. At 2 to 10°C the electrical activity of muscles increased before

shivering was visible. This occurred in both groups, but was greater in the non-acclimatised than in the cold-acclimatised animals. At 30°C oxygen consumption was higher in the acclimatised than in the non-acclimatised rats, but in the cold the oxygen consumption of the cold-acclimatised rats was of the same order as, but still slightly higher than, that of the non-acclimatised rats (Sellers et al. 1954).

Under barbital anaesthesia, rats acclimatised to 6°C for 4 to 6 weeks had higher oxygen consumption and rectal temperature at 30°C than warm-adapted rats. After 30 minutes in 6°C air, cold-adapted rats doubled their oxygen consumption, but the warm-adapted animals' oxygen consumption only increased 30 to 50%. Colonic and leg muscle temperatures fell in both groups, but more rapidly in warm-adapted rats (Heroux et al. 1956).

Electromyography of back and leg muscles showed pronounced continuous activity in the warm-adapted animals, but there was no change in the level of muscular activity in cold-adapted rats transferred to the cold at 6°C. This work was extended to show that the metabolic rate in both groups of animals increased in going from 30°C to 6°C, but that cold-adapted rats achieved the maximum increase in 3 minutes, as against 13 minutes for warm-adapted rats. During acclimatisation at 6°C, shivering disappeared in 4 weeks, but at −6°C shivering continued for 5 weeks. The absence of shivering in cold-adapted rats at 6°C, as measured by electromyography, yet with the retained ability to raise the metabolic rate in response to cold stress was taken to indicate that NST had developed in cold-adapted rats. Un-anaesthetised, restrained, cold-acclimatised rats showed bursts of shivering. During apparent sleep there were periods in which muscular activity fell to almost zero. Such periods of minimal activity were more frequently observed in cold- than in warm-adapted rats (Hart et al. 1956).

2.1.5 Regulation of non-shivering thermogenesis in rats

Regulation of heat production in cold-adapted rats was studied by comparing the metabolic response of cold-adapted (5°C) and warm-adapted rats which had been curarised to abolish shivering, the consequent paralysis necessitating artificial ventilation by endotracheal tube. The oxygen consumption was measured to determine the change in metabolic rate. Some of both groups had been adrenomedullated. Only the cold-adapted rats could increase metabolism enough to maintain rectal temperature. Adrenomedullation reduced, but did not abolish, the ability to maintain temperature. Warm-adapted rats, both intact and adrenomedullated, showed only a slight rise in oxygen consumption on initial exposure to cold (Cottle and Carlson 1956). The investigators concluded that heat production was maintained by adrenal and extra-adrenal adrenaline, and they postulated that there was another source of adrenaline apart from the adrenal medulla.

Earlier work had shown that the active material in hepatic and splenic 'sympathin' was not adrenaline, but probably its precursor noradrenaline. Stimulation of the hepatic and splenic nerves of cats caused the appearance in the plasma of an active substance identified biologically as noradrenaline, but smaller amounts of adrenaline were also sometimes liberated (Mann and West 1950). 'Sympathin' was further studied by stimulating the nervi accelerantes to the dog's heart which caused noradrenaline to be released into the coronary blood; however, adrenaline

was not present in detectable amounts (Outschoorn and Vogt 1952). These investigations showed that the active substance released by stimulation of the sympathetic nervous system is noradrenaline. It had already been shown that the adrenal medulla is the site of production of adrenaline, although some of its precursor, noradrenaline, is also present.

Cold- and warm-adapted Sprague-Dawley rats were curarised to abolish shivering, and artificial respiration was maintained through an endotracheal cannula, which made it possible to measure the oxygen consumption. Injection of noradrenaline had very little effect on the oxygen consumption of warm-adapted rats, whereas adrenaline caused it to increase in both cold- and warm-adapted rats, a little more in the former group. In cold-adapted rats noradrenaline was much more effective than adrenaline in increasing oxygen consumption, the response being quantitatively similar to cold exposure. As shivering had been abolished by tubocurarine, it appeared that NST had been established and that noradrenaline might play an important part in its regulation. The enhanced effect on cold-adapted rats suggested the possibility that the response of the tissues to noradrenaline had altered (Hsieh and Carlson 1957b).

Intravenous injection of the ganglionic blocking agent hexamethonium chloride given just before exposure to cold prevented a rise in oxygen consumption in cold-adapted curarised rats. Warm-adapted curarised rats showed no increase in oxygen consumption whether or not hexamethonium was given. Hexamethonium abolished the increased oxygen consumption of cold-adapted rats when given after 50 minutes cold exposure. Hexamethonium injection was followed by a decrease in rectal temperature. Adrenaline injected intramuscularly before hexamethonium was given did not prevent the fall in oxygen consumption in cold-adapted rats, but it was not as pronounced as in rats that had not been injected with adrenaline, and was followed by a steady rise in oxygen consumption. When noradrenaline was injected intramuscularly before hexamethonium, there was only a small, non-significant, fall in oxygen consumption. These findings gave further support to the view that noradrenaline may play an important part in chemical thermogenesis in the cold-adapted rat (Hsieh et al. 1957).

More work to clarify this new aspect of thermogenic regulation involved evisceration of rats acclimatised to 30°C and 6°C and then exposing both groups to 6°C. Anaesthetised, eviscerated, warm-adapted rats had a smaller increase in oxygen consumption than had sham-operated rats. Increases in both groups were abolished by tubocurarine, indicating that the increase in oxygen consumption was due to shivering. Anaesthetised cold-adapted rats showed similar increases in oxygen consumption on exposure to cold, whether eviscerated or sham-operated. This increased oxygen consumption was not abolished by tubocurarine. Thus heat production of cold-adapted rats was not due to shivering nor was it derived from abdominal viscera. Nevertheless eviscerated cold- and warm-adapted rats had similar oxygen consumptions at 30°C, but intact cold-adapted rats had a higher oxygen consumption than either eviscerated group. Therefore, at this warm temperature, viscera appear to contribute to the increased oxygen consumption observed in the cold-adapted rat (Depocas 1958).

Intravenous infusion of noradrenaline into anaesthetised rats acclimatised to 6°C produced a metabolic response linearly related to the logarithm of the amount of noradrenaline infused per minute. The thermogenic response to noradrenaline

increased with time of exposure to the cold environment. The infusion was performed at 20°C (200°C in the original paper, presumably a misprint). Rats kept at 6°C showed the maximal response after 30 days. The level of muscle electrical activity decreased with the time spent in the cold and increasing noradrenaline sensitivity. The noradrenaline metabolic response approached that obtained by placing rats in a −25°C environment. Rats kept at 30°C showed no metabolic response to noradrenaline infusion (Depocas 1960a). This confirmed earlier results and indicated the noradrenaline dose dependency of thermogenesis, as well as giving a measure of the time to develop noradrenaline-mediated thermogenesis.

Shivering and non-shivering heat production during cold acclimatisation of rats showed that cold-induced oxygen consumption is the sum of three mechanisms: shivering, which is abolished by curare; peripherally stimulated NST, which increases oxygen consumption (possibly due to increased tissue metabolism) and is unaffected by curare; and centrally stimulated NST, in which oxygen consumption is unaffected by acclimatisation (Davis et al. 1960).

The responsiveness to noradrenaline of both indoor and outdoor white rats, was linearly related to the temperature at which the animals were conditioned. Outdoor rats showed a greater sensitivity to noradrenaline in their oxygen consumption, blood pressure, and heart rate in the winter. They also had a higher oxygen consumption than indoor rats kept at 6°C. It was concluded that in both indoor and outdoor conditions, increased cold resistance is obtained through similar metabolic mechanisms (Heroux and Wright 1961). This is contrary to the thyroid response in indoor and outdoor rats.

The investigations discussed so far involved infusion of noradrenaline and other pharmacological and surgical techniques applied to the experimental animals. A complementary investigation was that of LeDuc (1961), who studied urinary excretion of endogenous catecholamines in intact rats kept in the cold for a prolonged period.

LeDuc found that rats kept at 22°C throughout the year, as well as those in the cold, had lower noradrenaline excretion in the summer months than in the winter. Evidently there is an automatic seasonal response, which is either inherited or perhaps acquired early in life. Adrenaline showed a similar, but less pronounced variation.

Rats kept at 3°C for 36 days showed a nearly maximal noradrenaline excretion after 12 hours in the cold. The maximum was reached on the tenth day. The excretion remained high but gradually declined. Adrenaline excretion gradually increased to a maximum in about one week in the cold and then decreased, but more rapidly than noradrenaline, especially during the second week. It was still higher than in the control group after one month at 3°C. The control group, kept at 22°C, showed no change in adrenaline excretion though noradrenaline showed a slight decrease over the period of observation.

Over this 36 day period, the cold rats lost weight over the first 4 days and then steadily gained weight, due to growth. The weight curve parallelled that of the warm controls, but remained at a lower level because of the initial weight loss.

Urine excretion increased abruptly on exposure to the cold, followed by a decrease to the fourth day. Thereafter the volume of urine increased gradually until by day twenty-four it was about the same as the level reached on the first day. It was always much greater than that of warm controls.

The colonic temperature dropped from the third to the sixth day and then gradually rose. From the twenty-fourth day it remained similar to that of the warm controls. Throughout the experiment it was within normal limits.

In long-term experiments the decline in noradrenaline excretion was more pronounced during the second month of exposure. Thereafter the noradrenaline excretion remained at about the same level while the rats were kept in the cold for up to 6 months. The steady level was 2.5 to 3.5 times as high as the values for the control group. Adrenaline excretion, after a fairly rapid decrease during the second week, remained 50 to 100% higher than in the control group over the 6 months.

Dopamine excretion was also determined. It was more variable, though there was always a peak output after about a week of cold exposure. This maximum was usually 30 to 59% above that of warm controls. Within a month dopamine excretion returned to a level close to that of controls.

Diuresis did not increase the output of catecholamines. Restriction of water, on the other hand, was associated with increased adrenaline output.

Recovery of exogenous catecholamines after injection into some rats at the time of maximal excretion (i.e. after 1 week and again after 1 month in the cold, when the decline in noradrenaline excretion was most pronounced) showed there was no impairment of degradation or subsequent reactivation of catecholamines in the cold. Thus the changes observed in excretion were due to changes in production. Also, injection of noradrenaline did not alter excretion of adrenaline and vice versa.

The decline in noradrenaline excretion was partly due to increasing weight. Over 1 month the weight factor was important, but in long-term experiments increased weight had little effect, as shown by changes in noradrenaline in warm rats.

LeDuc suggested an asymptotic relationship between the decline in noradrenaline excretion and time in the cold.

Adrenaline excretion increased less in going from 22°C to 3°C than from 3°C to −7°C, and was less for heavier rats. Noradrenaline excretion in lighter rats reached a plateau at about 3°C as the temperature was lowered; lighter rats reached a plateau more rapidly. The excretion of noradrenaline at 3°C depended on body weight, but the difference between the two groups was not large.

After 24 hours in the cold, the adrenal glands became depleted of adrenaline, but by the second day the content was normal and rose to a higher level in the animals kept in the cold. Noradrenaline content showed a slight increase after one week in the cold.

LeDuc also studied the effect of cold acclimatisation on catecholamine response. Rats that had had varying periods in the cold were re-exposed or were subjected to more severe cold stress. On re-exposure their catecholamine response was less than in warm rats on first exposure (see Chapter 3). Survival time at −7°C was prolonged by previous cold acclimatisation. Rats never exposed to cold before were dead within 8 days at −7°C. The length of time at 3°C improved survival: all those that had spent 12 weeks at 3°C were alive and normothermic after 12 days at −7°C, though some showed local tissue injuries.

After adrenalectomy rats were maintained on adrenal corticoid therapy, so in effect only the medulla was lost. The adrenaline response of adrenalectomised rats to cold was about 80% less than that of sham-operated rats, but excretion was higher than in adrenalectomised rats kept in the warm. Noradrenaline excretion was slightly higher than in the sham-operated group.

Ganglionic blockade in intact rats was achieved with mecamylamine. All warm-adapted rats kept in the warm survived, though the colonic temperature dropped then recovered. Warm-adapted rats at 3°C showed a rapid fall in colonic temperature after injection of mecamylamine and died within, on average, 125 minutes. They 'shivered tremendously'. Pre-treatment with noradrenaline had little effect (average survival time 137 minutes), but in the group pre-treated with adrenaline only two died, shivering was less, and the colonic temperature gradually returned to normal.

Cold-adapted rats injected with mecamylamine while exposed to 3°C showed a rapid fall of colonic temperature. All died; the average survival time was 260 minutes. Shivering was as intense as in the warm-adapted rats. When they were pre-treated with adrenaline, their colonic temperature pattern was similar to that of warm-adapted rats. One died after 3 hours, but in the others the colonic temperature recovered. The most pronounced effect was in cold-adapted rats pre-treated with noradrenaline: all survived; the colonic temperature dropped slightly but soon recovered; there was only a little intermittent shivering.

This part of LeDuc's work not only supports the earlier work on the effects of ganglionic blockade and adrenolytic drugs in acutely cold-exposed rats, but also strongly suggests that catecholamines are important in regulating heat production in the cold. Adrenaline appears to be almost equally effective in both warm- and cold-adapted animals, but noradrenaline, though more potent than adrenaline in promoting thermogenesis, is only effective in cold-acclimatised rats.

In rats with ganglion blockade, adrenaline excretion increased; in adrenalectomised rats, noradrenaline excretion increased. Evidently if one catecholamine is unavailable, the response in the other attempts to compensate.

Adrenalectomy experiments showed that the adrenal medulla is the chief source of adrenaline in exposure to cold, whilst ganglion blockade showed that adrenergic nerve endings are the source of noradrenaline. LeDuc concluded that noradrenaline plays the primary role in the response of cold-acclimatised rats to cold stress, whilst adrenaline appears to be a supplementary hormone in defence against cold and effective in both acclimatised and non-acclimatised rats.

Further evidence to support this was shown by injecting rats with reserpine. This drug depletes organs of noradrenaline (presumably by activation of monoamine oxidase). Adrenaline was also depleted as the rate of release from adrenal glands and other organs increased. Adrenaline depletion was of shorter duration than that of noradrenaline. Probably reserpine exerts a differential action on nerve storage granules and chromaffin storage granules. Adrenalectomised and intact cold-acclimatised rats had a 50% reduction in noradrenaline excretion during chronic administration of reserpine. Adrenaline excretion increased during reserpine administration in intact animals but decreased in adrenalectomised ones.

Warm- and cold-adapted rats could not withstand prolonged exposure to cold after restriction of food. Cold-acclimatised rats with clipped fur survived, but catecholamine response was more intense than in intact cold-acclimatised animals. Clipped warm-adapted rats rapidly attained maximal catecholamine excretions and died in hypothermia. Previous cold acclimatisation enhanced the ability to achieve a new equilibrium under a more severe cold stress. So, even though there is a maximum capacity to produce catecholamines, survival can be enhanced if time is given to develop this greater sensitivity to catecholamines. Under severe conditions when noradrenaline requirements were exceeded, adrenalectomised (with

cortical maintenance) rats showed impaired survival compared with intact animals. This is further evidence that adrenaline is a supplementary hormone of defence against cold.

2.1.6 Catecholamines in the cold response of other species

Warm-adapted mice kept at 0°C showed piloerection, shivering, and an increase in oxygen consumption which prevented serious hypothermia; the survival rate after 4 hours was 82%. When the experiment was repeated with the β-receptor blocking agent propranolol, the mice showed piloerection and shivering at 0°C, but a dose-dependent inhibition of oxygen consumption occurred. All such treated mice died within 3 hours at 0°C (Estler and Ammon 1969).

Cats kept at 0–5°C for 1 month, then anaesthetised (pentobarbital) and cooled had a 30–40% higher non-shivering oxygen consumption than had warm-acclimatised cats. The oxygen consumption of cats acclimatised to 10–15°C was the same as in warm-acclimatised cats (Hemingway and Stewart 1962). Rabbits showed a 50–60% increase in oxygen consumption in cold after acclimatisation (Bryck et al. 1969).

Hypophysectomised, adrenalectomised dogs resisted cold as well as unoperated dogs unless hypoglycaemia developed, in which case the core temperature dropped rapidly (Keller 1960).

Acute cooling of unacclimatised dogs caused a reduction in blood flow of the hind leg (due to vasoconstriction). Plasma noradrenaline and adrenaline levels dropped when dogs were cooled by 5°C, but in two of the seven dogs the catecholamines rose, which the experimenters interpreted as due to extra stress (Clauss et al. 1969).

Noradrenaline infused into the lateral ventricle of an ox (*Bos taurus*) had no effect at 30°C. At −1°C, shivering stopped and heat production (as measured by oxygen consumption) decreased, as did rectal and hypothalamic temperatures. Therefore it is unlikely noradrenaline mediated central temperature control. Acetyl choline, 5-hydroxy-tryptamine and tranylcypramine were also excluded. Intravenous noradrenaline had no effect on stopping shivering, heat production or rectal temperature. Adrenaline caused a brief increase in respiratory rate (Findly and Thompson 1968). The calf also appears to lack NST (Brück et al. 1969).

Cold exposure of monkeys led to cessation of shivering and return of muscular coordination. There were so few changes in cellular enzymes that it was thought rodent chemical thermoregulation did not apply to monkeys. The number and magnitude of oxidative enzymes show greatest increase in rodents and become progressively less with ascending species of monkeys. It was therefore considered that oxidative enzyme levels do not contribute significantly to NST in protoprimates and primates (Chaffee et al. 1969).

Animals have been divided into three groups according to the differences in their modes of cold adaptation (Jansky et al. 1969).

1. In rats and guinea pigs, NST is mediated by noradrenaline in the cold-adapted animal. Adrenaline is also thermoregulatory.

2. In white mice, hedgehogs, dogs and young fowl, the role of catecholamines is dubious. These animals show NST, but the mechanism is unknown. Heat production due to noradrenaline is additional to the heat production in the cold from

some other means. Metabolic increase after noradrenaline injection is the same for warm- and cold-adapted animals.

3. NST has not been observed after cold-adaptation in pigeons, chickens, miniature pigs and swine, including newborn pigs, which, when cold-adapted, have increased resistance to cold but noradrenaline had no calorigenic effect.

2.1.7 Catecholamines and thermoregulation in the newborn

In the foregoing, guineapigs were placed in the first group, but there is some doubt as to their mode of response. Hyman and Towell (1968) treated pregnant guineapigs with reserpine to deplete stores of adrenaline and noradrenaline. The young were still able to increase oxygen consumption in the cold, but their thermal stability was lowered. This was interpreted as suggesting catecholamines are not essential for the thermogenic response in the young guineapig, but may be important in facilitating a compensating vascular response to cold.

The earlier work of LeDuc (1961) cited above showed a different time sequence of adrenaline and noradrenaline depletion in the reserpinised rat. Furthermore, it is an assumption that catecholamine depletion of maternal stores will necessarily be associated with depletion of foetal reserves after the mother is injected with reserpine.

Moore and Underwood (1960a) observed that the subcutaneous injection of noradrenaline gave a 30–100% increase in heat production in the newborn kitten (0–37 days old). Adrenaline was relatively inactive, giving a 20% increase. Ganglion blockade with hexamethonium caused a depression in heat production. Moore and Underwood (1960b) also showed that hypoxia suppressed the thermogenic action of noradrenaline.

In the unanaesthetised newborn cat, rat, mouse and rabbit, noradrenaline causes rises in oxygen consumption and rectal temperature. At 30°C, after hexamethonium has been given, oxygen consumption decreased followed by a fall of deep and surface temperature; the primary cause is a drop in heat production not increased heat loss. Hexamethonium had little effect on oxygen consumption at 35°C, whereas noradrenaline had little effect at 30°C, but the maximal effect was at 35°C. Noradrenaline injection, however, reversed changes set in motion by hexamethonium at 30°C. Subcutaneous injection of adrenaline or saline control had no significant effect. The guineapig is atypical in that adrenaline also is calorigenic. The maximal effect of noradrenaline was observed in the first few days of extra-uterine life; there was little action by 5 to 7 weeks in the cat and 3 to 4 weeks in the rat (Moore and Underwood 1960c).

Subsequently, Sandler et al. (1961) determined the urinary excretion of 3-methoxy–4-hydroxy mandelic acid (VMA) of newborn infants was determined for different environmental temperatures. VMA excretion was 25% higher at an environmental temperature of 23.9°C than at 29.4°C. As VMA is a metabolite of both noradrenaline and adrenaline (see Methodology), it is not possible to determine the source of this additional VMA. Furthermore, it is not known if the infant excretes metabolites of catecholamines in the same proportion as in the adult. It was suggested that this extra VMA excretion may have been derived from increased secretion of noradrenaline.

Brück (1961) studied the temperature regulation of the newborn infant more closely. Some of the babies were full term, others were premature, but did not weigh less than 830 g. Premature babies were thought to be poikilothermic, and full-term babies to show lack of training of heat regulation centres. The thermoneutral zone for the human infant is 32–34°C. Bryck demonstrated that when the ambient temperature was lowered to 23°C, the newborn infant did show an increased metabolic rate. There was no obvious shivering, and heat production increased between 2.5 to 3 kcal/kg/h before restlessness developed. It remained above 3 kcal/kg/h during quiet phases. The metabolic rate was 100% higher than the basal level, apart from any increase attributable to restless episodes. Body temperature was measured by a copper constantan thermocouple 10 cm deep in the colon. The unclothed infant from the second day of life can maintain a body temperature of 36.5°C in an ambient temperature of 23°C. At 23°C a metabolic rate rise of 110% was achieved during the first few hours and 170% at the end of the first week. A metabolic rate rise of 42% was achieved by premature infants; though thermogenesis is impaired, they are therefore not poikilothermic. The control range in the pig, horse, and cow is greater than in humans. The rat, rabbit, dog, cat and chicken have inadequate regulatory processes, resembling those of a premature infant. No thermoregulating processes have been observed in the mouse, pigeon and ground squirrel; the newborn of these species are poikilothermic.

Noradrenaline infusion causes a large increase in oxygen uptake in newborn kittens, rats and rabbits. This response declines with age. In rats kept at a subneutral temperature of 25°C, this decline occurs between 30 and 40 days (Moore and Simmonds 1966).

Noradrenaline was infused into the umbilical vein of sleeping infants aged 2 to 8 days and weighing 1.97–4.16 kg at the rate of 0.2 to 1.0/μg/kg/min. Respiratory metabolism increased by between 13 and 101%. Four of the nine infants were aroused, but their metabolism had increased before this happened. No shivering was noticed. Rectal and skin temperatures were constant, though the latter showed a transitory fall in some cases (Karlberg et al. 1962).

In a complementary investigation of newborn infants' thermoregulatory mechanism, the urinary excretion of endogenous catecholamines was measured (Stern et al. 1965). The study confirmed that the thermoneutral temperature for newborn infants is 32–34°C, whilst that of adults is 25–30°C. Infants in an ambient temperature of 20–27°C showed increases in oxygen consumption and catecholamine excretion of 103% for noradrenaline and 64% for adrenaline compared with infants at the thermoneutral temperature. Further studies of eight newborn infants weighing between 2600 and 3500 g showed that rectal and skin temperature fall slightly at 18–20°C. Noradrenaline excretion increased and adrenaline excretion remained negligible (Schiff et al. 1966). Thus, contrary to previous belief, the human infant can regulate its thermal state, although this capacity is much reduced in premature infants. As newborn infants seem to be incapable of shivering, heat balance in a cold stress is by NST. This appears to be regulated by noradrenaline.

The normal colonic temperature range for newborn infants is 36.0–37.5°C, but there are wide variations in cold resistance among neonates. The deep body temperature in small neonates in thermoneutrality, is lower than in large infants, but the skin temperature is virtually the same (Silverman and Sinclair 1966); apparently skin temperature is more sensitive than deep body temperature in influencing metabolism.

Small (1001–2000 g birthweight) but asymptomatic neonates aged between 154 and 207 hours were paired for weight and gestational age. One group was kept in surroundings enabling the abdominal skin temperature to be controlled at 35.0°C, the standard recommended temperature, and the second group was kept with the abdominal skin temperature at 36.5°C. Both groups were placed in a 28°C environment, resting supine on a nylon mesh cradle, before and after 2 weeks at their respective skin temperatures. The ability to prevent a fall of deep body temperature was greater in the infants kept at the cooler temperature, but bodyweight and length increased more rapidly in the infants kept at the warmer temperature. A 'cold resistance index' was calculated from the difference between the colonic temperature after 60 minutes in the test temperature and in the incubator wall temperature, divided by the difference between the initial colonic and the incubator wall temperatures and then multiplied by 100. As the infants kept cooler grew less rapidly, their smaller size was less favourable for heat conservation. The temperature of their feet, taken at the end of the cold resistance tests before and after the 2 weeks' trial period, indicated that there was not a more efficient heat conservation mechanism due to restriction of peripheral blood flow. It seems likely, therefore, that there was a greater heat production in the babies kept at the cooler 'standard' temperature, the slower growth being due to a greater proportion of the caloric intake being diverted to heat production rather than tissue growth (Glass et al. 1968).

In the normal course of events, without introducing any special environmental conditions, NST normally declines in a number of mammals. NST is the chief mechanism of heat production in the newborn guineapig. NST almost disappears when the animal is reared at the neutral temperature of 30–32°C; its loss is slower in animals reared in a cold environment of 8°C. It was inferred from this that the ability to regain NST by cold-adaptation weakens with increasing age (Brück and Wünnenberg 1966). Newborn lambs have the ability to shiver, but NST is also a significant factor in their thermoregulation. Injection of noradrenaline (400 μg/kg subcutaneously) caused an increase in skin temperature, rectal temperature, and oxygen consumption in the newborn lambs. Similar effects were observed in 3 lambs aged 2½, 4 and 5 days. In an ambient temperature of 20°C, their oxygen consumption doubled after noradrenaline injection. Five lambs aged between 6½ and 12 days had either a reduced response to noradrenaline or did not respond at all. Three lambs aged 14, 15 and 20 days showed virtually no increase in oxygen consumption after injection with noradrenaline. There was thus a very rapid decline with age of that component of heat production mediated by noradrenaline (Thompson and Jenkinson 1969).

Further work has confirmed that the capacity for NST is much less in the adult animal than in the newborn of the same species. Taken as a percentage of the basal metabolic rate, the increased metabolism due to NST was found to be 40% in the cat, 100–200% in newborn kittens, and 100% in babies. The newborn miniature pig, common pig, and calf, however, lack NST (Brück et al. 1969). These authors believe that the high capacity of the rat for NST may be because it remains juvenile relatively longer than other species; ossification is not completed until the end of the first year. Cold experiments that produced conclusive proof of NST in the rat were done in the first year of life.

It thus appears that the human newborn infant has a well developed capacity for NST, but that in humans, as in many other mammals, the capacity is rapidly lost, being replaced by shivering. Whether the adult human can redevelop NST will be discussed in Chapter 3.

2.2 DEVELOPMENT OF FLUORIMETRIC TECHNIQUES FOR CATECHOLAMINE ASSAYS

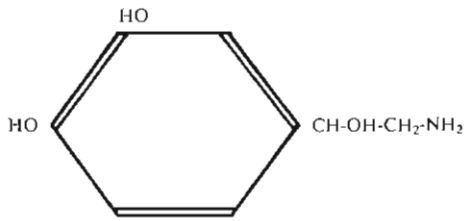
The catecholamines are derived from the metabolism of tyrosine thus (Schumann 1960):

Phenylalanine $\xrightarrow{oxidation}$ Tyrosine

Tyrosine $\xrightarrow{oxidation}$ 3, 4 dihydroxyphenylalanine (DOPA)

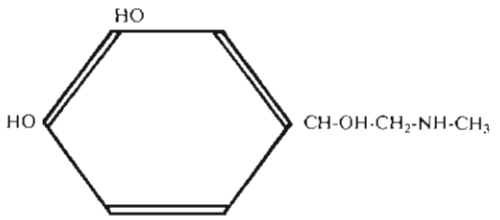
DOPA $\xrightarrow{decarboxylation}$ 3, 4 dihydroxyphenylethylamine (DOPAMINE)

DOPAMINE $\xrightarrow{oxidation}$ noradrenaline



i.e., β(3, 4-dihydroxyphenyl) β-hydroxy ethylamine

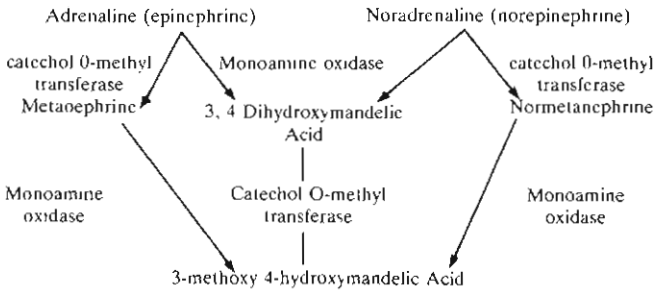
noradrenaline $\xrightarrow{methylation}$ adrenaline



i.e., β(3, 4-dihydroxyphenyl) β-hydroxy N-methylethylamine
(Vane et al. 1960).

Noradrenaline is liberated at the nerve endings of the sympathetic nervous system. Metabolism proceeds within the adrenal medulla and noradrenaline is converted by methylation to adrenaline. The adrenal medulla contains some noradrenaline, but 80% of its catecholamine content is adrenaline (Harper 1963). These are liberated into the blood stream.

Adrenaline and noradrenaline are finally katabolised to 3-methoxy 4-hydroxy mandelic acid by the following pathways and enzymes (Axelrod 1960):



The catecholamines and their katabolites are excreted in the urine in amounts depending on their concentrations in blood.

The term 'catecholamine assay' refers only to adrenaline and noradrenaline. Dopamine assay requires a special technique (Crout 1961).

2.2.1 Bioassay techniques

Catecholamines may be assayed by the response of various biological preparations. Contraction of the rat uterus is a very sensitive indicator for adrenaline. Apart from adrenaline and noradrenaline, biologically active catecholamines, such as dopamine, cannot be excluded by bioassay (von Euler and Floding 1956).

2.2.2 Fluorescence techniques

Fluorescence techniques are more convenient and permit more uniform standardisation than does a biological preparation. As interfering substances are more likely to affect results than with bioassay, specificity of extraction is important.

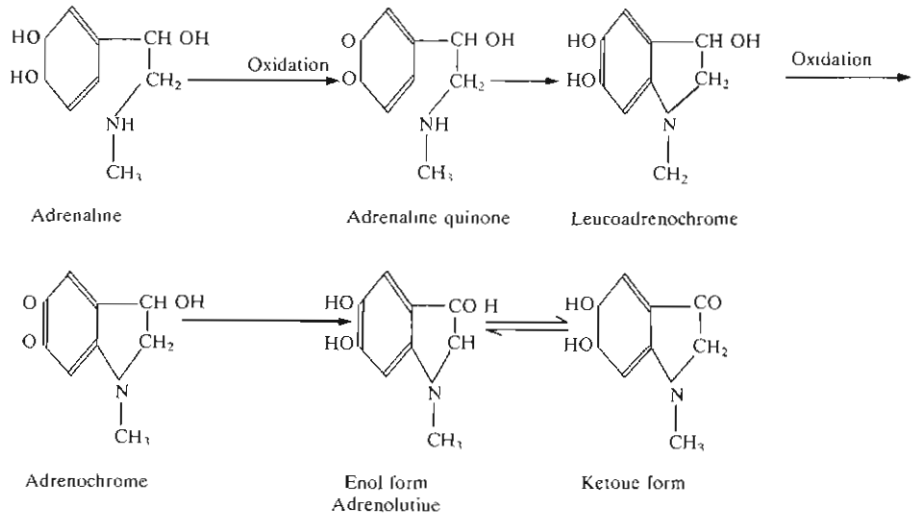
Colorimetric techniques are too insensitive and non-specific for quantitative work; therefore fluorescent techniques are preferred.

Sensitive methods for determination of adrenaline have depended on production of a fluorescent product. In 1948 Ehrlén used ferricyanide as the oxidant for the transformation of adrenaline to adrenochrome (von Euler and Floding 1955a). The strongly fluorescent compound formed by addition of alkali is very unstable in the presence of oxygen. Ehrlén showed that further oxidation could be prevented by addition of ascorbic acid. The product thus became stable and could be used for quantitative estimates. He was the first to identify the fluorescent compound as 1-methyl-3, 5, 6 trihydroxyindole.

Lund (1949a) crystallised this substance obtained by the tautomerisation of adrenochrome in strong alkali. Adrenolutine, as it is called, has a great affinity for oxygen, which is enhanced in an alkaline medium. Hence it is unstable unless a reducing substance such as ascorbic acid is added. Lund (1949b) used 9.2 mL of 1% ascorbic acid solution in 0.8 mL of 20% solution of sodium hydroxide. As ascorbic acid is also unstable in alkali, the alkaline ascorbate must be prepared immediately before use.

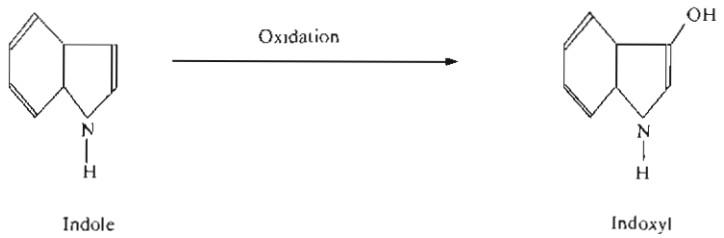
Adrenaline is easily oxidised to adrenochrome by a variety of oxidising agents. Soluble agents such as iodine, potassium persulphate, or potassium ferricyanide were considered unsuitable by Lund (1949b) because they had to be added in excess, interfering with the tautomerisation of adrenochrome to adrenolutine. Insoluble oxidising agents such as silver oxide, lead dioxide or manganese dioxide were preferred, Lund favouring manganese dioxide. Adrenaline was found to be adsorbed by aluminium oxide (alumina) in alkaline solution and could be subsequently eluted by acidification with acetic acid.

Chemical pathway in adrenolutine formation (Lund 1949a)



Adrenochrome is oxidised to oxoadrenochrome; adrenolutine oxidises to a somewhat similar compound. These two then form melanin by polymerisation.

The lutines are the 5:6 dihydroxyindoxyls derived from adrenaline and noradrenaline. Adrenolutine can be considered as 1-methyl-5, 6, dihydroxyindoxyl or as 1-methyl-3, 5, 6 trihydroxyindole (oxidation of indole gives indoxyl) (Harper 1963). Lund (1949b) showed that fluorescence is linearly related to the amount of lutine present.



(Harper 1963, pp. 164 and 169)

Noradrenaline is oxidised to noradrenochrome by a suitable oxidiser, such as manganese dioxide, and is tautomerised to noradrenolutine by sodium hydroxide.

The difference in oxidisability between adrenaline and noradrenaline permits the determination of each substance in the same sample.

Lund (1950) showed that adrenaline is oxidised quantitatively to adrenochrome within the pH range of 3 to 7 whereas only about 5% of noradrenaline is oxidised to noradrenochrome at pH 3, but quantitative oxidation takes place at pH 6.5. The acetic acid eluate was acidified with hydrochloric acid to pH 3 before shaking with manganese dioxide, to determine adrenaline content. Lund added sodium phosphate to another aliquot of eluate to give a pH of 6.5, then oxidised with manganese dioxide to convert both adrenaline to adrenochrome and noradrenaline to noradrenochrome. These were then converted to the lutines by sodium hydroxide in oxygen-free solution. The difference between the fluorescence intensities of the first and the second sample is thus a measure of the noradrenaline content of the eluate. Lund then showed that the adrenal medulla contained both adrenaline and noradrenaline, but that the latter was only about a quarter as much as the former (Lund 1949b).

Von Euler and Floding (1955a) used the difference in oxidisability of adrenaline and noradrenaline at different pH values to determine the amounts of each of these catecholamines in a mixture of the two. They used a method similar to that devised by Ehrlén for adrenaline, using ferricyanide as the oxidant in preference to iodine as it gave better differentiation between adrenaline and noradrenaline. Also, the blank faded more quickly (2–5 minutes as against approximately 20 minutes).

Adrenolutine and noradrenolutine have maximum fluorescence at different excitation wavelengths and the emitted fluorescence is of different wavelengths. Cohen and Goldenberg (1957a) utilised these phenomena to analyse plasma for adrenaline and noradrenaline simultaneously. On oxidation with manganese dioxide at pH 6.5–7.5, they found adrenolutine exhibited stronger fluorescence than noradrenolutine at excitation wavelengths of 340μ and 390–410μ. Roughly equal fluorescence was obtained from both lutines at excitation wavelengths of 360–375μ. Maximum fluorescence for adrenolutine was found at approximately 436μ and noradrenolutine at 405μ.

The wavelengths of emitted fluorescent light were 520–525μ for adrenolutine and 510–515μ for noradrenolutine. By using filter combinations of appropriate excitation and fluorescence emission wavelengths, the quantities of adrenaline and noradrenaline could be calculated from simultaneous equations.

Cohen and Goldenberg (1957b) found this technique more convenient than Lund's method of using two pH values. They considered that the hydroxyindoxyl method was more specific than the ethylene diamine procedure.

Cohen and Goldenberg worked with plasma. They noted that the small readings above blanks for normal plasma made the procedure unsuitable for observing fine differences in plasma levels of adrenaline and noradrenaline in normal subjects, but was useful when increased plasma levels might be anticipated, as in phaeochromocytoma.

Another procedure for assaying adrenaline-like substances in blood or plasma was adapted by Weil-Malherbe and Bone (1952) from a technique devised in 1949 by Natelson, Lugovoy and Pincus. Adrenaline in alkaline solution undergoes autooxidation to adrenochrome. This substance then forms a fluorescent condensation product with ethylene diamine that is more stable than adrenaline, with or without the presence of oxygen. Adrenaline was adsorbed from blood or plasma by

aluminium oxide and then the eluate was treated with an ion exchange resin to remove interfering substances.

Von Euler and Floding (1955a) considered this method was not sufficiently specific, because biological material contains catechol derivatives that also condense with ethylene diamine. Likewise, Cohen and Goldenberg (1957b) obtained much lower values than did Weil-Malherbe and Bone. As their recovery rates were good, Cohen and Goldenberg concluded the ethylene diamine technique was producing fluorescent compounds from substances other than catecholamines.

Assay of catecholamines in urine

The pioneering work on catecholamine assay was done on blood or tissue extracts. Attempts soon followed to apply these techniques to determine the urinary excretion of these catecholamines.

Von Euler et al. (1955) found that in applying Natelson, Lugovoy and Pincus's ethylene diamine condensation technique to urine, fluorescence values expressed as noradrenaline were up to 100 times higher than those obtained by biological assay (effect on the cat's blood pressure). This method therefore is not as specific as the lutine formation method, as it includes biologically inactive catechols excreted in urine.

Hydrolysis of urine was found to increase biologically assayed noradrenaline two or three times and biologically inactive catechols eight times which suggests a large proportion of inactive catechols is conjugated.

It was concluded that the ethylene diamine fluorescence reaction is not suitable for estimations of adrenaline and noradrenaline in urine.

Later, Weil-Malherbe and Bone (1957) adapted a combination of both methods for estimating catecholamines in urine. Oxidations with potassium ferricyanide at pH 6.0 and pH 3.5 were used to determine noradrenaline and adrenaline. The difference between these two results and that obtained from ethylene diamine method was used to calculate the amount of hydroxythyramine.

Thus, for urinary adrenaline and noradrenaline, the technique was similar to that used in the Scandinavian research.

To estimate amounts of adrenaline and noradrenaline in urine, von Euler and Floding (1955b) used a technique similar to that used for blood or plasma (von Euler and Floding 1955a). The catecholamines were extracted from urine by absorption onto aluminium oxide at pH 8.5 and eluted with 0.3N oxalic acid. In the presence of aluminium oxide, catecholamines are stable for 15 minutes at pH 8.5. The adsorption onto aluminium oxide probably depends on the formation of a coating of aluminium hydroxide on the surface to which catecholamines attach. During elution the acid dissolves this aluminium hydroxide layer, thus releasing the catecholamines (von Euler and Orwén 1955).

Von Euler and Floding (1955b) found that for the urine eluates at pH 6.0 both noradrenaline and adrenaline are completely oxidised in 2 minutes but oxidation is incomplete if pH is less than 6.0. For adrenaline, zinc sulphate was added to enhance oxidation. At pH 3.5 all adrenaline is oxidised to adrenochrome in 3 minutes, but only about 4% of noradrenaline. This technique gave values similar to those obtained by the biological method, particularly for noradrenaline, though adrenaline results were generally higher than biological ones.

Von Euler and Floding (1956) stated that addition of ethylene diamine tetra-acetic acid (EDTA) to the urine sample was essential for the differential estimation of adrenaline and noradrenaline. It had no effect on total catecholamines or in biological tests, but its presence prevented the 'often occurring' oxidation of noradrenaline at pH 3.5, which would give a too high apparent adrenaline fluorescence.

Von Euler and Lishajko (1959) added the disodium salt ethylene diamine tetra-acetate to the urine sample. Elution was performed with 0.25N acetic acid, as this gave less of the disturbing substances than sulphuric acid. The eluate was brought to pH 6.0–6.5 by using ammonia. Ammonia, lM sodium triphosphate or sodium hydrogen carbonate were found to be better than sodium hydroxide, which caused losses and increased blank values. The oxidant, ferricyanide, was omitted from the blanks. In von Euler and Floding's (1956) work the recovery rate was mostly 70% 'but may on occasions which seem difficult to control be markedly lower'; a recovery rate of 70–100% was claimed.

Von Euler and Lishajko (1961) made a further modification by adding ethylene diamine (EDA) to the alkali-ascorbic acid mixture. Instability of fluorescence can be prevented by allowing blanks to remain unchanged for several hours. The lutines were stable for approximately one hour. This addition of EDA has not been continued in later work. Reversed blanks were used; i.e., alkali-ascorbic acid and EDA were added to the sample, followed by ferricyanide.

Von Euler and Lishajko (1959, 1961) oxidised the catecholamines at pH 6.0–6.5 and used two separate sets of excitation and analysing wavelengths. The amounts of adrenaline and noradrenaline were then calculated by the formulae cited above (Cohen and Goldenberg 1957a).

2.2.4 Specificity

Only catecholamines that possess an hydroxy-group at the β-carbon (next to the ring) and hydrogen at the ∝-carbon in the side chain are able to form fluorescent compounds from the corresponding chromes. Isopropylnoradrenaline (Isuprel) gives a strong fluorescence, while dopamine and 3:4 dihydroxynoradrenaline (Corbusil) give a weak fluorescence. This weak fluorescence of the dopamine derivative contrasts with the high fluorescence obtained with the ethylene diamine reaction (von Euler and Floding 1955a).

The only chemical method of sufficient sensitivity and specificity for the assay of eluates from normal urine is the tri-hydroxyindole fluorimetric method. All subsequent modifications are variations of that described by Lund in 1949 (Crout 1961).

Crout (1961) added disodium ethylene diaminetetra-acetate (EDTA) to the urine aliquots and also to eluates in order to prevent the formation of gelatinous calcium magnesium-phosphate precipitate, which quenches fluorescence by scattering light and inhibiting formation of tri-hydroxyindoles. These precipitates mostly cannot be seen by the naked eye and cannot be removed by centrifugation.

The urine was collected over 15 mL of 6N hydrochloric acid to give a final pH of 3 or less, to stabilise catecholamines. The pH of aliquots of urine was adjusted to 8.4 to promote adsorption of catecholamines by alumina. After washing the alumina, catecholamines were eluted by 0.2N acetic acid.

Eluates were buffered to pH 6.5 for total catecholamines and to pH 3.5 for (chiefly) adrenaline (noradrenaline was considered to account for 2% of the reading). Oxidation at these two different pH values was found to resolve the two amines more completely than any other fluorimetric technique.

Crout (1961) used iodine as oxidiser, which necessitates adding sodium thiosulphate to remove the excess iodine. Oxidation takes 45–60 minutes in the presence of light. Oxidation with ferricyanide takes only 1–2 minutes. Laverty and Taylor (1968) give 3 minutes as a suitable oxidation time with iodine. Dopamine gives fluorescence to equivalent of 3 to 6 μg of noradrenaline. This error is reduced by using ferricyanide as the oxidant (Crout 1961).

Catecholamines are present in urine as free amines and also as conjugates, probably as glucuronides and sulphates. Total catecholamines can be determined by acid hydrolysis, the total being from one to three times that of free catecholamines alone (von Euler and Orwén 1955).

Determination of free catecholamines is preferable to that of total catecholamines (Crout 1961) because eluates from hydrolysed urine often have yellow pigment, which tends to quench tri-hydroxyindole fluorescence, and exogenous sources of catecholamines (chiefly in bananas), which are excreted as acid labile conjugates, contribute to total fluorescence after hydrolysis. Therefore, only free catecholamines were determined in the present work.

As the reagents give some fluorescence, blank values are necessary to determine the nett fluorescence due to tri-hydroxyindoles obtained from catecholamines.

Various types of blanks may be used: reagent (no amine sample), nonoxidised (no oxidant), reversed (oxidant, added after the anti-oxidant), faded (no final acidification), completely reversed (acid, anti-oxidant then oxidant), but none is entirely satisfactory (Laverty and Taylor 1968).

2.2.5 Standards

To determine the amount of catecholamines present, external standards containing a known amount of adrenaline and noradrenaline are oxidised. The galvanometer deflections of the samples are compared with those of the standard.

In addition, an internal standard is used. Here a known amount of the catecholamine is added to an aliquot of urine and the fluorescence of the oxidised eluate is compared with that of an aliquot of the same sample without any added catecholamine. The fluorescence of tri-hydroxyindoles in dilute solution is linearly related to their concentrations (Lund 1949b; Cohen and Goldenberg 1957a); therefore the difference in fluorescence corresponds to the known amount of catecholamine added to the internal standard. This fluorescence compared with that of the external standard gives a measure of the recovery rate of catecholamines from each particular sample of urine.

Even if all precipitates are removed by EDTA (see above), some quenching occurs due to contaminating compounds that absorb activating light. Self-quenching and absorption of fluorescent emission by coloured compounds may also occur (Crout 1961).

It is hoped these phenomena will affect the sample and internal standard equally, though self-quenching is reduced by dilution so the effect may be more appreciable in the internal standard, which has a greater concentration of catecholamine.

2.3 TWENTY-FOUR-HOUR CATECHOLAMINE EXCRETIONS

The results of the studies of animals and the human newborn suggested that catecholamines may be important in the cold acclimatisation of adult humans.

The literature on the relationship of catecholamines to human cold-acclimatisation is very scanty. Unacclimatised men subjected to an acute cold stress increased catecholamine excretion, chiefly adrenaline (Arnett and Watts 1960). While noradrenaline infusion initially had no effect on oxygen consumption, it caused an increase after cold-chamber acclimatisation (Joy et al. 1963). A similar effect of noradrenaline infusion was observed after several months in Antarctica (Budd and Warhaft 1966b).

It was decided to pursue a complementary line of investigation and see if dwelling in Antarctica affected the excretion rate of endogenous catecholamines.

2.3.1 Aim

The aim of this part of the study was to determine the urinary excretion of noradrenaline and adrenaline over 24 hours of all members of the wintering party before going to Antarctica, at monthly intervals in Antarctica, and of as many of the party as possible 5–10 months after returning to Australia. This investigation was made to see whether the Antarctic climate influenced the excretion rate of these catecholamines and hence whether they may be involved in adult human acclimatisation to cold.

2.3.2 Methods and materials

The experiment was designed to achieve the primary aim by assaying urinary excretion of endogenous catecholamines of a group of men in Antarctica, during their routine daily activities in Antarctica.

The men acted as their own controls: their catecholamine excretions were measured before they went to Antarctica and, for as many as possible, 5–10 months after their return to Australia.

In view of LeDuc's finding with rats (1961), in which noradrenaline excretion increased rapidly upon cold exposure and then gradually subsided, it was essential to obtain urine samples as soon as possible after arrival in Antarctica and to measure catecholamine excretions as often as practicable throughout the year.

When an Antarctic expedition arrives at the station, a procedure known as 'change-over' takes place. Here the new party takes supplies ashore, unloads and stacks them, while the old party runs the station and repacks crates to be returned to Australia. Change-over is a hectic time in which men work strenuously and for long hours; it generally takes about 10 days.

Clearly, this time is unsuitable for the initial Antarctic collection. Catecholamine excretion, chiefly noradrenaline, increases with physical exertion (Kärki 1956), and also increases (chiefly adrenaline) in states of anxiety (Levi 1969) or excitement. It would also have been virtually impossible to obtain accurate urine collections over 24 hours from men so busily engaged on other things. Therefore the initial Antarctic collection was made while the ship was in pack-ice, avoiding any stresses

due to rough water. In pack-ice the vessel proceeded slowly and the water was calm. The air was as cold as at the station. The men were physically relaxed, enjoying a new and most unusual experience. They would stroll on deck, often lightly clad in shorts and short-sleeved shirts to see how the new environment affected them. Nevertheless, in the colder conditions during change-over the new arrivals wore warmer clothing than the old party, who were mostly bare-headed. The 24-hour collection period finished just as the ship arrived off Casey.

To reveal any changes in catecholamine excretion that might reflect acclimatisation mechanisms, the assays were performed as frequently as practicable. Monthly intervals were deemed appropriate because of the time taken to perform all the assays, other phases of the investigation, and to fulfil general commitments to the running of the station. The 24-hour urine collections were somewhat tedious for the subjects, who had to carry their containers from place to place. Usually the containers were left near the urinals.

Collections over 24 hours were chosen as the most appropriate form of sample to eliminate such factors as diurnal variation, work and rest, all of which affect the catecholamine excretion rate (Kärki 1956).

Monthly 24-hour urine samples were collected from all twenty-four men of the wintering party in order to increase the likelihood of any changes being significant. Previous Antarctic experiments involved relatively few subjects; as few as four in the only other Antarctic catecholamine work (Budd and Warhaft 1970). Furthermore, by starting with all the men the effect of some subjects withdrawing from the experiment would be minimised. In the event no one withdrew.

The entire experimental program was designed in conformity with the ethical rules stipulated by the Declaration of Helsinki (1964). The author participated as a subject in all phases of the work.

Procedure

Urine samples were collected from fourteen of the men in December 1969 and January 1970, in Melbourne and Adelaide, and assayed for adrenaline and noradrenaline before sailing.

Specimens from the remaining ten were collected on board ship early in the voyage. The volumes were measured and aliquots stored in the ship's cold room at approximately 4°C. The collections were assayed at Casey, the results being incorporated with the previous results to provide pre-Antarctic basal results for subsequent comparison. These results are designated 'January'. There was only minimal physical exertion and the mode of living was quite relaxed when these specimens were collected.

In the 24 hours before arrival at Casey, another collection of urine was made from the twenty-four men. Volumes were measured and aliquots stored in the ship's cold room. They were assayed at Casey and designated the 'February' results, representing arrival in Antarctica. Thereafter the collections were made at monthly intervals up to December. In December, eight men in the field were not tested. As far as possible men going into the field had 24-hour collections made before departure (or after return to the station) and whilst in the field.

A follow-up study was made on nine of the men in June 1971, 5 months after returning to Australia. Two were residing in Canberra, one in Adelaide and the

remainder in Melbourne. Containers were distributed to all men and the interstate men promptly freighted them back to Melbourne for analysis. An attempt was made to study two of the expeditioners who were in Vietnam, to see whether a tropical climate influenced results, but the containers went astray. Two more subjects were studied in November 1971, and a repeat was made on two whose catecholamine excretions were assayed in June 1971. These results are designated the 'follow-up' series.

Subjects were instructed to empty the bladder (not into the container) and note the time. All urine passed over the next 24 hours was collected over 15 mL of 6N hydrochloric acid in plastic containers. The subjects were also instructed to finally empty the bladder 15 minutes before or after the 24th hour since the initial voiding. On the few rare occasions when it was not possible to void at the end of the 24-hour period, the time was recorded and the volume arithmetically adjusted to the 24-hour equivalent. Signs were displayed on the urinals as a reminder that a 24-hour collection was on that day.

All recent work is based on the trihydroxyindole method developed by Lund (1949a), in which catecholamines are oxidised then tautomerised to the corresponding lutines. The method used in the present study was essentially that of Crout's (1961) modification of that of von Euler and Lishajko (1961), EDTA being added to both the urine aliquot and to the eluate. The details were provided by Mrs Helene Anderson of the Department of Medicine, Austin Hospital, Heidelberg, Victoria (personal communication), but with the added refinement that extraction rates were determined individually for total catecholamines and adrenaline, since the extraction rate for adrenaline was often found to be rather different from that for total catecholamines. Sodium metabisulphite was added to the urine aliquot to stabilise catecholamines. Potassium ferricyanide was chosen as the oxidant to enhance specificity (von Euler and Lishajko 1959).

Separate samples of eluate were buffered at pH 3.5 for adrenaline and pH 6.5 for total catecholamines before oxidation. The fluorescence of the respective lutines was determined by the appropriate excitation and analysis of wavelengths. Though this method is more time consuming and cumbersome than buffering only one sample of eluate to pH 6.0 — 6.5 and calculating the quantities of adrenaline and noradrenaline from fluorescence at two different sets of wavelengths (Cohen and Goldenberg 1957a), it is considered to give greater resolution of adrenaline and noradrenaline (Crout 1961).

As 24-hour excretions were being measured, one tenth of the 24-hour volume was chosen as a convenient aliquot. Each aliquot was made up to 250 mL by the addition of distilled water with the volumes of reagents and standard concentrations being adjusted accordingly. For uniformity, similar volumes and concentrations were used for the cold-stress catecholamine determinations, but here the aliquot was often the entire volume of urine passed.

Fluorimetric analysis

Reagents.

Aluminium oxide, chromatography grade (Merck)
0.2M disodium ethylenediamine tetra-acetate (EDTA)
Sodium metabisulphite 30%

Sodium hydroxide 20% w/v
Acetic acid, 0.3M and 1.6M
phosphate buffer; 0.5M solution of Na_2HPO_4. $2H_20$ brought to pH 8.5 by 0.5M solution of KH_2PO_4
Potassium ferricyanide 0.25% w/v
Ascorbic acid 1% w/v and 0.5% w/v
Noradrenaline standard. 'Levophed for Infusion' (Winthrop) diluted with 0.3M acetic acid to 10 μg (of the base)/mL.
Adrenaline standard. Powdered adrenaline (Sigma) dissolved in 0.3M acetic acid to make stock standard 100 μg/mL. Working standard was made up to give 10 μg/mL.

Analytical procedure

The volume of urine passed over a 24-hour period was measured. Three aliquots, each one tenth of total volume, were taken and each made up to 250 mL with distilled water. If the total volume exceeded 2500 mL, then one twentieth of the volume was taken and the result of the assay was then doubled. One aliquot was used for analysing catecholamine content; to the second was added 10 μg of noradrenaline to provide an internal standard equivalent to 100 μg/24 hour; to the third aliquot was added 10 μg of adrenaline to provide an internal adrenaline standard equivalent to 100 μg/24 hour. Five mL of 0.2M EDTA and 5 mL of 30% of sodium metabisulphite were added to each aliquot. The pH was brought to 8.5 by adding 20% sodium hydroxide using a Metrohm pH meter and stirring continuously with a magnetic stirrer with Teflon-coated stirrer bars. Two grams of alumina were added to each sample as soon as the pH adjustment was completed and, using the magnetic stirrer, the sample was stirred for 5 to 6 minutes. Stirrer bars were removed by magnetic attraction of another bar outside the beaker. The bar was washed with distilled water when lifted clear of the sample, but still inside the beaker. The alumina was then allowed to settle and nearly all of the urine was decanted off, taking care than none of the alumina was lost. The aluminium oxide was then washed with distilled water into elution columns plugged with Pyrex glass wool. Washing with distilled water was continued until the washings were brought to pH 7 as shown by indicator paper immersed in washings as they left the nozzle of the columns. Stopcocks were closed and 10 mL of .3M acetic acid were pipetted into each column. The columns were plugged and agitated by hand for 30 seconds to elute catecholamines from the alumina. Each eluate was run into a plastic centrifuge tube and centrifuged at 3000 rpm for 15 minutes to remove any grains of alumina that may have passed through the glass wool. The supernatant was then decanted into a plastic tube, which was then stoppered. Eluates were frozen pending completion of the assay.

For the total catecholamine determination, to each test tube were added 0.25 mL of 0.2M EDTA, 1 mL of eluate (or for the external standard 1 μg of noradrenaline in 1 mL of 0.3M acetic acid) and 2 mL of 0.5M phosphate buffer, the final solution having pH 6.5.

For the adrenaline determination, to each test tube were added 0.25 mL of 0.2M EDTA, 1 mL of 1.6M acetic acid and 0.5 mL of the eluate (or for the external standard 0.5 mL of the adrenaline standard solution), the final solution having pH 3.5.

To each of the above solutions were added 0.25 mL of potassium ferricyanide and, 2 minutes later, 0.5 mL of alkaline ascorbate, which was prepared during the 2 minutes' oxidation by adding 0.5 mL ascorbic acid solution to 4.5 mL of 20% sodium hydroxide. The latter had been previously pipetted into a test tube. For total catecholamine assay, 0.5% ascorbic acid solution was used; for adrenaline assay, 1% solution.

A Hitachi 203 fluorescence spectrophotometer was used to determine fluorescence. Measurements were made 5 to 7 minutes after addition of the alkaline ascorbate to allow full development of fluorescence. The fluorescence of total catecholamines oxidised at pH 6.5 was determined by using excitation light of wavelength 405 mμ and analysing wavelength of 495 mμ. The fluorescence of adrenaline oxidised at pH 3.5 was determined at wavelengths of 435 mμ and 515 mμ. Noradrenaline standard oxidised at pH 3.5 gave zero reading. Therefore, values at this pH were deemed to be entirely due to adrenaline. Noradrenaline values were obtained by subtracting values obtained at pH 3.5 from those obtained at pH 6.5.

Urines were not hydrolysed; only free catecholamines were determined to avoid interference from endogenous and exogenous fluorescent compounds (Crout 1961). The fluorescence spectrophotometer was zeroed on the dark current. Then the reagent blank was read and the instrument zeroed on the reagent blank in which 0.3M acetic acid was used instead of an equal volume of eluate or external standard. The instrument was set to read 100 against the external standard of 10 μg of noradrenaline, i.e. equivalent to 100 μg/24 hour. For adrenaline it was set on the maximum deflection, which was just under 90, representing 100 μg/24 hour. Specimen readings were then arithmetically converted to μg/24 hours from the internal standard readings to correct for the recovery rate.

Checking the Hitachi 203 Fluorimeter

The instrument was equipped with two monochrometers, one for the excitation wavelength and one for the analysing wavelength. The accuracy of these monochrometers was checked periodically. Using distilled water in a cuvette with the analyser set on zero the maximum deflection should be achieved with the excitation monochrometer set on 365 mμ. Then with the excitation monochrometer set on zero, the maximum deflection should be obtained with the analyser set on 365 mμ. The same procedure was followed to set each monochrometer on 436 mμ.

The manufacturers set ±5 mμ as an acceptable variation. In fact the maximum deflections with distilled water were obtained with monochrometer readings of 366 mμ and 436 mμ, the former having an error of +1 mμ and the latter error being 0. These results were well within the set limits. The instrument's performance remained constant throughout the program and appeared to be unaffected by conveyance to and from Antarctica.

Reactivity of reagents

EDTA, potassium ferricyanide, and ascorbic acid solutions were made up freshly before each run. When not in use, ascorbic acid and ferricyanide solutions were refrigerated at 4°C. This also ensured that the ferricyanide was protected from

light, which may cause some decomposition. Standard solutions were thawed immediately before use and the external standards were freshly made for each series, being stored at 4°C between runs. Eluates were thawed on the day of assay.

2.3.3 Evaluation of the method

This method is designed to achieve specificity as well as sensitivity. The various steps help to exclude the influence of substances other than the catecholamines under study. Nevertheless, certain exogenous substances may affect fluorescence.

Common interfering substances are aldomet, methyl DOPA, tetracycline, quinine, quinidine, mandelamine and amphetamine (Dr Cameron Baird, personal communication). Bananas will interfere if the urine is hydrolysed (Crout 1961). The technique used did not involve hydrolysis; only free catecholamines were determined. Though there were no bananas available in Antarctica, pre-Antarctic and follow-up assays could have been affected had hydrolysis been done.

The subjects were healthy men, not requiring interfering drugs, such as aldomet or quinidine. As the researcher was also the expedition medical officer, tetracyclines were not prescribed on the rare occasions that called for antibiotic therapy. An important exception was in the follow-up series when, after completing his 24-hour collection, Subject 1 stated that he was taking a tetracycline for a minor infection. The specimen was processed with the others of that batch. It proved to be highly fluorescent, but no difficulties were experienced with the assay of another 24-hour specimen collected a few months later.

Quinine was a potential problem for 'Bitter Lemon' was sometimes issued in the soft drink ration. This drink contains a small amount of quinine. To avoid any possible interference, the Officer-in-Charge was asked not to issue 'Bitter Lemon' just before a monthly urine collection and the subjects were asked not to drink any saved from previous issues during a collection.

Even if both these precautions had failed, this drink is unlikely to have influenced the result. The author made two consecutive 24-hour urine collections, drinking two cans of 'Bitter Lemon' during the second 24-hour period. The total catecholamine excretion over the first 24-hour period was 54 μg, and over the second was 52μg. Thus the amount of quinine in 'Bitter Lemon' was of no significance.

Notwithstanding these precautions and the standard menus for everyone a few subjects showed very high fluorescence values when the eluate was buffered to pH 3.5 for the adrenaline determination, though no difficulty was experienced at pH 6.5 for total catecholamines. The cause of this high fluorescence could not be determined, but was probably an amine katabolite that, for some unknown reason, appeared in the urine in unusually large amounts. The fluorescence was so great that in some cases it completely masked the effect of added adrenaline in the internal standard.

Blanks

One of the problems of this technique is the fluorescence of the reagents themselves or of the non-lutine compounds they may form. Lund (1950) used the faded blank, which is prepared in the same way as a test sample but with the anti-oxidant ascorbic acid omitted from the sodium hydroxide used for tautomerisation. The mixture is

allowed to stand so that all lutine decomposes and fluorescence is read, but it does not allow for the possibility of fluorescence due to ascorbic acid or compounds of ascorbic acid and other reagents. A reagent blank may be preferable (Cohen and Goldenberg 1957a).

Three types of blanks were evaluated in the present work:

1. Reagent Blank, in which all reagents are added in the same amounts and in the same order as in the test except that 0.3M acetic acid is added instead of the acetic acid eluate. The monochromators were set on the appropriate wavelengths and the fluorimeter was zeroed on dark current (the instrument was fitted with a shield that could be interposed to completely darken the photoelectric cell compartment). This was done after about 15 minutes warm-up to allow the instrument to stabilise.

The galvanometer deflection was observed with the blank in the cuvette and the fluorimeter was then zeroed on the blank. A fresh blank was prepared from time to time through the run to ensure that the instrument zero had not drifted. Similarly, the external standard was freshly oxidised from time to time through the run to ensure that the instrument zero had not drifted and to check the instrument reading of the standard. This also served to ensure that the reagents were working.

2. Reversed Blank, in which the alkaline ascorbate is added first and then the oxidant (potassium ferricyanide). The alkali destroys catecholamines. This type of blank has the advantage that all the substances for the test sample are in the blank, so their own fluorescence and the fluorescence of any formed substances can be determined.

3. Faded Blank, in which the eluate is treated as for the test sample, but ascorbic acid is omitted from the sodium hydroxide. The lutines are not stabilised and the mixture is allowed to stand so that fluorescence fades. At least 2 minutes are required for fading (von Euler and Floding 1955a) and fluorescence due to ascorbic acid cannot be checked. Even if ascorbic acid is added after fading, fluorescence due to the ascorbic acid reacting with other components in the mixture might be missed.

Theoretically the reversed blank is best because it contains everything that the test sample does, but the catecholamines have been decomposed before oxidation to lutines can occur. Not only is each chemical component present, but there is the opportunity for any fluorescent reaction product to be formed. However, both the reversed and faded blanks require an aliquot of eluate from each sample so that any fluorescent substance peculiar to a particular eluate can be determined, which adds to the complexity of the procedure in that a blank must be prepared for each eluate.

A careful evaluation of these three types of blanks showed no difference in the galvanometer deflection. This finding is in accord with the opinion of Laverty and Taylor (1968).

At pH 6.5 the blank readings were mostly one scale division of the galvanometer. At pH 3.5 the blank reading for the three types of blanks was three divisions. To reduce this high background fluorescence at pH 3.5, volumes were reduced, only 1 mL of 6.6M acetic acid being used instead of the 2 mL of phosphate buffer and 0.5 mL of eluate instead of 1.0 mL used for total catecholamines at pH 6.5. 1% ascorbic acid was used instead of 0.5% for total catecholamines.

Noradrenolutine has been found to contribute 2–5% of the fluorescence at pH 3.5 (Lund 1950, von Euler and Floding 1955b, Crout 1961) and therefore can be

ignored. In the present work the external standard noradrenaline when oxidised at pH 3.5 and examined with excitation wavelength of 435 mμ and analysing wavelength of 516 mμ the galvanometer deflection equalled that of the blank. Fluorescence of the eluate under these conditions was therefore taken to be due entirely to adrenolutine.

To ensure that the ascorbic acid had not been decomposed by long exposure to strong alkali, it was added to the sodium hydroxide during the 2-minute oxidation period.

Stability of eluates

After the mixture had oxidised for precisely 2 minutes and alkaline ascorbate had been added, it was allowed to stand for about 7 minutes, by which time fluorescence was at a maxiumum. Fluorescence gradually deteriorated after another 15 minutes. This contrasts with a stability of an hour observed by Lund (1949b) and by von Euler and Floding (1955b).

Catecholamines were found to be stable indefinitely in the frozen eluate. Von Euler and Orwén (1955) found eluates were stable for 48 hours at 'room temperature', a week at +3°C and no loss of activity was detected after a month at −23°C.

Stability of catecholamines

Acidified urine (approximately pH 3) aliquots from some specimens were assayed in March. The remainder of the urine was put outside and remained frozen until brought in for re-assay in October. No loss in catecholamine content was observed over this period.

Other samples of acidified urine left at 10°C were found to have lost almost all catecholamine content over one month. Total catecholamines assay produced at most two divisions of galvanometer deflection. Urine with pH up to 3 was found to show no loss of catecholamines after a week at 10°C confirming the findings of von Euler and Floding (1956) with urine kept at 'room temperature'.

Recovery

A method standard was compared with the external standard. In this procedure, 10 μg of the appropriate catecholamine (equivalent to 100 μg/24 hour) were added to 250 mL of distilled water. The sample was then extracted, eluted and oxidised as for the urine samples. The fluorescence value was 70% of that of the external standard.

The recovery rate of the internal standard varied with each specimen of urine. It was mostly 40–60%, but occasionally was only 25%. This rate was low compared with the 70% or better obtained by von Euler and Lishajko (1959), though they also obtained some unexplained 'markedly lower' recoveries, as did H. Anderson (personal communication).

The recovery rate of catecholamines in distilled water was comparable with that obtained from urine by von Euler and Lishajko (1959), but Crout (1961) claimed average recoveries from urine of 91% for noradrenaline and 83% for adrenaline. In the present study, the final result was corrected for the recovery rate, which was not done in previous work (Cohen and Goldenberg 1957a, Crout 1961). This

variability in the recovery rate is very puzzling, for the subjects were all from a closed community living under fairly uniform conditions, the chief difference being in the amount of cold exposure, which is not related to low recovery. Most importantly, everyone ate very nearly the same sort of food. Some would omit certain items and perhaps eat more of another item available to all, but no foods were available to one person but not to another.

Another interesting aspect was the effect of the length of time the urine specimen stood at 10°C before catecholamines were extracted. If extraction was done on the day the 24-hour collection was completed, the recovery rate of added noradrenaline was about 25% in many of the specimens. If the specimens stood for a week at 10°C, the recovery rate increased to 40–60%. On the other hand, the eluate from a urine aliquot without any added noradrenaline varied only to within one or two galvanometer scale divisions.

Clearly freshness of urine did not affect the extraction of endogenous noradrenaline, but fresh urine appeared to have some binding effect on exogenous noradrenaline. It seems unlikely to be a quenching effect, for if fresh urine contains such a substance, which deteriorates after standing for a week, fluorescence of noradrenolutine obtained from the endogenous noradrenaline should be equally quenched. It may be that fresh urine of some subjects contained a substance to which exogenous noradrenaline became conjugated and subsequently decomposed over several days. It is not clear, however, why such a substance should bind only the exogenous noradrenaline, leaving the endogenous noradrenaline unaffected. This phenomenon was not observed with the adrenaline internal standard in which the eluate was oxidised at pH 3.5.

Because of this feature, specimens were routinely left at 10°C for a week before extraction, but occasional samples still gave low recovery rates.

Von Euler and Lishajko (1959) stated, without explanation, that 'It is not certain that recovery of added catecholamines always runs parallel with the yield of amines already present in urine. The use of internal standards may give useful hints but these should not be taken as giving a quantitative indication of the yields of the amounts originally present'. It was certainly true of a number of specimens in the present work.

Crout (1961) believed internal standards are desirable, but did not correct his observed results for the recovery rate. H. Anderson (personal communication) obtained the recovery rate at pH 6.5 for total free catecholamines and used this value to correct both total free catecholamines and adrenaline. In the present work, the extraction rate at pH 6.5 was rarely identical to that at pH 3.5, so the recovery rates for noradrenaline plus adrenaline and adrenaline alone were used to correct the final value; no better alternative is suggested in the literature. Correcting for internal standard recovery rate gave more consistent results than if the extraction standard was used or no recovery rate correction was applied.

Reproducibility

Each eluate was oxidised in duplicate. Fluorescence was within two scale divisions for higher total free catecholamine values and were either identical or differed by, at most, only one scale division at the lower readings obtained with adrenaline alone.

Results obtained by simultaneous equations

A comparison was made of results obtained by direct analysis after oxidation at pH values of 3.5 and 6.5, and with noradrenaline and adrenaline calculated from the fluorescence value at two different sets of excitation and analysing wavelengths after oxidation at pH 6.5 by the method of Cohen and Goldenberg (1957a). The values obtained for catecholamine excretion (μg/24 hour) are shown in Table 3.

The methods gave a similar value for the total of adrenaline plus noradrenaline. Adrenaline values were virtually the same in two cases; the calculated value was slightly lower in the other two. Noradrenaline calculated from the equations had consistently slightly higher values.

2.3.4 Results

The monthly mean values of urinary excretion of noradrenaline are illustrated in Figure 5. They increased significantly ($P<0.001$) when the subjects arrived in Antarctica and then decreased significantly ($P<0.001$) in the next month to a level not significantly different from the pre-Antarctic value. In the first half of the year the excretion rates oscillated from month to month, but were higher than in the pre-Antarctic series. In the latter half of the year the values in each series were similar to each other, except for a decrease in the November excretion rate to a level almost the same as that of the pre-Antarctic series. Also, the July and September values were not significantly higher than that of the pre-Antarctic series. The follow-up series conducted 5 to 10 months after returning to Australia showed that the excretion rate had returned to a level close to that of the pre-Antarctic series.

Analysis of variance confirmed that noradrenaline excretion in February was higher (at the 1% level) than in other months in Antarctica, which were, in turn, higher (at the 5% level) than in the pre-Antarctic series in January.

Adrenaline

The monthly mean values are illustrated in Figure 6. The adrenaline excretion rates from July to December inclusive are significantly greater than in the January series. Analysis of variance showed that the adrenaline excretion in the series July to December was higher, at the 1% significance level, than in the series from February to June. This increase showed a linear regression on time.

	Total		*Noradrenaline*		*Adrenaline*	
Subject	*Analysis*	*Calculated*	*Analysis*	*Calculated*	*Analysis*	*Calculated*
1	60	61	52	53	8	8
2	26	27	15	20	11	7
11	57	61	43	50	14	11
14	54	56	45	48	9	8

Table 3. Comparisons of catecholamine values obtained by analysis at pH 3.5 and 6.5 with those calculated from fluorescence at different sets of wavelengths

At follow-up the adrenaline excretion rate had virtually returned to the pre-Antarctic level.

Effects of field work

In subjects participating in field traverses, noradrenaline excretion tended to increase at first then return to levels similar to those obtained at the station (Table 4). The patterns of the autumn and spring traverses were similar.

Adrenaline excretion was at first similar to that on the station, but subsequently showed a rising trend (Table 5). The spring pattern was similar to the autumn pattern, but the excretion rates were of greater magnitude. However, although the rates showed consistent trends, they are not significantly different.

2.3.5 Discussion

The noradrenaline excretion pattern closely resembled that of rats kept for a prolonged period in the cold: an early pronounced increase followed by a gradual decrease, but remaining higher in the cold than in warm conditions (LeDuc 1961). The reduction may have been due to increased sensitivity, though its pressor effect becomes less in Antarctica (Budd and Warhaft 1966b). Alternatively, cold acclimatisation may effect a more efficient utilisation of noradrenaline.

The human pattern differs from that of rats in that the rate for each month early in the year was significantly greater or smaller than in the preceeding month (Figure 5). This hunting pattern suggests an imprecisely adjusted negative feedback, with the oscillations progressively damping over the first half of the year.

Subject	*Station*	*Station*	*Field*	*Field*	*Field*	*Station*	*Station*
Autumn							
4	19	20	27	—	—	24	19
5	52	51	29	76	—	63	54
15	60	66	27	—	—	26	55
18	32	32	68	—	—	59	67
24	23	24	93	55	—	28	38
Spring							
5	67	78	92	48	47	51	—
10	50	26	30	—	—	47	50
17	41	46	108	—	—	87	41
19	41	53	72	—	—	69	24
21	18	52	40	—	—	56	—
24	34	35	28	—	—	32	—
Total							
Mean	39.7	43.9	60.1			48.7	43.5
P		>0.30	>0.10			>0.10	>0.50
Significance		ns	ns			ns	ns

Table 4. Comparisons of noradrenaline excretion in the field and at the station. Autumn and spring traverse results are pooled (μg/24 h). Maximum field values are taken for statistical comparisons for both noradrenaline and adrenaline.

Unlike the rats, which were kept continuously at a constant temperature, the men were intermittently exposed to increasingly colder conditions in the earlier months. It is postulated that this caused noradrenaline excretion to increase in 1 month, then to decrease when a higher degree of cold acclimatisation was developed; the rate in the second Antarctic month was not significantly different from the pre-Antarctic rate. A further increase in cold stress then led to a repetition of the hunting pattern. The July excretion rate was not significantly greater than the pre-Antarctic rate, probably because of improved cold acclimatisation combined with a reduction in outdoor activities resulting from the short period of daylight.

Subsequently noradrenaline excretion rose, but varied little from August to December, except for a pronounced reduction in November to a level close to that of the pre-Antarctic series. This appeared to be due to the relatively higher air temperatures combined with indoor preparations for the final summer activities. Also in November, the weather pattern changed, blizzards becoming more frequent and lasting longer, reducing the amount of outdoor work that was possible. Outdoor activity increased in December and so did the noradrenaline excretion. The post-Antarctic follow-up showed a noradrenaline excretion rate similar to that of the pre-Antarctic series.

The pattern of the mean monthly adrenaline excretion differed considerably from that of noradrenaline (Figures 5 and 6). It did not increase on arrival in Antarctica, but rose significantly in the latter half of the year. Possibly the adrenaline response to a cold environment is slower than that of noradrenaline, but with a tardiness much greater than that observed in rats (Le Duc 1961), and possibly has a cumulative effect.

It seems more likely, however, that some non-climatic factor may have influenced adrenaline excretion during the latter half of the year.

The rate of adrenaline excretion in Swedish aircrew was greater when flying as a passenger than as a pilot and was greatest during ground activity. Changes in noradrenaline were much less, but the rate was highest when the subject was the pilot (von Euler and Lundberg 1954). The adrenaline excretion of paratroopers was highest just before jumps, but there was little alteration in noradrenaline (Bloom et al. 1963). Excretion of catecholamines during space flight showed great individual variation, but adrenaline was the catecholamine most frequently excreted in increased amount (Weil-Malherbe et al. 1968). These findings indicate that adrenaline is the catecholamine most likely to be increased by anxiety. Furthermore, anticipation (in the present study, thoughts of approaching home-coming) may be more anxiety-provoking than the event itself.

Many other activities cause rises in catecholamine excretion. Levi (1969) reported that sorting ball bearings under distracting conditions caused a rise in both catecholamines, as did watching aggressive, erotic or comical movies. No change in the mean excretion occurred while the subjects were watching a bland natural scenery film, though it increased in many individuals — who were perhaps bored and anxious for the film to end.

It therefore seems likely that the rising adrenaline excretion over the second half of the year in Antarctica was chiefly due to anxieties associated with station life. Anxiety in association with a cold climate increases the adrenaline excretion, and smoking further increases adrenaline responsiveness to cold (Feller and Hale 1964).

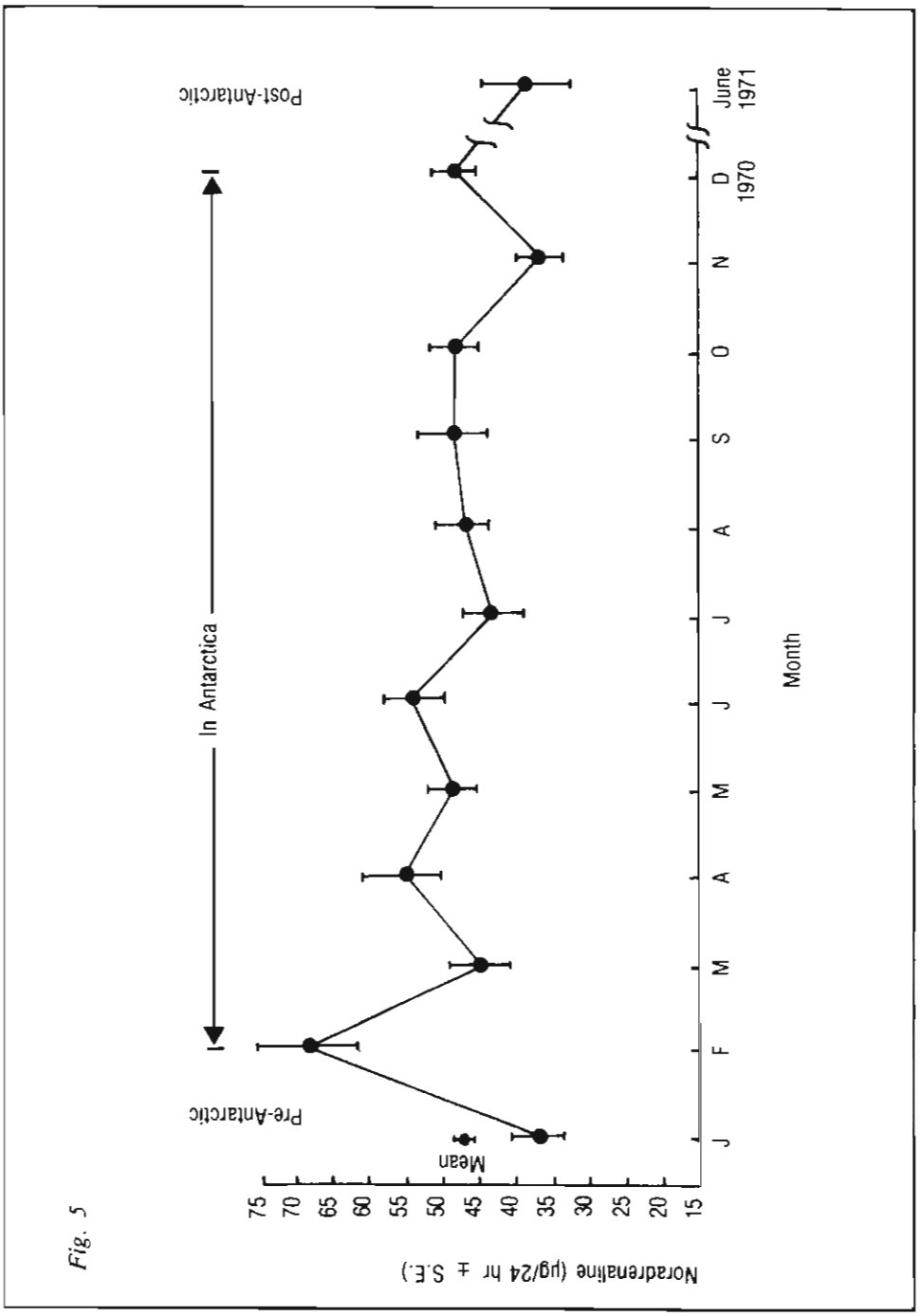


Figure 5. Mean monthly urinary excretion of noradrenaline in pre-Antarctic, Antarctic and post-Antarctic series. Vertical bars represent ± SE

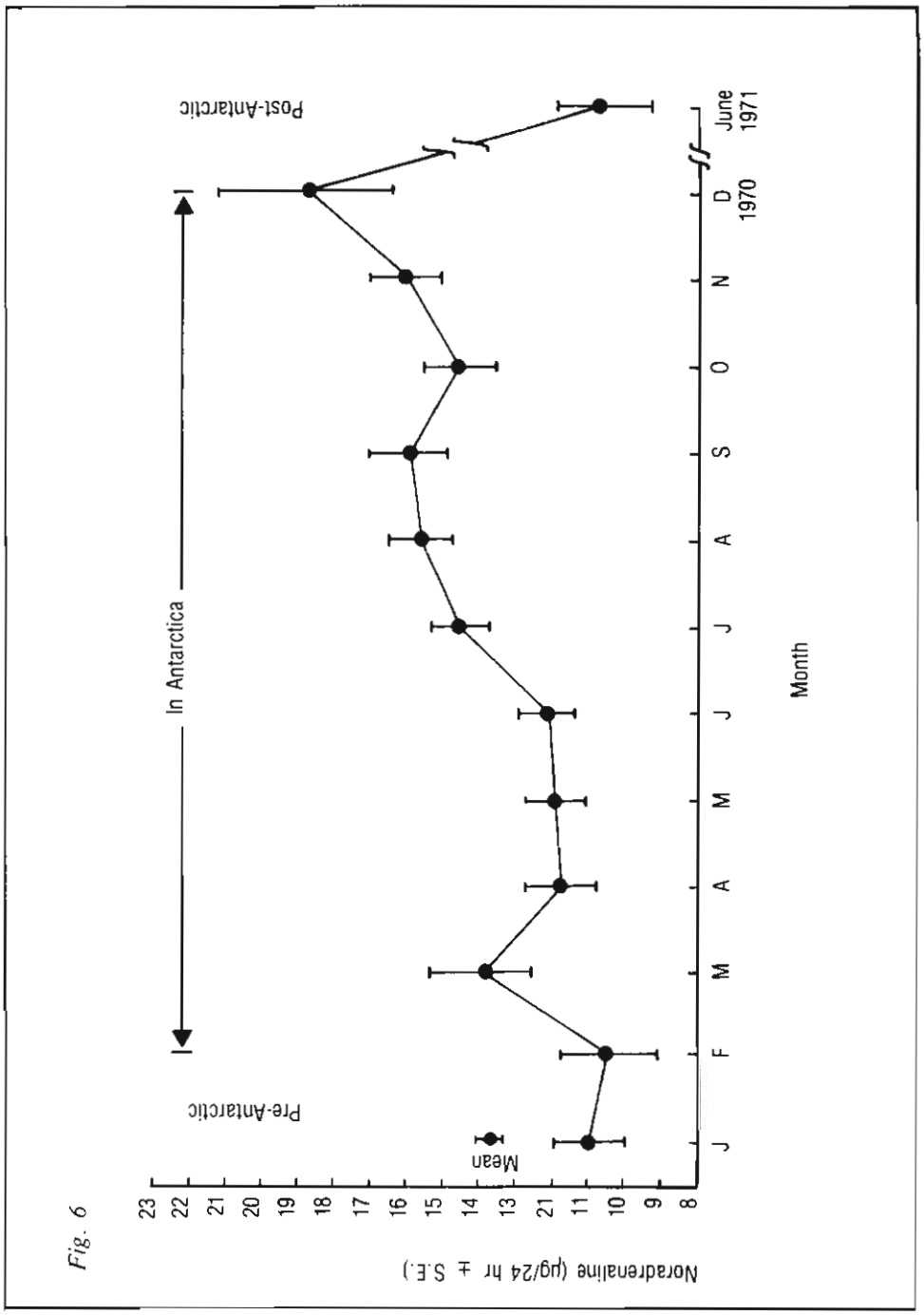


Figure 6. Mean monthly urinary excretion of adrenaline in pre-Antarctic, Antarctic and post-Antarctic series. Vertical bars represent ± SE

The low adrenaline excretion in the initial Antarctic series excludes anxiety or anticipation as causes of the high noradrenaline excretion. The increase in noradrenaline associated with piloting an aircraft (von Euler and Lundberg 1954) suggests this catecholamine is associated with an alerting reaction, which was unlikely under the relaxed conditions prevailing when the initial Antarctic urine specimen was collected. High noradrenaline levels are associated with psychotic depression (probably as the cause rather than the effect), due to an excess of the metabolite noradnamine (Bunney et al. 1967), but there was no evidence of psychotic depression in any subject at any time, though some subjects did have episodes of less severe forms of depression. Every subject showed much variation in both noradrenaline and adrenaline excretions between series, which probably reflects differing degrees of stress, particularly cold stress, from month to month.

Subject 1, who had considerable responsibility, showed a large increase in noradrenaline excretion in the initial Antarctic series, and in the following series in March, when he had much organising to do, his noradrenaline excretion remained high, though there was a pronounced decrease in the mean. That the high noradrenaline excretion was due to mental tension is supported by his high adrenaline excretion in March. This subject showed the highest overall average excretion of noradrenaline as well as a high adrenaline excretion.

Subject 2 showed an increased noradrenaline excretion in February, which was the general pattern, but the peak excretion occurred in April when his adrenaline excretion was also close to the peak for the year. This increase in April may have been due to anxiety associated with the large amount of laboratory work that had to be done in that month. His adrenaline excretion subsequently reached its peak in September and October. In these months there was some additional stress associated with another aspect of his responsibilities, as well as some anxiety about the completion of his research project.

Subject 5 showed a slight decrease in initial Antarctic noradrenaline excretion, even though he went on deck a good deal whilst lightly clad. Throughout the year his monthly noradrenaline excretions remained fairly constant, but increased in the field.

Subject 15, who injured his foot during rough weather on the voyage, was unable to go on deck during the initial Antarctic collection period and his February noradrenaline excretion was much the same as his pre-Antarctic level. In March, however, when he was able to move about outdoors, his noradrenaline excretion increased markedly at a time when it was decreasing in most other subjects.

Subject 23 showed only a slight increase; possibly this was related to his recreation of swimming in cold water though it was some months since he had done this. His peak excretion occurred several months later. His occupation involved a positive thermal load and he was unable to get outdoors as much as most. He showed peak adrenaline excretions in the November and December series, which may be related to anxiety associated with his job chiefly in September and October. In the following months the situation was resolved, but he was subjected to the opprobrium of his fellows. His noradrenaline excretion was unaffected.

Subject 24, who did not go on deck as much as most had an initial Antarctic excretion rate that was lower than the pre-Antarctic rate.

An investigation of catecholamine excretion published after the present study was made (Budd and Warhaft 1970) found no differences between catecholamine

excretions of four subjects in Melbourne and during the late Antarctic winter. The observations were not frequent enough to reveal the pattern observed in the present study, the early noradrenaline response being missed. Random samples of noradrenaline excretions in the winter were in many cases close to the pre-Antarctic value. Furthermore, the winter collection may have been too early to reveal the late rise in adrenaline excretion. The excretion rates calculated on a 24-hour basis were 24 μg of noradrenaline and 7 μg of adrenaline; which is lower than the mean pre-Antarctic values in the present work, but the collections were made over 13 hours of rest and 3½ hours that included a 2-hour cold stress. It is most unlikely that the rates were representative of a 24-hour period. The urine samples were 'chilled' and returned to Australia for analysis. Catecholamines are stable in frozen acidified urine, but stability in chilled urine is uncertain.

A diurnal variation in the excretion rate of both noradrenaline and adrenaline has been observed. The lowest rates were during the 8 hours of night, probably due to recumbency; the highest were in the 8 hours after rising. Physical exertion increases catecholamine excretion, adrenaline rising to twenty-five and noradrenaline to seventeen times the resting value in a 40 km ski race (Kärki 1956). Collecting urine over a full 24-hour period in each series eliminated the effects of diurnal variation. Intermittent exertion averaged over a day was similar in each series, days of unusual activity being avoided for urine collections. The general level of exercise was constant, as shown by the fitness indices (Section 3.4). Crout (1961) found the mean 24-hour free noradrenaline excretions of twenty-four ambulatory hospital patients to be 30 ± 13 μg ± one standard deviation which is close to the pre-Antarctic excretion rate of this investigation. However, the adrenaline rate of 5.6 ± 3.1 μg was lower. Ambulatory hospital patients are likely to have less physical exertion than healthy men leading a normal life. The anxieties associated with preparations for the expedition may have promoted increased excretions, particularly of adrenaline.

Excretion of the catecholamine metabolite 3-methoxy-4-hydroxymandelic acid (VMA) was reported by Davies (1970) to be higher in the Antarctic winter than in March, when four of the subjects had just arrived and five had already wintered. VMA excretion does not distinguish between the separate amounts of noradrenaline and adrenaline produced, and the specimens were collected too infrequently to reveal fine variations. The acidified specimens stored at 4°C were assayed on return to England; the stability of VMA is uncertain, so the lower March values could have been due to some degradation.

Field studies involved too few observations to permit statistical confirmation of the trends. Nevertheless the pattern for noradrenaline excretion was a prompt increase followed by a return to values close to those at the station. This pattern resembles that found for rats subjected to an increased intensity of cold exposure (Le Duc 1961). The delay in the increase in noradrenaline excretion shown by Subject 5 was probably because the field conditions were no more severe than at the station for this subject, who spent much time outdoors. His increase in noradrenaline excretion occurred in the second month in the field, by which time the environment was becoming much colder. Subject 17, who avoided cold exposure as much as possible at the station, showed a very pronounced increase in noradrenaline excretion on going into the field in the spring traverse (Table 4).

The mean adrenaline excretion showed scarcely any change in the field, but in the few subjects who spent more than one month consecutively in the field there was a gradual increase in adrenaline excretion (Table 5). Not only was the environmental temperature lower in the field than at the station, but other non-climatic stresses, such as rather cramped living conditions and intensified interpersonal reactions, were experienced. The mean adrenaline excretions in the spring traverse were greater than in the autumn traverse (Table 5), which conforms to the overall trend observed in all subjects. Most subjects showed virtually no decrease on returning to the station, which gives further support to the suggestion that the increasing adrenaline excretion in the latter half of the year was due chiefly to non-climatic stresses. The responses of both catecholamines to increases in the respective stresses appear to be additive.

Experiments involving anaesthesia and curarisation are ethically impermissible under the prevailing conditions in Antarctica (Declaration of Helsinki). Human mechanisms can therefore only be inferred from the results of those investigations that can be conducted, and compared with similar investigations on the rat, a species that has been exhaustively studied. On this basis, the close similarity between noradrenaline excretion patterns of men in Antarctica and rats maintained in a cold chamber suggests that noradrenaline plays an important part in the acclimatisation of adult humans.

Furthermore, there is strong evidence that newborn infants maintain thermal balance by noradrenaline-mediated non-shivering thermogenesis (NST) (Brück 1961; Karlberg, et al. 1962; Stern et al. 1965; Schiff et al. 1966; see Section 6.1), but that it disappears during post-natal development (Brück 1961). This process can be retarded but not entirely inhibited by rearing the newborn in a cold environment (Brück and Wünnenberg 1966). It has been postulated that in adults it is dormant (Joy et al. 1963).

Subject	*Station*	*Station*	*Field*	*Field*	*Field*	*Station*	*Station*
Autumn							
4	6	8	14	—	—	8	11
5	6	6	5	7	—	8	9
15	30	9	19	—	—	11	10
18	15	11	9	—	—	12	12
24	6	10	7	10	—	8	9
Spring							
5	20	15	8	12	17	10	—
10	11	21	18	—	—	17	17
17	12	15	11	—	—	13	20
19	15	16	11	—	—	14	15
21	20	12	24	—	—	32	—
24	20	10	19	—	—	12	—
Total							
Mean	14.6	12.1	14.5			13.2	12.9
P		>0.30	>0.20			>0.40	>0.10
Significance		ns	ns			ns	ns

Table 5. Comparisons of adrenaline excretion in the field and at the station. Autumn and spring traverse results are pooled (μg/24 h).

2.3.6 Conclusion

Of the various factors that can influence noradrenaline excretion, climatic stress appears to be the only one that could account for the observed pattern in the present study. From the results of rat and human infant studies it seems likely that the infantile mechanism of regulatory thermogenesis, which depends on noradrenaline, is partially re-activated in the cold acclimatisation of adult humans. Adrenaline is involved in responses to acute cold exposure, but does not appear to be involved significantly in the acclimatisation process. The adrenaline excretion pattern observed here appears to be due to one or more non-climatic factors, probably anxieties and tension related to the conditions of life on an Antarctic station, intensified under field conditions by the potentiating effect of the cold climate.

2.4 TWENTY-FOUR-HOUR URINE EXCRETIONS

It is a commonplace observation that one urinates more on a cold day than on a mild to warm day. If the reduction in urine volume on a warm day is due to increased water loss from sweating, it could be expected that a reduction in fluid intake on a cold day would compensate for this reduction in sweat volume. Cold weather diuresis appears to be a direct response to cold. Reduction in the volume of body water may be an aspect of cold acclimatisation (Dr K.E. Hicks, personal communication). The amount of catecholamines excreted over a given time may be related to the volume of urine voided in that time.

2.4.1 Method

The 24-hour urine volumes were measured as part of the determination of the urinary excretion of catecholamines. The procedure and precautions to ensure an accurate 24-hour collection of urine were described in Section 2.2.3.

2.4.2 Results

The mean volumes of urine excreted over a 24-hour period each month are shown in Figure 7. The maximal excretion rate was in the initial Antarctic series, but it was not significantly different from the January (pre-Antarctic) mean volume. Thereafter the urinary volume decreased, becoming significantly less than the January volume in July ($P<0.05$), and decreasing slightly further to the lowest volume in September. The urine volume then increased over the remainder of the year. The volumes of the December and follow-up series were very close to that of the pre-Antarctic series.

2.4.3 Discussion

A short-term cold exposure of one hour causes a reduction in plasma water, as shown by increases in plasma protein concentration and the haematrocrit (Suzuki

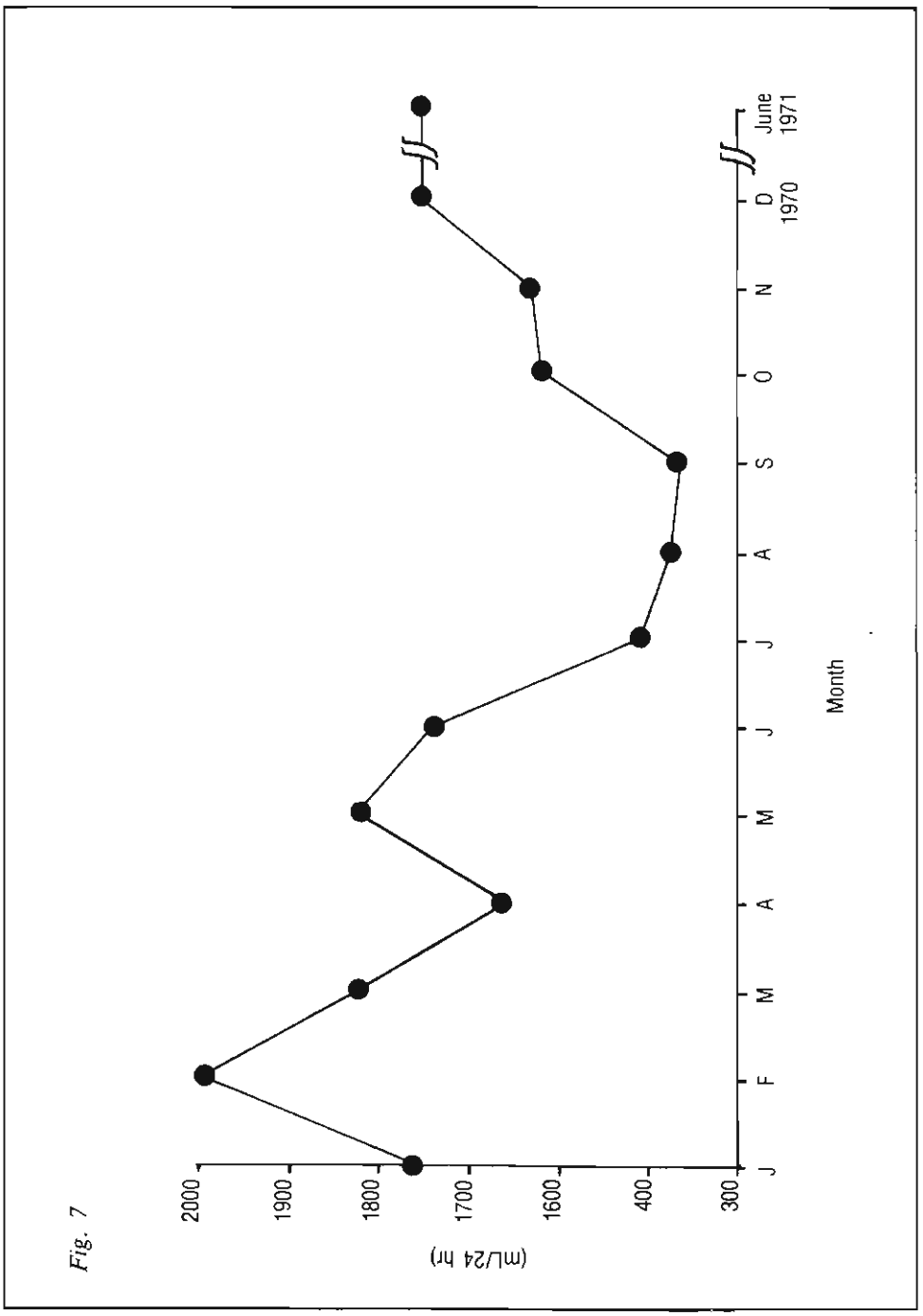


Figure 7. Twenty-four hour urine excretion

et al. 1967). Although not significant, the larger urine volumes excreted in most of the early Antarctic months compared with the pre-Antarctic volume are examples of cold diuresis. The urine volumes fell below the pre-Antarctic value in winter and early spring, the lowest volume being in September. This reduction in urine output may have been due to a re-expansion of plasma water volume related to some de-acclimatisation associated with reduced outdoor activity during the shorter daylight hours. The regular issue of beverages and the constancy of drinking habits of each subject rendered the fluid intake fairly constant throughout the year, though occasionally an individual would drink more than was his usual custom, which gave rise to a very large volume out of keeping with his usual pattern. Some tended to be consistently low; others were high-volume excreters, whilst others again showed much variation. Overall, the large individual variation resulted in the differences between series being generally not significant.

After September, urine volumes rose progressively, probably due to increasing cold acclimatisation associated with the increased outdoor activity, except in November, at which time there was also a reduction in noradrenaline excretion (see 2.2.3). The lowest urine volume, in September, occurred at about the same time as the pattern of other variables changed (see 1.3).

In previous studies, no difference had been found between urine flow rates in Melbourne and Antarctica (Budd and Warhaft 1970). Urine excretion was not measured over a full 24 hours, but most of the urine was excreted at night, when the cold stress was absent. Furthermore, as seen from Figure 7 a random Antarctic mean volume may be very similar to the mean pre-Antarctic volume.

Comparison of the shapes of the curves shows no relationship between urine flow rates (Figure 7) and noradrenaline (Figure 5) or adrenaline (Figure 6) excretion rates. This absence of relationship is in accord with earlier findings (Kärki 1956).

It may be inferred from the urine volume pattern over the year that a reduction in body water volume occurred; this seems to be part of the mechanism of cold acclimatisation. How this renders the body more efficient in coping with a cold stress is uncertain. Water has a high specific heat, so perhaps by reducing the proportion of water, less heat is required to maintain body temperature. On the other hand this high specific heat would tend to stabilise body heat content in fluctuating environmental temperatures, thus establishing a thermal inertia. This concept may be illustrated by a geographical analogy; coastal areas have a smaller variation between summer and winter temperatures than inland regions at the same latitude.

Nevertheless, survival may be enhanced not by thermal inertia but rather by the ability to return a cooled body to its normal temperature with the least amount of heat. Furthermore, water does not generate heat. Reduction in body water leaves a greater proportion of the body's mass consisting of heat producing tissue.

2.4.4 Conclusion

Cold diuresis noted in short-term observations appears to persist over months during acclimatisation to cold in Antarctica, with a reduction in urine output once acclimatisation is achieved. There was a biphasic pattern, with the minimum volume occurring in September, probably due to some de-acclimatisation resulting from

more time spent indoors during the winter months with their short daylight hours. Increasing urine volumes towards the end of the year suggest subsequent reacclimatisation.

The urine volume pattern suggests a resetting of body water volume. The advantages of a smaller body water volume may lie in the reduction of the overall specific heat of the body, enabling rewarming to be achieved with less heat and causing a relatively larger proportion of body mass to consist of heat-producing tissue. Nevertheless, the survival value of cold-induced diuresis in the establishment of cold acclimatisation is at this time still speculative.

3. Responses to standard cold stresses

3.1 RECTAL AND SKIN TEMPERATURES

3.1.1 Background

Human adaptation to cold has been studied in (1) indigenous inhabitants of places with cold climates; (2) Caucasians temporarily dwelling in cold climates, often under field conditions and (3) cold-chamber experiments (Carlson and Hsieh 1965). The present study involves (2) and (3), with the latter component being used as an index for acclimatisation.

The intellectual aspects of mankind's adaptation to cold protect him from the environment, minimising true physiological acclimatisation. Nevertheless, Western clothing does not provide as good insulation as does that worn by people indigenous to cold climates. Furthermore, indigenous people shelter from the weather as much as possible, whereas Western people go to cold climates to perform specific tasks that motivate them to go out in more severe conditions than would indigenes. If climate causes changes in response to cold, whether by inheritable features induced by enhanced survival or by changes wrought in the developing child, then there ought to be racial differences in response to cold in the former case, and in the latter, differences within members of one race, depending on where they were reared.

Ethnophysiology

The blood flow of the hand and forearm at an ambient temperature of 20°C was found to be greater in Eskimos than in Caucasians. Rectal temperature was lower and hand temperature warmer than that of controls. The basal metabolic rate of Eskimos was higher than that of Caucasians. When the forearm was immersed in water at 5°C and 10°C, the deep-muscle temperature of the Eskimos fell more rapidly than in the Caucasians, but skin temperatures remained higher. Eskimo rectal temperatures were better maintained (Brown et al. 1954).

With regard to subjective appreciation of cold, the Eskimo experienced no pain at 10°C, though Caucasians felt this temperature to be painful. At 5°C the Eskimo felt pain but endured it for 2 hours, whilst Caucasians experienced severe pain at this temperature and could endure it for only 1 hour.

In another study, nine Alaskan natives (Eskimos) were compared with eight Caucasians (Meehan 1955a). The subjects wore cotton underwear in a temperature of 6° to 7°C for 1½ hours. It was found that the Eskimos' basal metabolic rate was not significantly higher than that of Caucasians. Eskimos shivered more and showed a greater metabolic response. The Caucasians' mean rectal temperature rose from 37.8°C to 38.03°C after 90 minutes, whilst the Eskimos' dropped from 37.67°C to 37.26°C. These differences were not significant. The Eskimo periphery, however, started cooler and finished warmer than did that of the Caucasians. Caucasians also showed a greater total loss of body heat.

In earlier work on five Caucasian men deliberately cold-adapted by 16 to 18 hours exposure to cold daily for 14 days (Carlson et al. 1953) it was found that heat

production by metabolism was less in the cold-adapted. The men had thermocouples attached to underwear, but were clothed during measurements to prevent them being uncomfortably cold in outside air temperatures of −5°C to −17°C. The cold-adapted person's skin vessels cycle (i.e. undergo intermittent cold-induced vasodilatation) at higher temperatures than in the unadapted. The adapted person kept the extremities warmer but called less on metabolism. The deep peripheral temperature was lower in the cold-adapted, suggesting that the 'core' tends to withdraw during adaptation. Thus, artificially cold-adapted Caucasians' responses accord well with those of Eskimos. This withdrawal of the core leads to a less steep temperature gradient, and less energy is required to keep the outer layers of tissue at the now lower temperature. Comfort and resistance to cold injury is improved by higher skin temperature.

Scholander et al. (1958a) studied the effect of cold exposure on young Caucasian males. The subjects carried out various physical activities, such as hiking during the day for 6 weeks above the tree-line in Norwegian mountains under conditions of snow and sleet, dressed in summer clothing. At night they used a single blanket sleeping bag with hydrophobic cover and wore a string singlet, underpants and socks. The ambient temperature was 3°C to 5°C. They experienced bouts of shivering, but were able to sleep while shivering. Control subjects could not sleep because of chilling of the periphery, especially the toes, but the metabolic rate of the acclimatised men was higher than that of the controls. The rectal temperature of both groups fell to 1–1.5°C in the first 3–4 hours then remained steady for the rest of the night, but the acclimatised group maintained a warm surface; though the toe temperature fell slightly, though not as far as in the unacclimatised controls. During exertion at constant rectal temperature the cold-acclimatised men used as much oxygen as did the controls.

It was concluded that neither at rest nor during exercise did acclimatisation result in increased insulation by shell cooling.

When Eskimos, Caucasians and Negroes were subjected to a cold stress of 17°C for 2 hours, Adams and Covino (1958) found that Eskimos had higher rectal and average skin temperatures. Eskimos and Caucasians shivered at a higher skin temperature and increased their metabolic rates to a greater extent than did Negroes (see Section 3.3). The Australian Aborigine lowers both peripheral and core temperature, resembling a poikilotherm (Scholander et al. 1958b, Hammel et al. 1959), when sleeping in an ambient temperature of about 0°C.

In further work comparing Eskimos with Caucasians, Andersen et al. (1963) found, contrary to previous work cited above, that Eskimo heat production was no higher than that of Caucasians. However, this study was done during light work. As in most of the previous studies, Eskimos did not maintain rectal temperature as well as Caucasians, but their hands rewarmed quicker after cooling, which suggests they have a different vasomotor control.

Arctic Indians did not maintain rectal temperature as well as Caucasians during a night at 0°C. The Indians were 15% lighter than Caucasians (Irving et al. 1960). Nomadic Lapps in 1 clo* sleeping bags for 8 hours in freezing ambient temperatures

*The clo is the unit of clothing insulation and is defined as $0.18 \frac{°C}{kcal./sq.m/h}$ (Burton and Edholm 1955).

had a metabolic rate close to basal during the night, whilst villagers and controls shivered. The nomads' mean rectal temperature declined from 36.4°C to 36.0°C, but that of villagers and controls remained at 36.5°C during the night (Andersen et al. 1960). The reactions to cold by Negroes and Caucasians, all reared in North America, were compared by Iampietro et al. (1959). These two groups are descendants of races that evolved in warm and (presumably) cold environments, respectively, but were reared in a similar climatic milieu. The men (sixteen Negroes and seventeen Caucasians), wearing shorts, sat for 2 hours at 10°C. The mean weighted skin temperature was lower in the Negro after 100 minutes, but this difference was barely significant (P=.05). From 60 to 100 minutes, the rectal temperature of the Caucasians was slightly higher, and from 20 to 60 minutes their metabolism was slightly higher, but these differences were not significant. The Caucasians were slightly fatter. Digital cooling was induced by immersing their fingers in water at 0°C for 45 minutes. Caucasians had higher finger temperatures and more pronounced hunting of the digital vascular system. They also had a shorter period to the first vascular dilation.

The different peripheral vascular reaction of Eskimos, Arctic Indians, Caucasians and Negroes to immersion in ice water appears to be of racial origin, and is a likely explanation for the much higher incidence of cold injury in Negro than in Caucasian troops of the United States Army during the Korean War. Caucasian finger temperatures showed a spread of results ranging from the high Eskimo to the low Negro values (Meehan 1955b).

A comparison of Bushmen, Bantu and Caucasians in South Africa showed that at temperatures from 5°C to 27°C the metabolic rate rose in all groups but was greater in Bantu and Bushmen, though the rectal temperature of Caucasians rose and fell in Bantu and Bushmen. This was considered to be due to the greater subcutaneous insulation of Caucasians (Wyndham et al. 1964a). The toe and finger temperatures of Bushmen were higher than in the other two groups. The lack of significant difference between Negro and Caucasian temperatures in Iampietro's work was attributed to hybridisation of American Negroes (Wyndham et al. 1964c). Observations of Wyndham's groups at various temperatures showed that the temperature selected by Iampietro, viz. 10°C, should have clearly revealed any racial differences in physiological reaction to cold.

The reactions of Bantu and Caucasians to 27°C, 20°C, 17°C, 10°C and 5°C were compared (Wyndham et al. 1964c). The subjects, clad only in light cotton shorts, lay for 2 hours at these temperatures. Comparisons were made using the 1 hour values. The Bantu were shorter, lighter and had smaller skinfold thickness than the Caucasians. The Bantu metabolic rate was greater than that of Caucasians at 27°C, 20°C and 5°C. The rectal temperatures of the Caucasians increased with lowered ambient temperature but decreased in the Bantu. This was thought to be due to the thinner skinfold of the latter. At 10°C the average rectal temperature of the Caucasian was 37.2°C, and of the Bantu, 36.5°C. Caucasian finger and toe temperatures were higher than those of the Bantu at 27°C and 20°C, but from 15°C to 5°C the temperatures were much the same for both groups. From 27°C to 15°C, the average skin temperatures of the two racial groups were not significantly different, but at 10°C and 5°C, they were slightly lower in the Caucasians than in the Bantu.

These comparisons suggest that race may influence human reaction to cold, but that both the thermal history and state of nutrition of the individual may be very important.

San Juan Puerto Ricans were compared with Puerto Ricans who had been reared in Natick, Massachusetts (Newman 1968). When subjected to 5°C for 60 minutes whilst 'lightly' dressed, Natick Puerto Ricans showed a drop in skin temperature but increases in shivering, heat production and rectal temperature. The San Juan group showed similar reactions, except that the rectal temperature dropped. After acclimatisation (5°C for 4 hours daily for 6 weeks) San Juan subjects showed no change, while Natick Puerto Ricans and Caucasians showed no significant changes in rectal temperature or heat production, but skin temperature increased and shivering decreased. The mean rectal temperature after being exposed to 5°C for 60 minutes, plotted against the mean body weight for various races, was a straight line; those above 65 kg showed an increase in rectal temperature when subjected to a cold stress. Newman concluded that differences in the state of nutrition was the main cause of different responses in the two groups of Puerto Ricans.

In Caucasian females the average skin temperature was found to be 2°C lower than that of males at an ambient temperature of 5°C. This was probably due to enhanced subcutaneous insulation, as shown by the skinfold thickness of the former. The metabolic rate was the same for males and females, and there was no significant difference in rectal, finger and toe temperatures at this ambient temperature (Wyndham et al. 1964b)

Observations on a Nepalese pilgrim in the Himalayas (Pugh 1963) showed that this man, poorly clad and with bare hands and feet, was able to sleep in temperatures of −10°C or lower. His rectal temperature dropped in the first 2 hours from 37.1°C to 35.9°C but thereafter remained constant, though the ambient temperature dropped from −5.5°C to −6.5°C in 2 hours, and to −9.5°C after 10 hours. In the first 2 hours, his hand temperature changed from 26.7°C to 27.8°C, and his foot temperature from 22.2°C to 26.7°C. Final temperatures for hand and foot were 22.7°C and 22.2°C respectively. When walking barefooted on snow at −6°C, his skin temperature between the second and third toes fell to 8°C. Thus, this man showed the features, noted by others in cold-adapted ethnic groups, of good maintenance of the extremities temperature but with a lower rectal temperature than has been observed in people accustomed to a warm or temperate climate.

Peripheral vascular responses to local cooling

Vascular reactivity of fingers immersed in ice water was greater in Japanese and Chinese who lived in cold areas than in their compatriots in warm areas. Digital reactivity of Japanese and Chinese living in Manchuria was compared with that of Manchurian Mongols and native-born Orochins. a nomadic tribe. The Mongols and Orochins were superior to Japanese and Chinese, the Orochins having the highest reaction index of the four. In childhood, however, there was no significant difference in vascular reactivity between the four groups, although Orochin children were slightly superior to others. It appears therefore that environmental conditioning in early childhood is important in establishing the different responses to cold of four groups of racially similar subjects (Yoshimura and Iida 1952).

Yoshimura and Iida attempted to improve reactivity by regularly immersing the feet of boys and Japanese soldiers in ice water. Only in the boys did an appreciable

improvement occur, which suggests that the individual must be subjected to severe conditions during childhood to gain improvement in vascular reactivity to cold. Training consisting of 30-minute immersions was less effective than 15-minute immersions. Thus overtraining may be harmful, which could account for the findings in some Antarctic expeditions.

On immersing the hand in water at 6°C Korean diving women (Ama) showed greater vasoconstriction of finger blood vessels than did the controls (Paik et al. 1972).

Whole-body immersion in cold water

A different approach to adaptation to cold is to study people immersed in cold water. The intimate contact with water, which is much better than air as a conductor of heat, imposes a greater thermal stress at relatively higher temperatures than in air. Of importance in this context are Korean Ama diving women who were tested by whole-body immersion in cold water (Hong et al. 1969).

Artificial cold-acclimatisation

Ten subjects wearing only shorts were exposed for 8 hours a day for 31 days to an air temperature of 11.8 ± 0.5°C in winter. Another group of six was exposed for 28 days to 13.5 ± 1.5°C in summer, when cold acclimatisation should be minimal. In both groups rectal temperatures were maintained at a lower temperature after the exposure period. Peripheral temperature did not change significantly in the winter group, but foot, head and mean skin temperatures decreased in the summer group after exposure to cold. The study concluded that humans can be artificially cold-acclimatised (Davis 1961).

In another cold-chamber investigation, the rectal temperature in warm air did not change significantly following a period of cold-chamber exposure, but after 7½ hours of cold stress, the rectal temperature dropped below that of the pre-exposure response (Keatinge 1961).

Reactions of men in polar regions

Eight unclothed men in Antarctica were exposed to 17°C for 2 hours on three occasions in autumn, winter and spring (Milan, et al. 1961). The mean body, skin and foot temperatures increased after 3 months, but rectal and finger temperatures did not change over the year. Although their basal metabolic rate did not change, the metabolic responses to the standard cold stress associated with a delay in the onset of shivering decreased after three months in Antarctica. The apparent paradox, viz. higher peripheral temperature, rectal temperature unchanged and lower metabolic rate in response to a standard cold stress, was explained by the investigators as being due to less convective heat being lost because shivering was reduced. The decreased metabolic response was thought to be part of an adaptation to cold.

A group of Caucasian South Africans was studied before going to Antarctica and again after 6 and 12 months in Antarctica. They were compared with a control group of Caucasians who remained in South Africa. Both groups were subjected to a standard cold stress of 5°C, 10°C and 15°C for 60 minutes. Another set of

readings was taken at the thermoneutral point of 27°C. Skin temperatures were measured with copper-constantan thermocouples and rectal temperatures were measured with a clinical thermometer (Wyndham et al. 1964). Rectal temperature was not significantly different in the pre-Antarctic and Antarctic series after an exposure of 1 hour at ambient temperatures ranging from 27°C to 10°C, although the rectal temperature tended to drop during cold exposure in Antarctica. After all exposures to 5°C, the rectal temperature at the warm (27°C) phase level in Antarctica dropped to a significantly lower temperature than in the pre-Antarctic series. The average rectal temperature after 60 minutes at 10°C was 37.2°C in the pre-Antarctic series and 36.9°C in Antarctica. These values, however, were not significantly different from those of the control group who stayed in South Africa. The average skin temperature progressively fell in Antarctica for all ambient air temperatures. Finger temperatures of the Antarctic group fell below their own pre-Antarctic values as well as the values of the control group. Toe temperatures were higher at 10°C and 5°C in Antarctica than in the pre-Antarctic series and were higher than those of the control group.

Wyndham and Loots (1969) found that, at the end of a year in the Antarctic, the rectal temperature of both fat and thin men after 60 minutes at 10°C (and 5°C) was higher than at 20°C and 27°C, but that rectal temperature scarcely changed when the cold stress was imposed soon after arrival in Antarctica. The fat men had higher rectal temperatures than the thin men, and their rectal temperature pattern in response to cold stress changed less after a year in Antarctica than it did just after arrival. One man, regarded as average, had a response pattern intermediate between the fat and thin group. In the series at the end of the year, the average rectal temperature of thin men after 60 minutes at 10°C; was 36.4°C, and of fat men was 37.1°C.

At air temperatures below 20°C, the mean skin temperature of the fat men was lower than those of the thin men. At the end of the year, the mean skin temperature of the thin men dropped, but did not change in the fat men. The finger temperatures of fat and thin men were very little different either at the beginning or the end of the year, irrespective of air temperature, and there was no trend in finger temperatures over the period in Antarctica. Toe temperatures of the thin men were lower than those of the fat men at 10°C, and in both fat and thin men, toe temperatures tended to be lower at 10°C after a year in Antarctica. At 20°C, the toe temperatures of fat and thin men were much the same at the beginning and the end of the year in Antarctica.

Budd (1964) found that the mean rectal temperature after 60 minutes at 10°C was lower than the basal value in the pre-Antarctic series, but was higher than the basal value in all Antarctic series, being best maintained (0.3°C above basal value) in the September series, but with a slight reduction (+0.2°C above basal value) in the December series. Rectal temperature maintenance was poorest in the follow-up series. The test was continued for 100 minutes, at which time the various parameters in each series remained in the same relative positions. The warm phase rectal temperature varied between 36.59°C and 36.80°C, the lowest value being in December series. The two earlier Antarctic series had higher values than in the pre-Antarctic series, and were close to the follow-up values. The differences, however, were not significant. The highest 60-minute rectal temperature was in the September series (37.1°C). The December series 60-minute temperature (36.7°C)

was close to that of the pre-Antarctic series, but as basal level was lower in the December series, temperature maintenance was better than in the pre-Antarctic series.

No consistent variation of skin temperature between series was observed, but extremity temperatures tended to be lower in Antarctica than in Melbourne.

Budd and Warhaft (1966a), using a 10°C cold stress, showed the enhanced rectal temperature maintenance in the Antarctic series conducted in July corresponded to approximately 24 weeks in Antarctica. The changes in rectal temperature at the 60-minute phase were −0.2°C and 0 for the pre-Antarctic and the Antarctic series respectively. The corresponding series at the 120-minute phase showed changes of −0.35°C and −0.15°C respectively. The warm phase rectal temperature was 37.05°C in the pre-Antarctic series and 36.68°C in Antarctica.

Toe and finger temperatures were very slightly higher at the 120 minute phase in Antarctica than in the pre-Antarctic series. Average finger temperatures were 12.5°C and 13.5°C for pre-Antarctic and Antarctic series respectively, and toe temperatures were about 10°C in each series, i.e. the toe skin temperature had dropped to the ambient air temperature.

In a study conducted on subantarctic Heard Island (53°S, 73°E), Budd (1965) found that rectal temperature and shivering did not change significantly after 2 hours of whole-body cooling at 10°C, despite loss of fat insulation. Finger temperatures fell more rapidly at Heard Island, and skin temperature gradient between elbow and finger increased, which suggests counter-current heat exchange increased and vasoconstriction was enhanced. The Heard Island results had a similar pattern to those obtained by Budd (1964) at Mawson on the Antarctic continent.

Finger numbness is measured by the ability to detect various gap widths (Massey 1959) at different skin temperatures. Newcomers and those who had spent a year in Antarctica showed no difference in the resting finger temperature, but over the year there was a significant fall in the temperature in both groups. The newcomers had less resistance to numbing than those who had already spent a year in Antarctica. After 6 weeks the newcomers' resistance to numbing improved and remained similar to that of the second-year men. During the test, frostbite sometimes occurred; eighteen cases in the first-year group and only four cases in the second-year group. These results agree with those reported in Budd's later work. Despite the lower resting temperatures, there was reduced numbness and increased resistance to frostbite after residence in Antarctica.

Finger blood flow as measured by plethysmography, both resting flow and the increased flow due to cold-induced vasodilatation during immersion in ice water, was found to be reduced during residence at Wilkes, Antarctica and expeditions therefrom (Elkington 1968). This contrasts with the response in Eskimos (Meehan 1955b) and Orochins (Yoshimura and Iida 1952).

3.1.1 Methods and materials

A subgroup of twelve men was subjected to periodical standard cold stresses. The initial series of cold stress observations was made on ten of the men in January before sailing. The whole group of twelve was studied in Antarctica about every

3 months. In addition, four of the group were tested in April just after returning from a field trip of 1 month's duration. Five of the group were retested in June, 4 months after returning to Australia. Two of this group were flown from Canberra and one drove a car 119 km before the test. Two more of the subjects were retested in November, 9 months after returning to Australia.

Before the cold stress the subject undressed, leaving on only light underpants. He was weighed, and skinfold thicknesses just below the left scapula and over the left triceps, and the arm circumference were measured (see 3.2.2).

The subject, covered by blankets, then lay on a couch. The ambient temperature was kept at 20°C–21°C.

A rectal thermometer was inserted to a distance of 10 cm from the anal verge. Skin thermistors were attached by adhesive tape to the chest over the precordium and over the pulp of the right index finger and of the right big toe. Temperatures were recorded every 15 minutes, during the preliminary warm phase. After about 15 minutes, blood pressure and pulse rates were determined. In accordance with the procedure laid down for physiological measurements at Australian stations, diastolic blood pressure was taken as that pressure at which the Korotkow sounds become muffled.

Oxygen consumption was then measured only in the Antarctic series, using a Sanborn 'Metabulator'. Following this, 40 mL of blood were withdrawn from the antecubital vein and put in plastic tubes, cooled in crushed ice or snowdrift, then centrifuged, and the plasma was withdrawn.

When the rectal temperature was constant for two successive readings, the subject stood up and passed urine into a plastic container with 10 mL of 6N hydrochloric acid. The time of the previous urination was noted.

The subject then walked to an adjacent room with a temperature of 10°C and lay as motionless as possible for 2 hours on a bed with mesh mattress. The upper limbs were kept away from body and the lower limbs were kept slightly apart. Skin and rectal temperatures were measured every 10 minutes. The time of onset of shivering was noted and the magnitude of the shivering was assessed by visual observation. The cold stress of 10°C was chosen to provide conditions comparable with other Australian work (Budd 1964, Budd and Warhaft 1966a). Also, Wyndham et al. (1964c) considers 10°C as best suited to show changes in cold response.

Blood pressure and pulse rate were measured 10 minutes after the start and 10 minutes before the end of the cold stress. Oxygen consumption was measured in the last 10 minute period.

After 120 minutes, the subject walked to the warm room and passed urine into a receptacle containing 10 mL of 6N hydrochloric acid. As with the 24-hour specimens, the urine specimens were kept at 4–10°C for a few days until assayed.

The subject lay on a couch and was covered with blankets, and 40 mL of blood were withdrawn from the antecubital vein, cooled and centrifuged as before. The plasma was withdrawn and frozen, as was the pre-stress plasma, until biochemical analysis could be effected.

Skin and rectal temperatures were measured every 15 minutes until rectal temperature was constant for two consecutive readings. Blood pressure and pulse rates were measured during the rewarming phase. Ambient temperatures were noted each time the skin and rectal temperatures were measured.

A similar procedure was followed for twelve different subjects in Melbourne in the following November. Some of this control group had previously been in Antarctica for at least 18 months, but most had never lived in cold climates.

In the series of cold-stress tests conducted in Australia before going to Antarctica, blood-sugar levels were determined before and after the stress, using Lavco true glucose kits. As results showed no relationship to the cold stress, this investigation was discontinued.

Calibration of thermistors

Each thermistor was immersed in water and a standardised mercury-in-glass thermometer was placed near the thermistor. The water was stirred constantly by a magnetic stirring bar. The temperature was slowly changed by adding warm or cold water so that a calibration table was compiled for each thermistor over the working range.

The Wheatstone bridge reading was compared with the temperature reading of the mercury-in-glass thermometer. As the resistance characteristics at a given temperature may change, the thermistors were calibrated initially in Melbourne, at mid-year, at the end of the year in Antarctica, and in conjunction with the follow-up series. The resistance characteristics did not alter over the period of the investigation, but the wiring of one thermistor used on the toe broke off and another thermistor was substituted at this site.

The Wheatstone bridge was designed and constructed in the workshops of the Australian Department of Health, School of Public Health and Tropical Medicine. It featured a switch whereby each thermistor could readily be substituted for one arm of the bridge.

Measurement and maintenance of ambient air temperature

The ambient air temperature was measured by the same mercury-in-glass thermometer as that used for calibrating the thermistors. In Melbourne the test temperature of 10°C was obtained by setting the thermostat of a cold chamber located at the Antarctic Division Head Office. Fine temperature adjustments were made by opening and closing the door as required.

In Antarctica the cold stress was performed in the hospital sick bay (which was otherwise unoccupied). Here the room had to be heated to bring it up to the required temperature. The radiator thermostat was set to 10°C and fine adjustments were made by opening or closing a door to the exterior. A mattress was placed between the subject and the heater to avoid any direct heat radiation. A baffle was placed between the subject and the external door so that when it was ajar no cold air currents flowed over the subject. The thermometer was placed at the level of the subject to avoid error from vertical temperature gradients.

Analysis of results

The pre-Antarctic series results were compared with the Antarctic and follow-up series by the two-tailed paired t test. Regression analysis was applied to the principal variables in the Antarctic series. The equation parameters were tested for significant variation by analysis of variance (see Appendix).

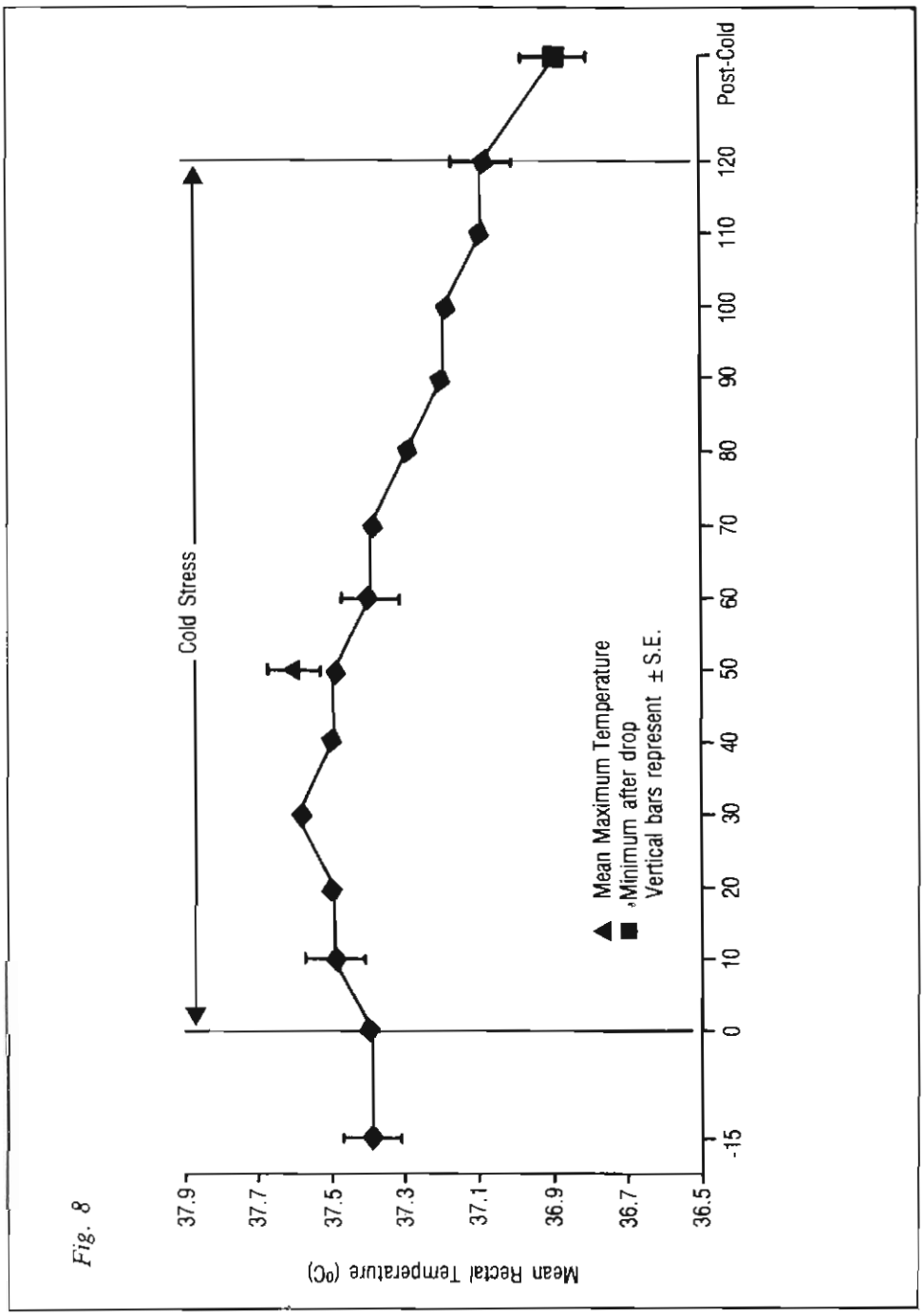


Figure 8. Mean rectal temperature changes due to cold stress, January

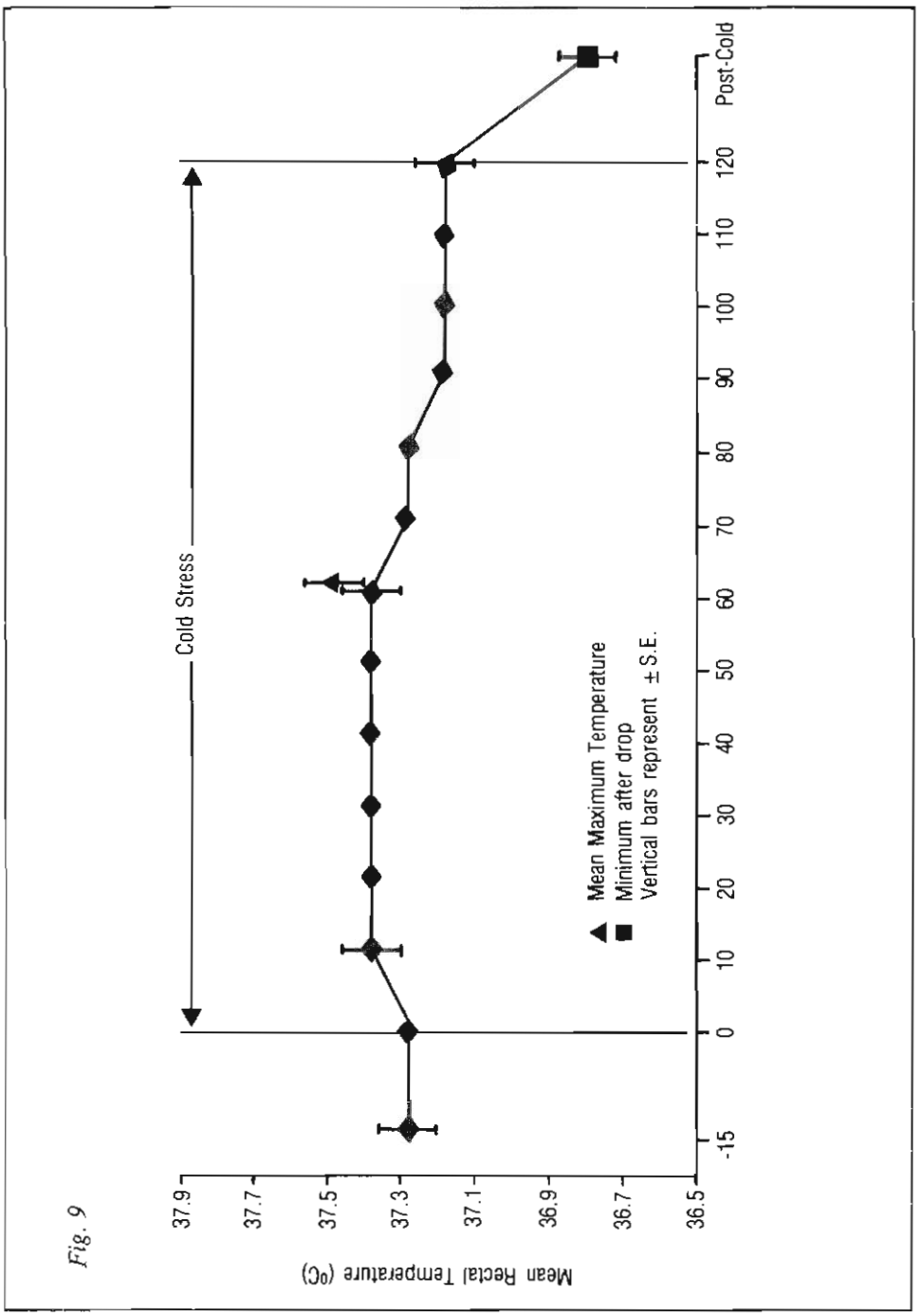


Figure 9. Mean rectal temperature changes due to cold stress, March

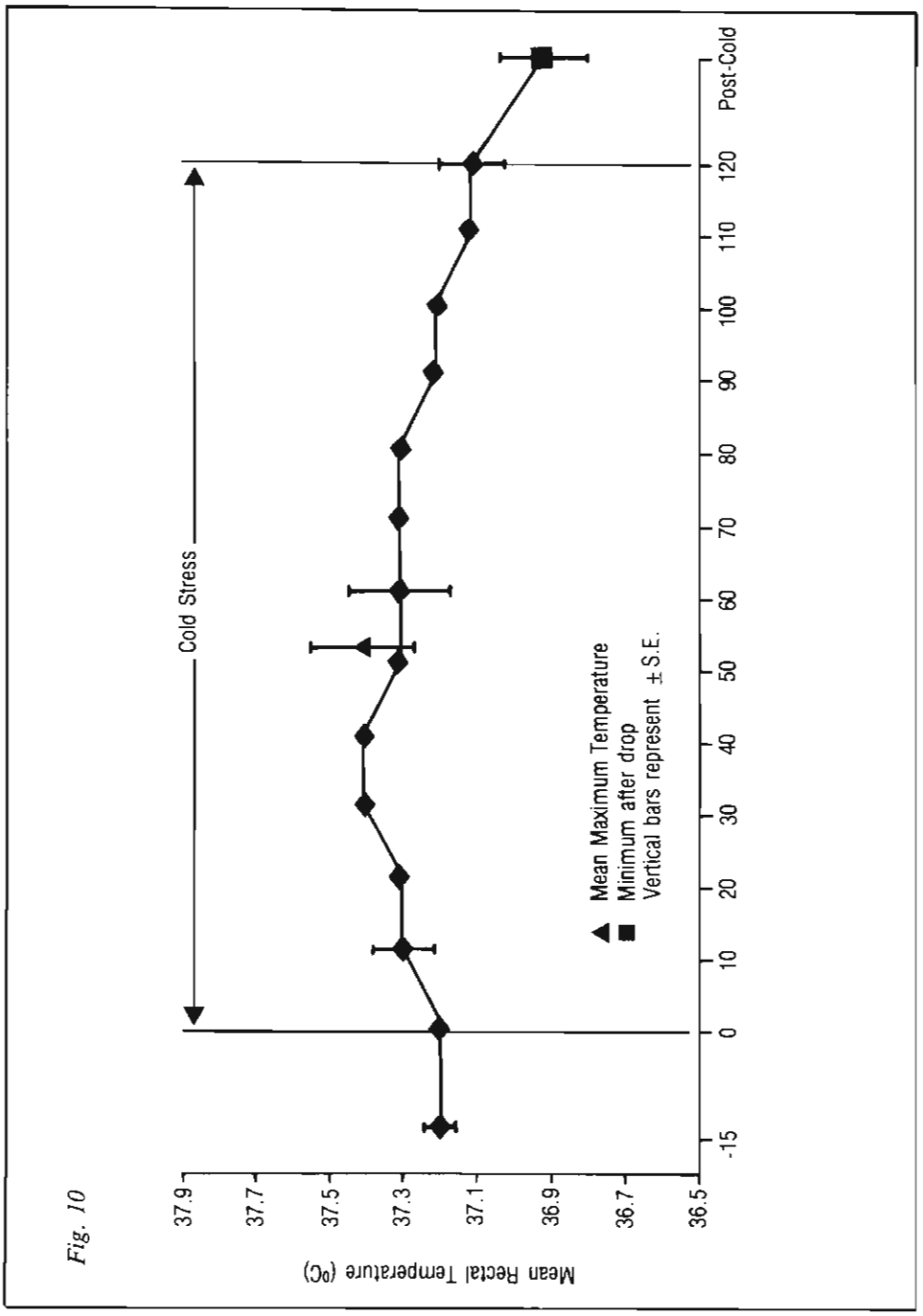


Figure 10. Mean rectal temperature changes due to cold stress, April

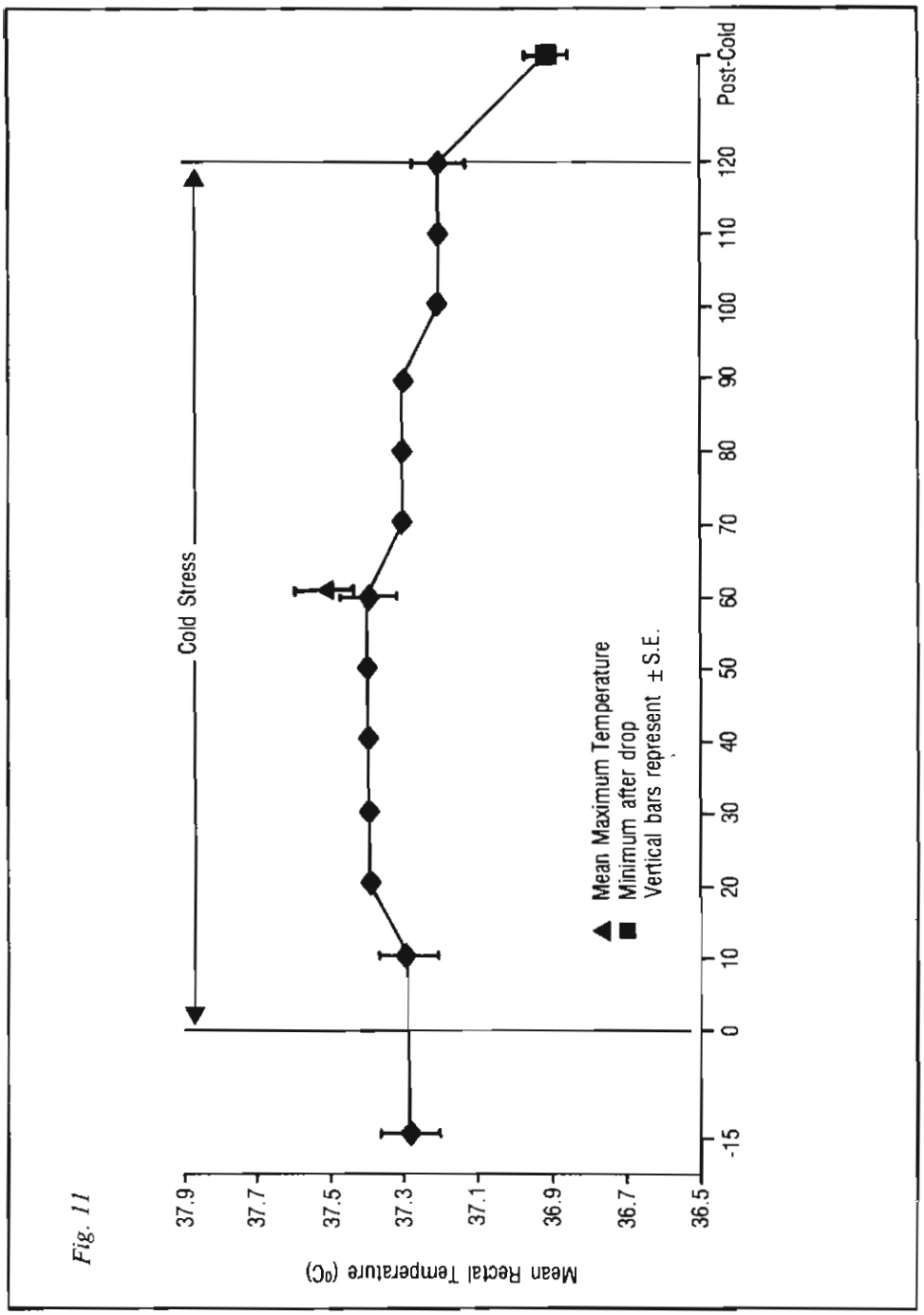


Figure 11. Mean rectal temperature changes due to cold stress, June

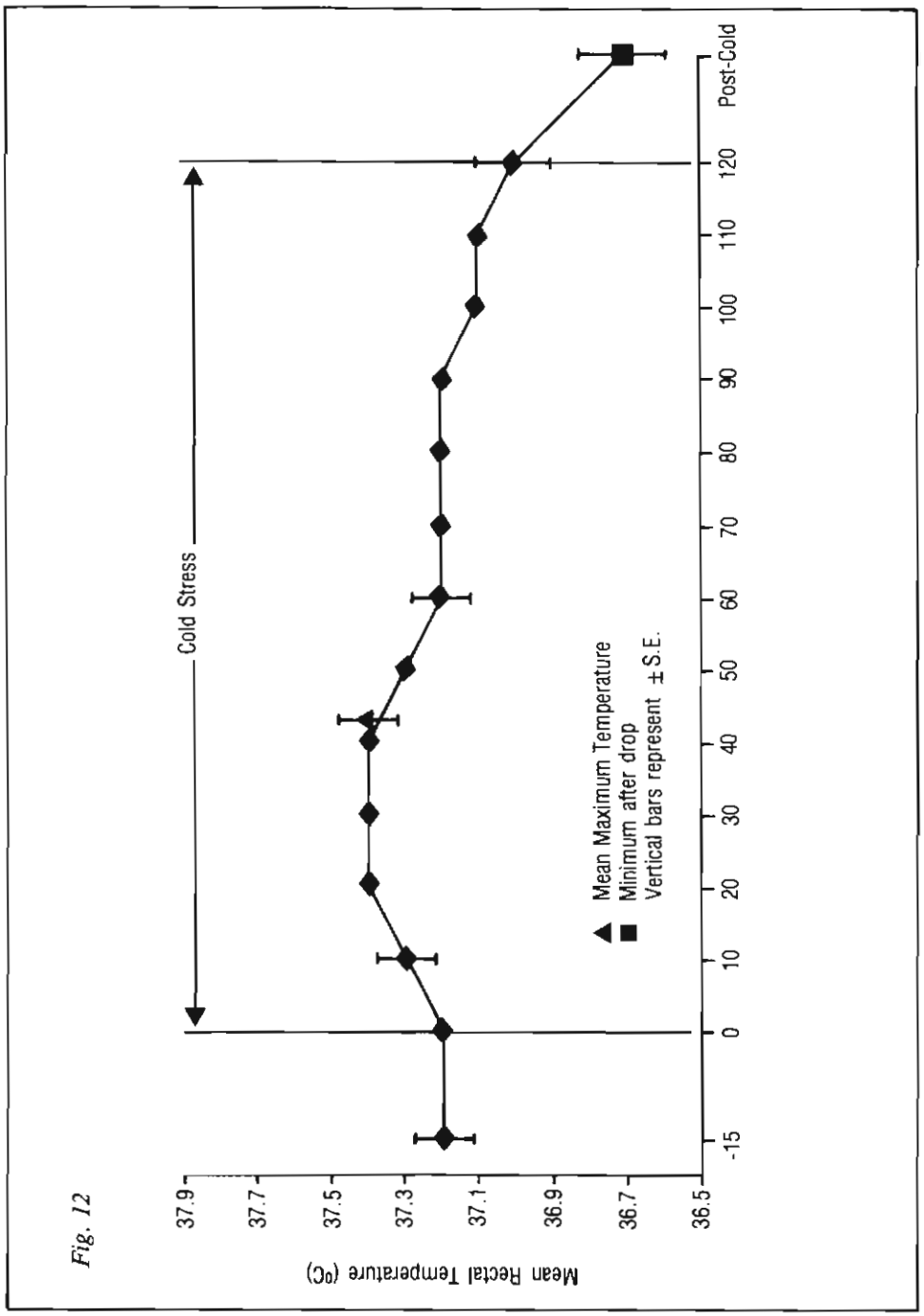


Figure 12. Mean rectal temperature changes due to cold stress, September

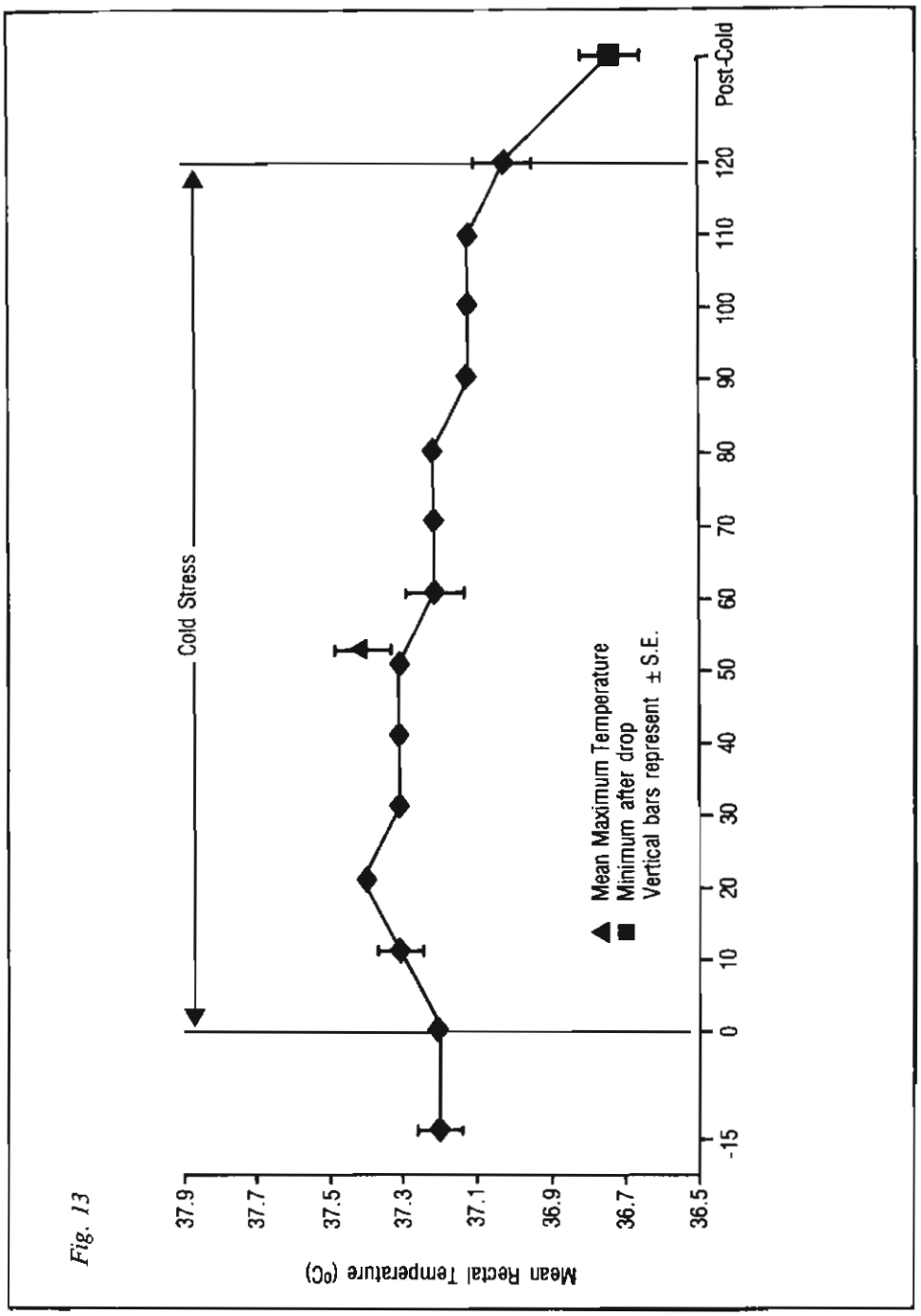


Figure 13. Mean rectal temperature changes due to cold stress, November

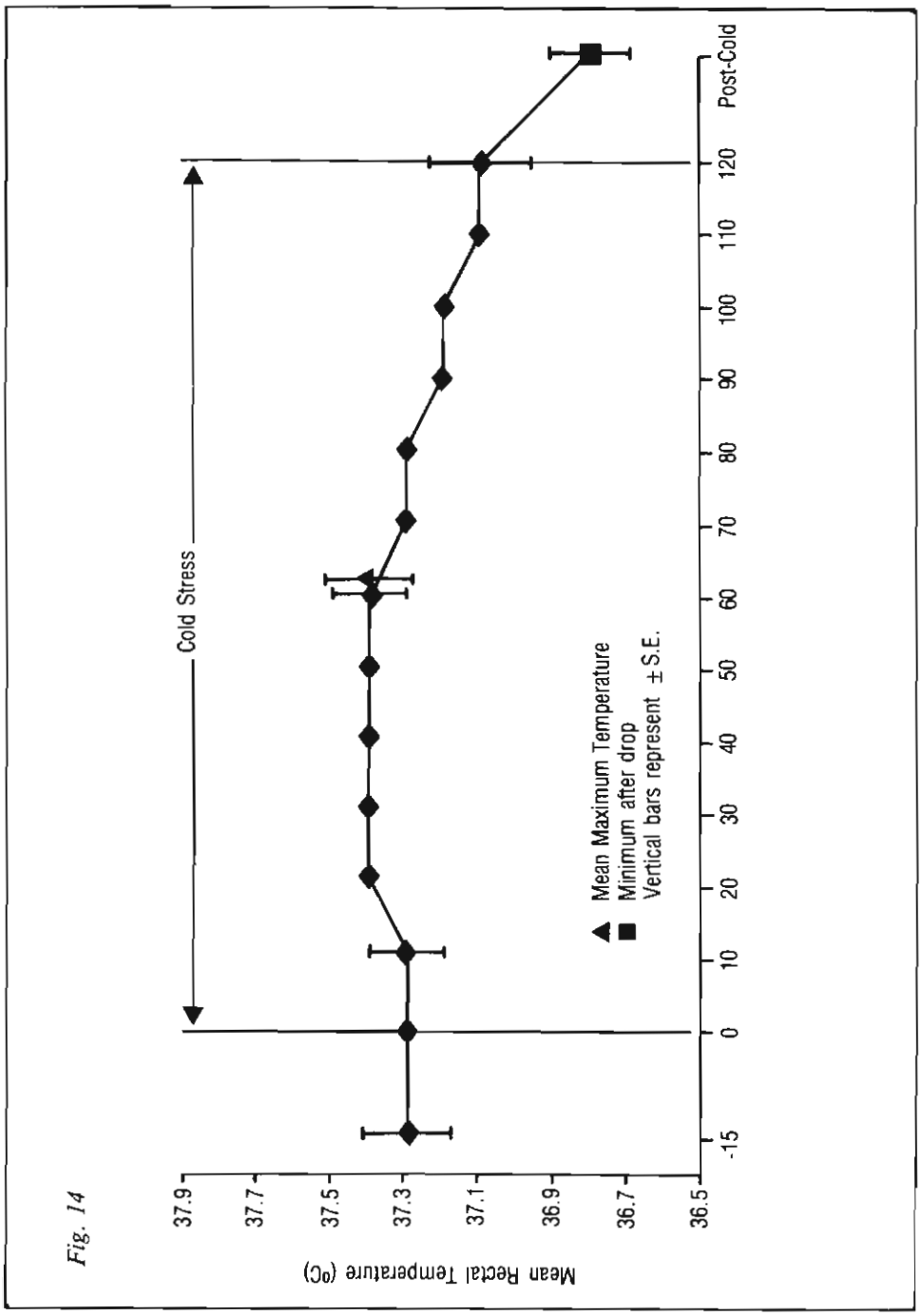


Figure 14. Mean rectal temperature changes due to cold stress, post-Antarctic group

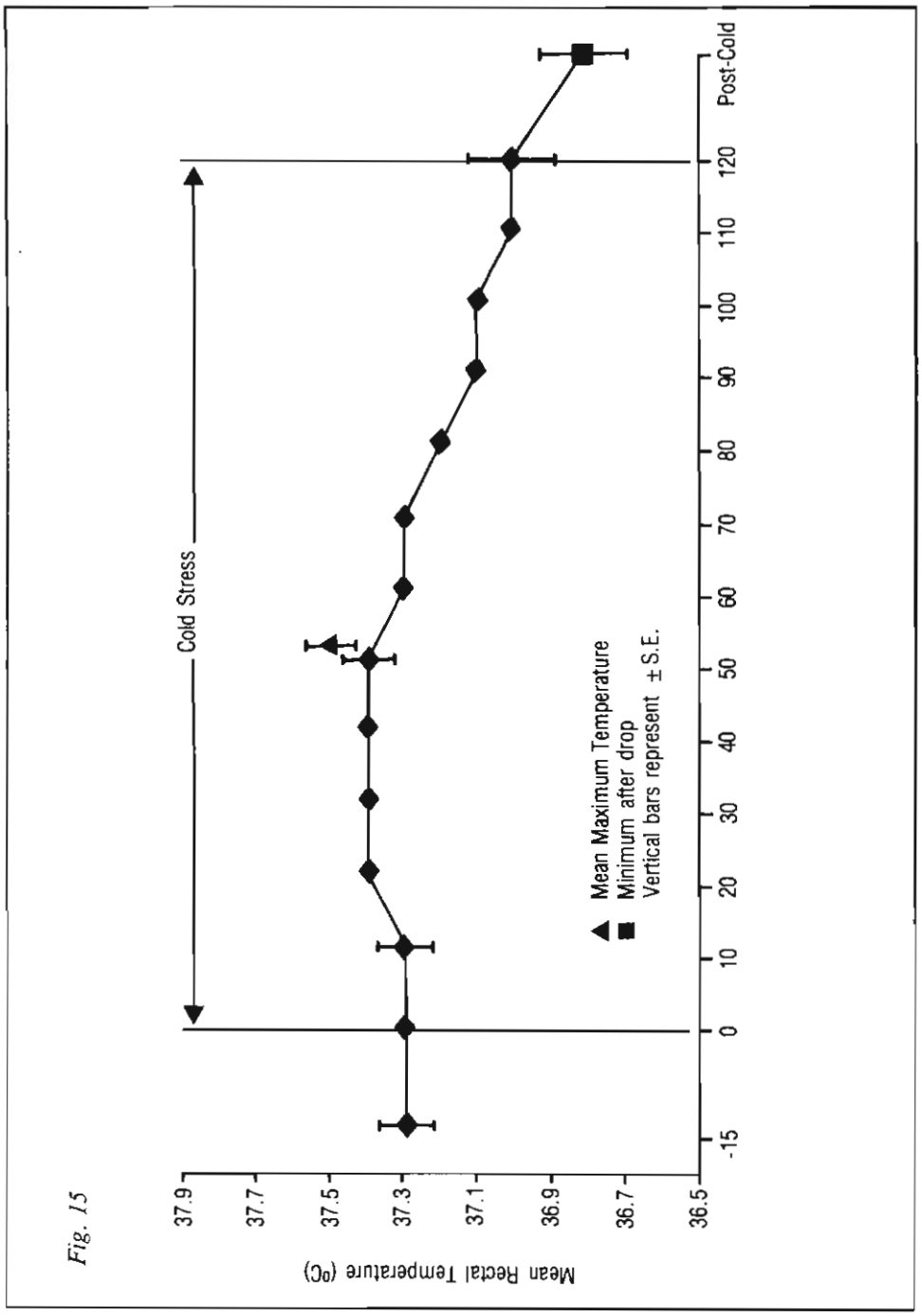


Figure 15. Mean rectal temperature changes due to cold stress in the control group

Fig. 16

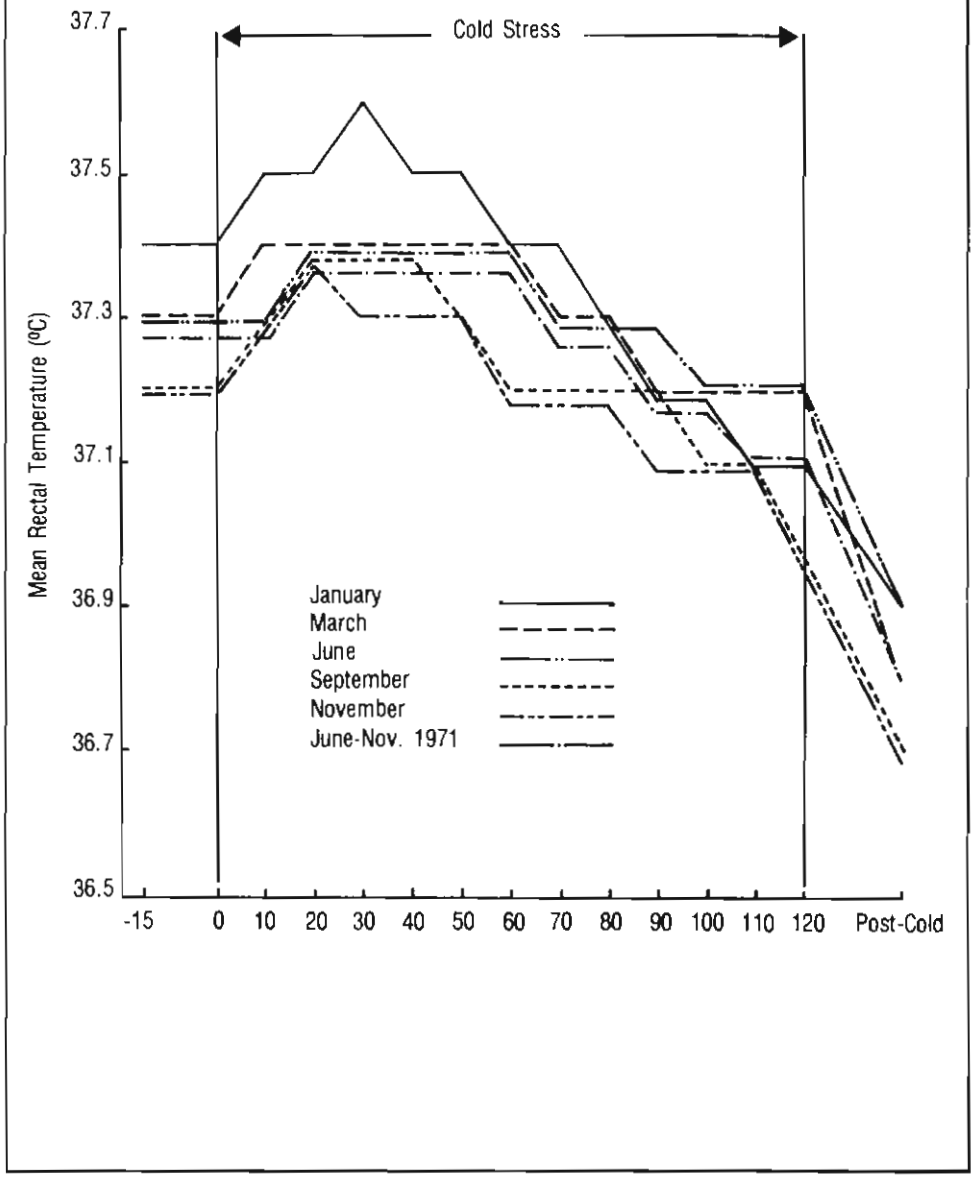


Figure 16. Mean rectal temperature changes due to cold stress, superimposed for pre-Antarctic, Antarctic and post-Antarctic series

3.1.2 Results

Rectal temperature

Rectal temperatures during each cold stress showed an initial rise and a subsequent decrease (Figures 8–15). The maximum temperature was not significantly greater than the basal value in all the main series. During the rewarming phase the rectal temperature showed a further decrease, the minimum value being significantly lower than either the basal or the final cold-stress temperatures in all series.

The rectal temperatures showed only slight differences between series at corresponding phases (Figure 16). Regression analysis did not indicate altered rectal temperature responses in Antarctica, but because of the lower ambient temperature in the warm phase in the June series, paired t tests were applied and these were consistent with the hypothesis that there were no trends in the rectal temperature response patterns while the subjects were in Antarctica. The quadratic regressions for the Antarctic series, however, were significantly different from that of the January series, the decrease in the rectal temperature over the second hour of the cold stress being greater in the January series than in Antarctica (Figure 17).

The maximal cold-phase rectal temperatures were lower ($P<0.05$) in the September and November series than in January. The time elapsed in the cold stress before the rectal temperature started to decline did not alter significantly in Antarctica. Changes in rectal temperatures between various phases of each series show that the after-drop was greater in the Antarctic than in the January series, but the total drop from the basal to the minimum cold-phase values was 0.5°C in all the main series except for June, when it was 0.4°C. The follow-up series and the control group gave results very similar to those of the pre-Antarctic series (Figures 8, 14 and 15).

Chest-skin temperature

Chest-skin temperatures during each cold stress showed on average a steady cooling, though some individuals showed some temperature hunting over the second hour. The January series did not differ significantly from the Antarctic series. Regression analysis of the Antarctic series showed a cooler chest-skin initially, then a warmer chest-skin towards the end of the expedition. This observation is supported by between-series comparisons. There was no evidence of any change in the rate of decrease in chest-skin temperature during the cold phase (Figure 18).

In the rewarming phase (Figure 21) neither the temperature nor the rate of rewarming showed any significant differences between series.

Index finger skin temperature

The temperatures of the skin of the index finger showed pronounced cooling in each series (Figure 19). Excluding the June series, in which the warm-phase ambient temperature could not be adequately maintained, the warm-phase skin temperatures were not significantly different from that of the January series. The 120-minute value in the Antarctic series gave a quadratic regression showing a decrease, with a minimum in early June, followed by an increase (Figure 19). This pattern is further illustrated by comparisons of the Antarctic series with the pre-Antarctic

Fig. 17

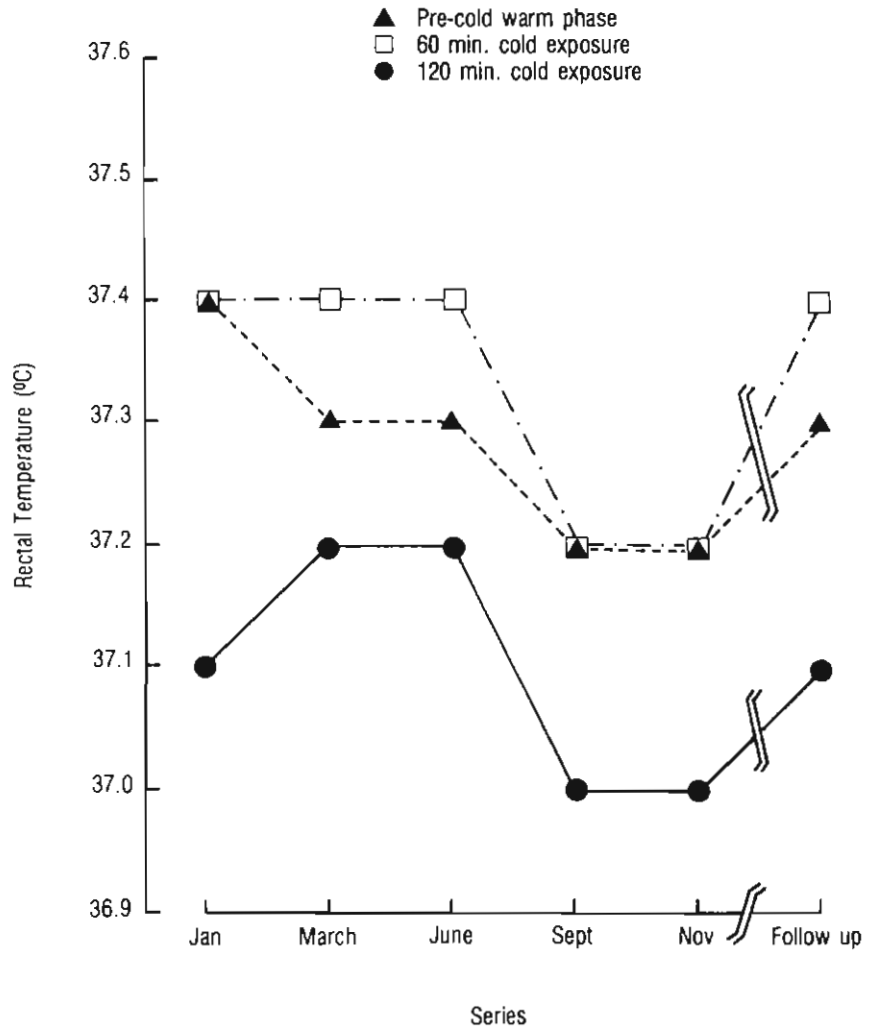


Figure 17. Comparison of rectal temperatures at pre-cold warm phase, 60 minute cold exposure and 120 minute cold exposure in pre-Antarctic, Antarctic and post-Antarctic series

Fig. 18

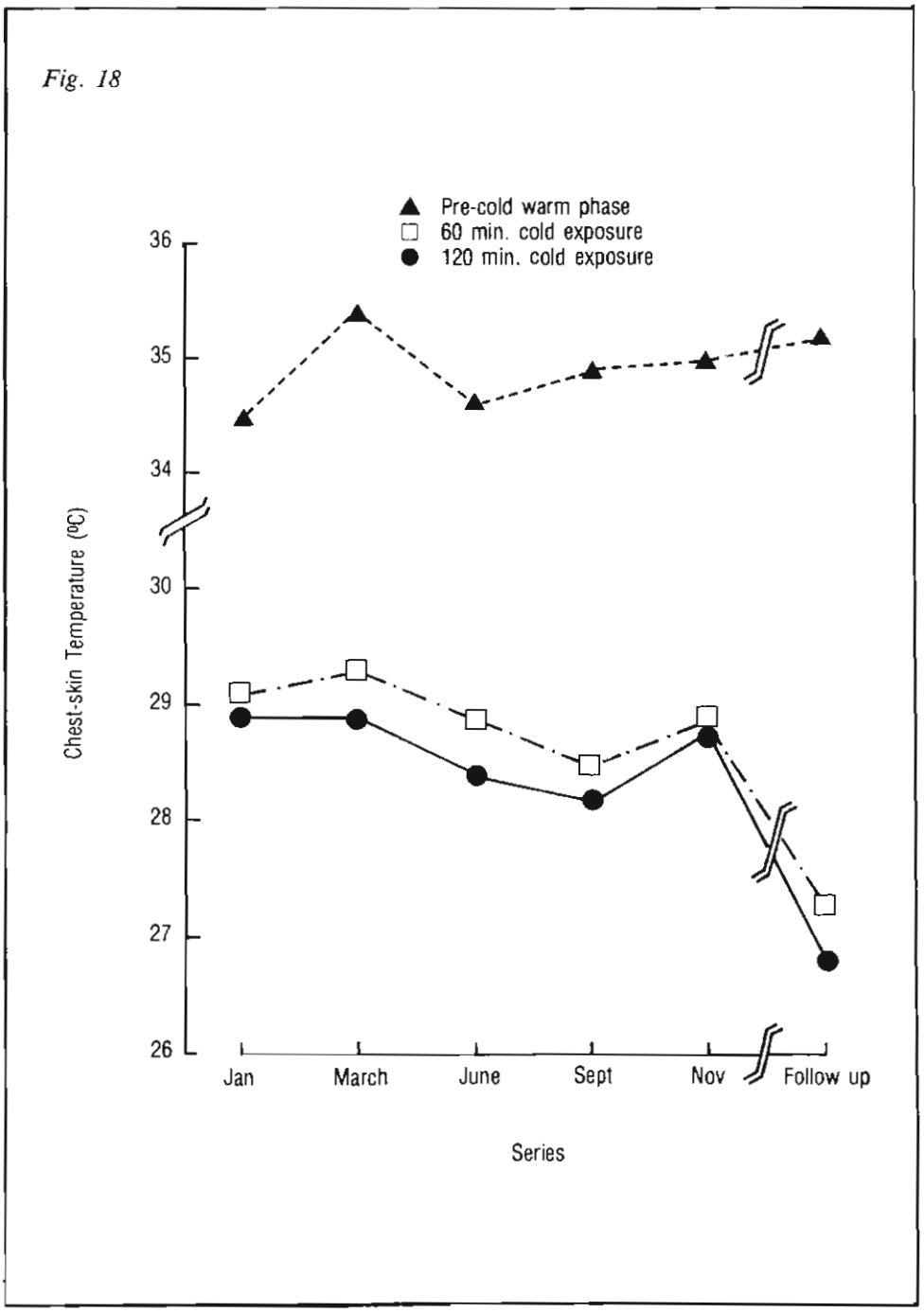


Figure 18. Comparison of chest-skin temperatures at pre-cold warm phase, 60 minute cold exposure and 120 minute cold exposure in pre-Antarctic, Antarctic and post-Antarctic series

Fig. 19

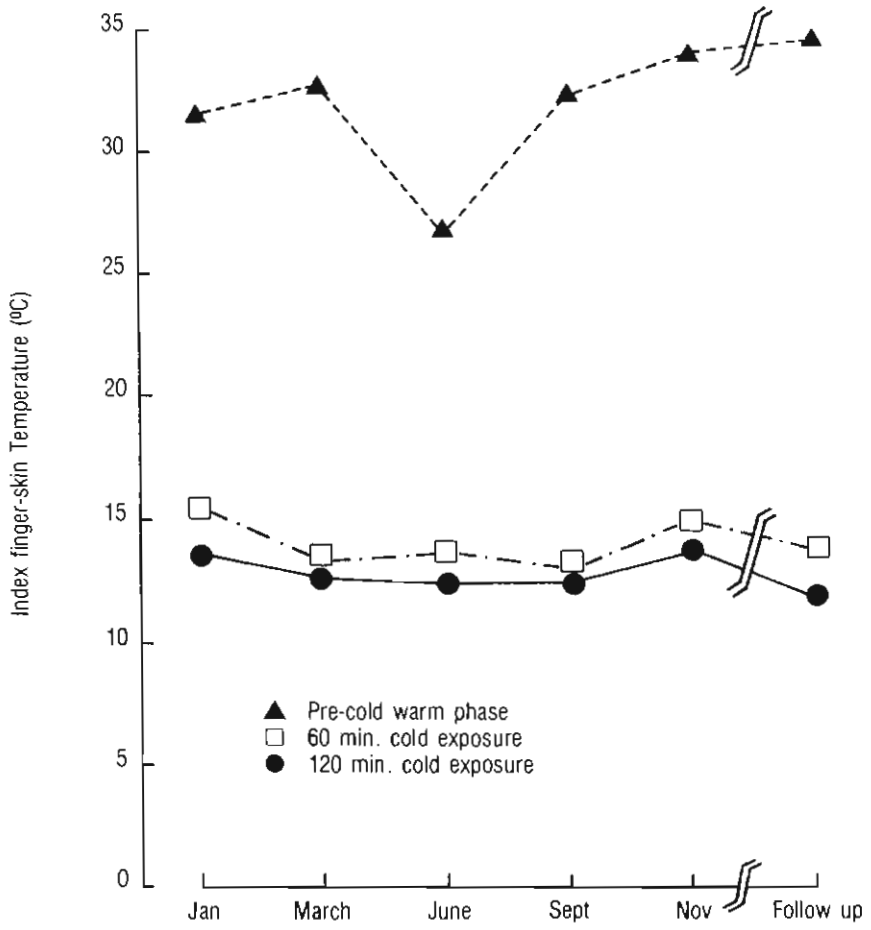


Figure 19. Comparison of index finger-skin temperatures at pre-cold warm phase, 60 minute cold exposure and 120 minute cold exposure in pre-Antarctic, Antarctic and post-Antarctic series

Fig. 20

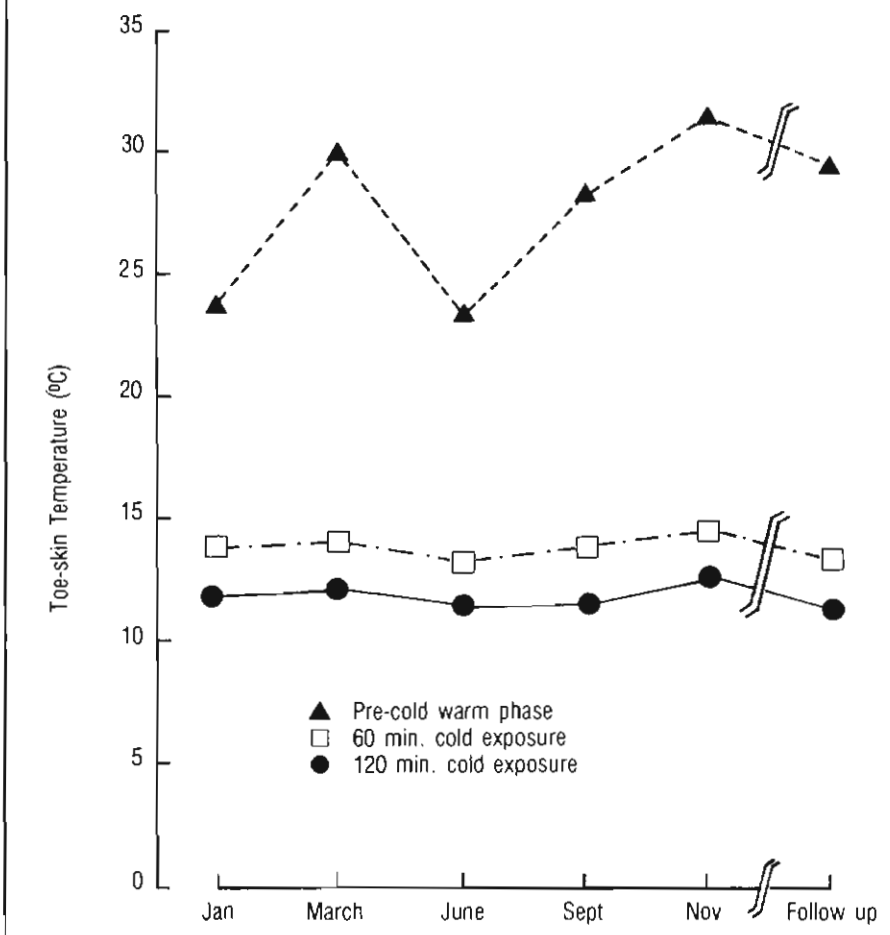


Figure 20. Comparison of toe-skin temperatures at pre-cold warm phase, 60 minute cold exposure and 120 minute cold exposure in pre-Antarctic, Antarctic and post-Antarctic series

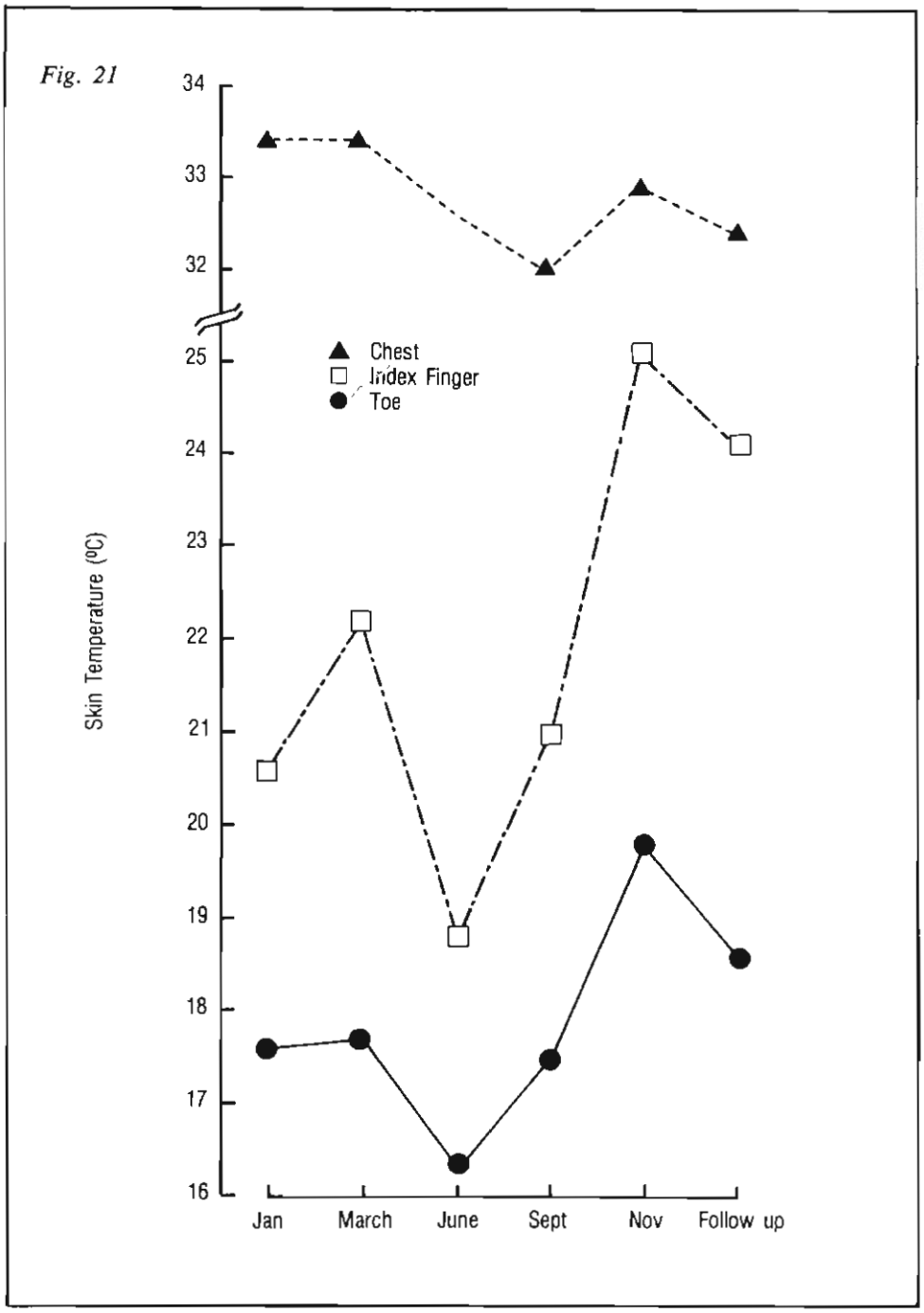


Figure 21. Chest, index finger and toe-skin temperatures in rewarming phase in pre-Antarctic, Antarctic and post-Antarctic series

series: the June and September series gave lower ($P<0.02$) finger temperatures, with the November value being warmer ($P<0.005$) than the September value. The follow-up series and control group responses were similar to those of the pre-Antarctic (January) series.

The skin of the index finger showed greatest rewarming ability (Figure 21) after the cold stress in the November series: it reached the warmest temperature after 30 minutes, the fastest rate in the Antarctic series.

Toe-skin temperature

The toe-skin shows even more pronounced cooling than the finger-skin during the cold stresses (Figure 20). The warm phase toe-skin temperature became warmer in Antarctica (except for June) and at follow-up than in the pre-Antarctic series. In Antarctica, the 120 minute value showed a quadratic regression with the minimum in early July, rising to the maximum in the November series (Figure 20). Comparison between series showed a drop from March to June ($P<0.05$) and a rise from September to November ($P<0.05$) at 120-minute cold exposure. The follow-up series and control group responses were similar to those of the pre-Antarctic series.

Maximal toe-skin rewarming ability (Figure 21) after the cold stress was exhibited in the November series: it reached the warmest temperature after 30 minutes, and had the greatest rate of rewarming in the series.

3.1.3 Discussion

There were no significant differences in the warm-phase rectal and skin temperatures between the pre-Antarctic and Antarctic series (apart from the June series, when the warm-phase ambient temperature could not be adequately maintained, due to a fault in the station heating system). There was, however, a slight tendency for the rectal temperature to become lower and the skin temperature higher in Antarctica than in Melbourne. Similar findings were obtained in warm air for Caucasians in Antarctica (Budd 1964 and 1965, Budd and Warhaft 1966a), and when artificially cold-adapted (Carlson et al. 1953), and on comparing Eskimos with controls (Brown et al. 1954). A reduction in rectal and skin temperatures had been observed in Antarctica (Wyndham et al. 1964; Wyndham and Loots 1969). Eskimos had lower rectal and skin temperatures than Caucasians (Meehan 1955b). The opposite, however, was found by Adams and Covino (1958) in which rectal and skin temperatures of Eskimos were higher than those of Caucasians. They found the temperatures of these sites to be higher in Caucasians than in Negroes. The variability in observations on Eskimos is probably due to different levels of acclimatisation in the samples tested, as the native life-style of Eskimos is now disappearing.

The quadratic regressions obtained for the skin temperature at various sites during the cold stress indicate a biphasic acclimatisation pattern: an early form with increased cooling of the periphery (compared with that of the pre-Antarctic series), and a late form with a higher skin temperature throughout the cold stress than in the earlier series. This enhanced maintenance of skin temperature was associated with a lower rectal temperature. The rate of rectal cooling was similar for all Antarctic series and was less than that of the pre-Antarctic series.

Cold-stress response patterns resembling those of the early Antarctic series were observed in Caucasians who spent up to a year in Antarctica (Budd 1964 and 1965,

Wyndham and Loots 1969). By contrast, no change in rectal temperature and a reduced cooling of the skin were observed in response to a mild stress of 17°C after 3 months in Antarctica (Milan et al. 1961).

Alterations in responses similar to those found in the November series were observed after 24 weeks in Antarctica (Budd and Warhaft 1966a). Also, Caucasians after 8 weeks in a cold climate kept the periphery warmer at night than did the controls (Scholander et al. 1958a). This late response pattern, compared with that of the early series, was similar to that reported in comparisons of cold-climate indigenes, such as Eskimos (Meehan 1955b, Andersen et al. 1963), Arctic Indians (Irving et al. 1960), nomadic Lapps (Andersen et al. 1960), and a Nepalese pilgrim (Pugh 1963), with controls, who were presumably Caucasian members of the various expeditions whose exposure to the cold climate would have been of a similar duration to that of the subjects in the early Antarctic series of this investigation.

Caucasians, who after a year in Antarctica had lower rectal and skin temperatures at the end of the cold stress than previously (Wyndham et al. 1964d), appear to have been in a transitional stage between the two forms of acclimatisation corresponding to that of the September series.

Individual skin temperatures at various sites showed hunting over the second hour of the cold stress, due to cold-induced vasodilatation. This varied markedly between series and between subjects but there was no apparent seasonal variation.

Enhanced peripheral rewarming, as observed in the final Antarctic series and persisting to a lesser degree at follow-up, is considered to be an index of cold acclimatisation (Kreider et al. 1959). This is supported by the finding that the hands of Eskimos rewarmed quicker than those of controls (Andersen et al. 1963). It is not due to reactive hyperaemia, as rewarming was maximal in November when the extremities had cooled least. It therefore seems likely to be another manifestation of cold acclimatisation.

The chest-skin, however, showed diminished rewarming ability in Antarctica compared with the pre-Antarctic series. Similar reductions in rewarming ability have been found for the abdomen and thigh-skin (Budd and Warhaft 1966a). The reduction in the rewarming rate at these sites is probably due to increased subcutaneous fat insulating the skin from the well-vascularised underlying tissue. Individuals who did not increase subcutaneous fat thickness of the trunk showed improved chest-skin rewarming in the late Antarctic series.

The rectal temperature after-drop and the total decrease in the rectal temperature from the basal value to the minimum during the rewarming phase remained fairly constant throughout. On entering the cold chamber, vasoconstriction reduces the amount of cooled blood passing into the external iliac veins from the lower limbs (Bazett et al. 1948). There may also be a compensatory vasodilatation of intestinal blood vessels (Grayson 1951). The reverse occurs during rewarming.

The change in the vascular responses to whole-body cooling in the early form of cold-acclimatisation appears to be essentially an enhanced peripheral vascular constriction, whereas the late form is more complex. The reduced rectal temperature seems to result from a lower 'setting' of the body's thermostat (Le Blanc 1956). This reduces the temperature gradient between core and shell and also the total heat content, so less energy is required to maintain or restore core temperature.

A warmer periphery reduces the risk of cold injury. Though vasoconstriction remains the main means of peripheral heat conservation, countercurrent heat

exchange between limb arteries and their venae comitantes may be improved, as suggested from observations on Korean diving women (Hong et al. 1969). Re-adjustment of blood flow, with the skin remaining warm, but deep muscle tissues becoming cooler rhan in the unacclimatised subject during cold exposure, may occur as in Eskimos (Brown et al. 1954).

The different time sequences in the alteration of the various response components found in various investigations are probably due to different levels of cold exposure of the subjects. There was considerable subject variation in this investigation, in part because the degree of cold exposure depended on the subject's duties and inclinations, as well as inherent differences in the way an individual responds to a cold environment. Nevertheless, racial difference affect the vascular reaction to immersion of the hand in ice water — Negroes show later or non-existent cold-induced vasodilatation (CIVD) (Meehan 1955b), whereas descendants of North Chinese show earlier CIVD than do those of South Chinese ancestry (So 1975), both groups of subjects living under similar conditions in the United States of America. Racial differences may also be another source of variation in response to whole body cooling. In Bantu and Bushmen, temperature regulation appears to be inferior to that of Caucasians (Wyndham et al. 1964a). Australian Aborigines, who are subjected to hot day and cold night temperatures, showed pronounced rectal and peripheral cooling during the night (Hammel et al. 1959). Rennie (1971) observed that the different responses of the Eskimo and the Ama of Korea are favourable to the different kinds of cold stress to which each type of person is subjected: the Eskimo who is normally warmly clad but immerses the hands in freezing water has peripheral circulation that maintains warm finger and hand temperature, whereas the Ama diving women have good peripheral circulation without an increase in heat flux, probably due to counter-current heat exchange (Hong et al. 1969), and thus conserve heat during whole-body immersion in cold water without undue vasoconstriction (see 3.2).

3.1.4 Conclusions

From the variations in rectal and skin temperatures in warm surroundings, during periodical cold stresses, and rewarming, it is concluded that the environment at Casey induced cold-acclimatisation. As in heat acclimatisation (Adam et al. 1953, Macpherson 1960), it was manifested in early and late forms, though taking much longer than in the case of heat acclimatisation. This may be a consequence of mankind's presumed origins in a hot climate (Morris 1967). In the early form, survival is enhanced by increased peripheral vasoconstriction leading to greater cooling of the periphery in response to cold stress and a higher core temperature than in the pre-Antarctic cold stress. The late form of cold acclimatisation is characterised by a lowered core temperature but enhanced core-temperature maintenance. The periphery showed less cooling and more rapid rewarming than in either the pre-Antarctic or the early Antarctic series. The late form of acclimatisation resembles the pattern of cold response noted in cold-climate indigenes of the northern hemisphere, whereas the early response is similar to that of Caucasian members of the expeditions concerned, who had had a period in the cold comparable with that of the present subjects by the time the early Antarctic cold stresses were applied.

3.2 SKIN-FOLD THICKNESS, BODY WEIGHT, ONSET OF SHIVERING, AND OXYGEN CONSUMPTION

Subcutaneous fat thickness

Increased thickness of subcutaneous fat is thought to enhance resistance to a cold stress by virtue of its insulating properties. The chief characteristic enabling English Channel swimmers to remain in water at about 15°C for 12 to 20 hours is probably their thick layer of subcutaneous fat (Pugh and Edholm 1955). Men with an average skin-fold thickness less than 10 mm are liable to die after prolonged immersion in water as warm as 20°C; men with average skin-fold thickness of over 20 mm may survive indefinitely in water as cold as 12°C (Keatinge 1969).

Subcutaneous fat thickness is considered to be an important factor in the ability to maintain rectal temperature during whole-body cooling in air. The differences observed between ethnic groups in their response to a cold stress have been interpreted as due to different levels of nutrition. The rectal cooling of Bantu and Bushmen during cold stress compared to the rise in rectal temperature of Caucasians during a similar stress is regarded as due to the thicker subcutaneous fat of the latter (Wyndham et al. 1964a).

During whole-body cooling in air at 15°C for 2 hours (Baker and Daniels 1956) it was found that the skin temperature varied inversely with thickness of the subcutaneous fat, which insulated the skin from the warmer, well-vascularised, deeper tissues. The rectal temperature was proportional to the percentage of body fat. Taller stature and greater fat-free weight were associated with a tendency to lower rectal temperature, probably due to the surface area being relatively larger than in more rotund subjects. Wyndham and Loots (1969) also found that fat men had higher rectal temperatures than thin men after a 60 minute standard cold stress at 10°C and at 5°C. On exposure to cold air, thin men showed more individual variation than fat men.

An increase in subcutaneous fat has been observed in men living in Antarctica (Wyndham et al.). Body weight increased in Antarctica and this mostly correlated with increased fatness (Budd 1964). In later studies, only the thin subjects showed an increase in fatness in Antarctica (Wyndham and Loots 1969). It is thought that an increase in thickness of subcutaneous fat may be a specific cold-acclimatisation mechanism. A lean Nepalese pilgrim maintained body temperature during a night in which the ambient temperature fell to −.5°C. He survived temperatures down to −15°C in the Himalayas, without ill effect, while wearing his usual clothing, which was estimated to give an insulation of only approximately 1.5 clo, which is regarded as quite inadequate, by Western standards, for such severe conditions (Pugh 1963). It has been estimated that the average thickness of tissue participating in insulation may range from 5 to 73 mm, depending on the severity of the cold stress. The latter figure mostly exceeds the thickness of subcutaneous fat even in obese people (Buskirk 1966).

The skin insulation for a given thickness of subcutaneous fat was found to be 60% greater for Ama diving women than for other Korean women, and Korean men had significantly less insulation than non-diving Korean women. The Ama are thinner than American women of comparable age (Rennie et al. 1962). Rather than developing a more active peripheral vasoconstriction, limb blood flow was actually greater in the Ama than in controls. Although subcutaneous fat thickness

was less in the Ama than in controls, insulation was similar in both groups. Studies of limb heat-flux showed that the Ama lost relatively less heat from circulation than did the controls; it is postulated that this improved conservation is due to a more efficient counter-current heat exchange being established in the limb vasculature of the Ama. This enhanced heat exchange is, at least in part, acquired rather than racial, as the Ama are superior to other women of similar stock. The untrained women are, however, superior to their menfolk, so this feature may have a sex-linked inheritance favouring the female. It is probably for this reason that women have traditionally performed these diving tasks rather than men (Hong et al. 1969).

Shivering

Body heat is produced by exothermic chemical reactions of metabolism. One of the main sources of heat is muscular activity. Mostly the heat produced is just part of the thermal inefficiency of any system producing mechanical energy, in that some of the energy put into the system will be converted to heat, according to the Second Law of Thermodynamics, from which is derived the concept that, in any real system, entropy always increases; i.e. energy forms are degraded to the least orderly form, namely heat energy (Hercus 1947).

The most obvious form of thermogenesis is that of shivering. Here the muscle fibres contract and relax in a disorderly way so as to minimise the nett movement of the limbs. Initially, before fully developed shivering occurs, fasciculation of certain muscles (e.g. pectoralis major or quadriceps femoris) may occur. In fully developed shivering, agonists and antagonists are brought into action, thus reducing nett mechanical work. This reduction in mechanical work enables a larger proportion of the fuel energy to be converted into heat. Thus it is the opposite to that desired in heat engines, where one seeks to minimise the proportion of energy lost as heat.

Shivering is a cumbersome and uncomfortable way to produce heat. It would be preferable for the energy of food to be converted directly into heat by exothermic chemical reactions, which would avoid unwanted mechanical work that impairs the precision of other muscular activity. In addition, there is increased circulation to the active muscles and hence increased heat loss by conduction and radiation from the overlying skin. Furthermore, shivering stirs the layer of air next to the skin, so that heat is lost by convection. This is probably of much more significance in the nude person, the chief function of clothing being to reduce convection of air next to the skin.

A combination of these factors renders the efficiency of shivering very low. In experiments involving acute cold exposure of unacclimatised men, the mean total decrease in body heat content was 222 Cal. From increased carbon dioxide in expired air, it was calculated that shivering produced 28 Cal of heat. Therefore, had there been no shivering, heat loss would have been 250 Cal. Thus efficiency of shivering in protecting against heat loss is only 11% (Horvath et al. 1956). In the same study, it was found that the first tremors began at an average skin temperature of 29.2°C and generalised shivering at 27.1°C. The first tremors occurred before any fall in rectal temperature. In fact rectal temperature increased during a cold stress of −3°C. On the other hand, Carlson and Hsieh (1965) believe shivering occurs when the deep body temperature drops below a critical level. Observations on the Nepalese pilgrim (Pugh 1963) showed that on one occasion he shivered under similar conditions to those of a previous occasion when he had not shivered.

He began shivering when his rectal temperature fell from 36.50°C to 36.38°C, which was lower than on the previous occasion.

Cold-climate indigenes generally shiver less than Caucasian controls, and begin shivering at a lower temperature. This has been found in the Australian Aborigine (Scholander et al. 1958b, Hammel et al. 1959), in Lapps (Andersen et al. 1960), and in Ama women, where the water bath temperature to induce maximum vasoconstriction without shivering was 30°C or less for Ama women and 33 to 34°C for controls (Rennie et al. 1962).

Puerto Ricans living in a cold climate showed a reduced shivering response to a cold stress than those living in Puerto Rico (Newman 1968). Wyndham and Loots (1969) found men in Antarctica shivered less during a cold stress later in the year than they did just after arrival. Budd and Warhaft (1966a) found no change in the shivering response to a cold stress in Antarctica compared with that in Australia. In artificial cold-chamber acclimatisation, shivering became less in response to the cold stress (Davis 1961).

Contrasting with these findings are those of Irving et al. (1960). They reported that, during a cold night, Arctic Indian men shivered just as much as Caucasian controls, but slept more soundly. Caucasians and Eskimos both shivered when the average skin temperature reached 29.5°C, but Negroes did not shiver until it reached 28°C (Adams and Covino 1958). Meehan (1955a) found Eskimos shivered more than Caucasians in a 90 minute cold stress at 6°–7°C.

Oxygen consumption

The metabolic response necessary to maintain thermal balance or minimise changes from the basal level during a cold stress requires increased oxygen consumption. The amount of heat produced can then be calculated from the measured oxygen uptake. Allowance for subjects being of different size can be made by quoting the metabolic rate per square metre of surface area. This allowance is important when comparing different subjects, especially when they are of different races that may differ considerably in size.

The findings in the works cited above is that the metabolic rate generally increased most in subjects who showed the greatest shivering response, which is to be expected from the increased energy consumption of the shivering muscle fibres. On the other hand, Ama women who did not shiver under the conditions of the experiment had a higher metabolic rate than the controls, and this difference was more pronounced in winter (Rennie et al. 1962, Hong et al. 1969).

Reactions of clothed subjects to ambient temperatures of −5° to −17°C showed that at any given skin temperature, the heat production by metabolism was less in the cold-adapted than in the warm-adapted person. The cold-adapted person kept the extremities warmer, but called less on metabolism (Carlson et al. 1953).

It has been observed that in both men and women the metabolic response to cold is inversely related to body fat (Buskirk et al. 1963), but the metabolic response of Caucasian females to cold when corrected for surface area was the same as that of male Caucasians, although the women were fatter than the men (Wyndham et al. 1964b).

The metabolic rates of Bushmen and Bantu were similar when corrected for surface area. Both were higher than that of Caucasians at 27°C and 5°C, but the metabolic response was not significantly different between the three groups at

intermediate air temperatures (Wyndham et al. 1964a). Meehan (1955a) found that the basal metabolic rate of Eskimos was not significantly higher than that of Caucasians, but Eskimos showed a greater metabolic response to a cold stress of 6° to 7°C for 90 minutes than did the Caucasians.

In comparing Eskimos, Caucasians and Negroes, Adams and Covino (1958) found Eskimos had a higher basal metabolic rate than Caucasians, who in turn had a higher basal metabolic rate than Negroes. The metabolic responses of Eskimos and Caucasians to a cold stress of 17°C while nude were similar and were greater than that of Negroes. During light work, heat produced by Eskimos was no higher than that of Caucasians (Andersen et al. 1963).

Aborigines of Central Australia are accustomed to sleeping on the ground unclothed between two fires. Air temperature at night falls to about 0°C in the winter. The Aborigines showed little or no metabolic response and slept soundly, but showed pronounced rectal and skin cooling. Caucasian controls exhibited bouts of shivering and their metabolic rate averaged 30% above basal (Scholander et al. 1958b).

Another expedition in the summer showed that the responses of Aborigines and Caucasians were still different. The response of tropical Aborigines was intermediate between that of Central Australian Aborigines and Caucasians. The similarity of the pattern of response in summer and in winter suggests that the Aborigines' response to cold is due to an inborn ability to tolerate cold. As this was more pronounced in the Central Australian Aborigines than in the tropical Aborigines, it was thought that prolonged exposure to cold enhanced this inherent tolerance to cold (Hammel et al. 1959). The metabolic rate of nomadic Lapps during a cold night was close to basal; it increased in village Lapps and was greatest in controls (Andersen et al. 1960).

When the Nepalese pilgrim was sitting in cold air, his metabolism was 35% above the expected level, but without any visible shivering (Pugh 1963). This was thought to indicate the presence of non-shivering thermogenesis (NST). The progressive increase in his metabolism to 2.7 times the calculated basal level was associated with moderate sustained shivering, which did not give discomfort, in contrast to the vigorous paroxysmal shivering and fluctuating metabolism shown by unacclimatised subjects.

Young Caucasian males acclimatised to cold over a period of 6 weeks had a higher metabolic rate (50–55% above basal) than non-acclimatised controls, whose metabolic rate was 30–35% above basal. These metabolic rates were determined while both groups were resting at night, the former sleeping but with bouts of shivering. The controls could not sleep and were restless due to cold periphery and bouts of shivering, yet their metabolic rate was lower. Exercising on a bicycle ergometer just vigorously enough to maintain rectal temperature in an ambient temperature of 5°C whilst wearing only shorts and socks, the cold-acclimatised men used just as much oxygen as the controls (Scholander et al. 1958a).

In Caucasians dwelling temporarily in polar regions, their basal metabolic rate did not alter over a 2-year period in the Arctic, nor was any seasonal variation apparent (Lewis et al. 1961). Although their basal metabolic rate in Antarctica did not change, their metabolic response to a cold stress of 17°C for 2 hours whilst nude diminished over the first 3 months, and was lowest in winter (Milan et al. 1961).

Wyndham et al. (1964c) found that the metabolic rate increased with decreasing skin temperature, though to a much lesser extent in Antarctica than in Johannesburg. At an ambient temperature of 27°C the metabolic rate did not change in Antarctica. However, after 1 hour at 5°C, the metabolic rate was lower in Antarctica than it had been at this temperature before the subjects went to Antarctica, and was lower than that of a control group who remained in Johannesburg.

Wyndham and Loots (1969) also observed this reduced metabolic response to a cold stress in Antarctica, but only in thin men. The fat men showed only a small increase in metabolism with a fall in air temperature, and there was no trend over the period spent in Antarctica, although the metabolic response was slightly reduced just after arrival in Antarctica. Thin men showed a sharp increase in metabolic rate when the air temperature fell below 20°C soon after arrival in Antarctica, whereas after a year in Antarctica the sharp increase in metabolic rate occurred only after the air temperature dropped below 15°C.

Budd (1964) did not find any significant differences between the warm-phase oxygen consumption in Antarctica and that in Melbourne. In the warm phase the subject lay wrapped in blankets at a 'comfortable' temperature. In the standard cold stress at 10°C, the average oxygen consumption during the first half hour of the exposure was lower in Antarctica than in Melbourne. However, of the four subjects, two showed a small decrease in oxygen consumption throughout the study, but the other two did not.

In cold-chamber acclimatisation experiments conducted in summer and winter (see 3.1), no difference was found in the basal metabolic rate of the winter and summer groups. Cold-elevated metabolism did not decrease in the winter group, but this group showed a highly significant decrease in shivering as measured electromyographically. Davis (1961) concluded that this showed the existence of NST in adult men, which he related to winter conditions. Another investigation into the effects of artificial cold exposure (see 3.4) of shorter duration but colder conditions than in Davis's study, found no evidence of NST (Keatinge 1961).

3.2.1 Aim

The aim of this part of the investigation was to see whether: skinfold thickness and bodyweight were related to skin temperature and rectal temperature patterns during a standard cold stress; onset of shivering due to a cold stress was related to skinfold thickness, bodyweight, skin temperature and rectal temperature; oxygen consumption was related to a cold stress and shivering; any fluctuations in bodyweight were due to changes in subcutaneous fat; any changes in the pattern of these parameters could be attributed to acclimatisation.

3.2.2 Methods and materials

Body weight

Each subject was weighed before reclining for the warm phase preceding the cold stress. The subject, wearing light underpants, stood on the platform of a beam balance and counter balances were added to the nearest kilogram, then a small weight was slid along the calibrated beam to give his weight to the nearest gram.

Skinfold thickness

The skinfold thicknesses were measured just below the left scapula over the triceps, halfway between acromion and olecranon, by means of Harpenden skinfold calipers.

A large proportion of the body fat lies in the subcutaneous tissues which, in many parts of the body, are only loosely attached to the underlying tissues and can be picked up between the thumb and forefinger to form a fold, the thickness of which depends on how much subcutaneous fat is present. The measurement depends on how the skinfold is picked up and upon the design of the calipers. If the jaws of the calipers are approximated by a spring, the reading will depend on the strength of the spring, since the tissue is compressible.

In evaluating different types of skinfold calipers, it was found that reproducibility of results depended greatly on the pressure exerted by the caliper faces on the skinfold. The best reproducibility was obtained with a working pressure between 9 and 19 g/mm^2. Even so, variation of pressure within this range gave great differences in readings. Therefore, calipers that produce a constant pressure at all jaw openings were required. The effect of face area on reproducibility was negligible, but an area of 90 mm^2 was found to be practicable. Too small an area caused discomfort to the subject.

The most suitable calipers were ones made by British Indicators Ltd for measuring the thickness of wood, metal and leather. These calipers were used in the Harpenden Growth Study and were subsequently known as the Harpenden Skinfold Calipers. This instrument has an accuracy of 0.1 mm. The differences between duplicate readings were found to be proportional to the mean value. A transformation equation: $x = 100 \log$ (reading in 0.1 mm $-$ 18) was suggested to overcome this non-normal frequency distribution of results having a long tail to the right (Edwards et al. 1955).

The transformed skinfold thicknesses were derived from tables supplied by the manufacturer of the calipers. This table was prepared from the above formula.

No skin calipers can give accurate results unless the skinfold is picked up in a standard fashion. The thumb and forefinger of the left hand should be placed just sufficiently far apart so that a full fold is pinched up firmly and held between the fingers all the time the measurement is being taken. The calipers are applied to the fold a little beyond the fingers so that the pressure on the fold at the point measured is exerted by the caliper faces and not by the fingers. The dial of the Harpenden Calipers is then read to the nearest 0.1 mm. Above 20 mm the value registered may gradually decline. This can usually be stopped by a firmer pinch with the left hand and, if that fails, the reading should be taken immediately the calipers are applied. The sites selected should be ones where a proper skinfold can be raised. Tanner (1959) advocates over the triceps, midway between the acromion and the head of the radius. In the present work, the olecranon process of the ulna, rather than the head of the radius was chosen as the lower point of reference because it is a more conspicuous landmark. The other site favoured by Tanner is just below the angle of the left scapula. This site was used in the present work.

Most surveys have utilised these two sites, though if only one site is to be measured, Tanner suggests that the skin over the triceps should be used. In the present work, as subcutaneous fat over the trunk was deemed to be of considerable potential importance, the subscapular site was used as well. Measurements were

made on the left side. Tanner states that, by international agreement, all anthropometry has been carried out on the left for 'the last 70 years', as of publication of his paper in 1959.

In the present work, skinfold thicknesses were measured in triplicate at each site in each series and the mean obtained. The Harpenden skinfold calipers used were constructed to exert a constant pressure of 10 g/mm^2 over the faces at all jaw openings.

Arm circumference

Arm circumference was measured with a flexible, non-extensible tape measure, midway between acromion and olecranon on the left arm.

Shivering

Ideally, shivering should be assessed quantitatively by electromyography. This apparatus was not available, so shivering activity was quantified by recording the time at which shivering began during the cold stress. Attempts at assessing intensity are fraught with error, as the observer cannot truly compare intensities observed at three-monthly intervals.

Pilo-erection (gooseflesh) and fasciculation of muscle bundles were disregarded. The time at which shivering of a muscle group, even if mild, was considered to have begun was the onset of shivering.

Oxygen consumption

Oxygen consumption was measured by a Sanborn Metabulator, with the subjects resting at the end of the warm phase and again at the end of the cold phase. In this instrument a bellows is completely compressed to exclude air and is then filled with oxygen. The subject breathes this oxygen, the fluctuations in the bellows due to inhalation and exhalation being recorded on pressure-sensitive paper on a rotating drum. Exhaled carbon dioxide is absorbed by soda lime; thus as the oxygen is used the bellows gradually collapses. The rate of oxygen utilisation can then be determined from the slope of the oscillating line drawn by a pressure point connected to the bellows. Calibrated rulers are supplied with the instrument for this purpose.

Gas leakage was prevented by a mouthpiece with a rubber flange between the lips and cheeks on the outer side and the gums on the inner side. On the inner surface are rubber projections, which the subject gripped with his teeth. In this way an air-tight seal is secured. In earlier work (Budd 1964), difficulty was experienced with gas leaks around face masks when worn by bearded subjects.

The drum was rotated at constant speed by a synchronous motor designed to operate on 110 volt 60 Hz. The station power supply was to Australian standards of 240 volt 50 Hz. The Metabulator was operated with a step-down transformer, but the lower frequency caused a slower rotation of the drum, with a consequently steeper oxygen-consumption curve. The results were therefore corrected by taking 5/6 of the measured value.

Actual oxygen consumption was recorded rather than metabolic rate, because the point of interest was to observe change in oxygen consumption due to cold stress in each particular subject. His surface area would not appreciably change

over the duration of the 2-hour cold stress, nor change much over the whole year. Furthermore, introduction of derived values may obscure changes in oxygen consumption (Budd 1964).

3.2.3 Results

Skinfold thickness, body weight, and arm circumference

The thicknesses of the scapular and triceps skinfolds, body mass and arm circumference, respectively, are shown in Tables 6a, b, c, d. Omitting Subject 2, who deliberately reduced weight, the transformed scapular skinfold thickness and body mass increased linearly, with slopes of 0.81 and 0.23 respectively.

Regression analysis of the transformed triceps skinfold showed that, although each subject adapted differently, it tended to increase. The triceps skinfold was at its minimum in the March series, but the arm circumference showed no reduction in that series and was greater ($P<0.01$–0.005) in the later Antarctic series than in January (Table 6d).

The rectal temperatures during the cold stress at 60 and 120 minutes were unrelated to the scapular skinfold thickness, but whereas the skinfold showed a random scatter in the January series, in the later series the subjects coalesced into two groups: one with medium and the other with thick subcutaneous fat (Figures 22 and 23). Likewise the change in rectal temperature from the pre cold-stress level was unrelated to skinfold thickness beneath the scapula. The chest-skin temperature tended to be inversely related to the scapular skinfold thickness (Figures 24 and 25). No relationship was found between index finger temperatures and transformed scapular and triceps skinfold thicknesses. Likewise, toe-skin temperatures were unrelated to the transformed scapular skinfold thickness.

Onset of shivering

The time elapsed in the cold chamber before shivering was established was later in the November series than in January ($P<0.05$) or March ($P<0.02$) (Table 7a). After the March series, the delay in the onset of shivering continued to increase, but regression analysis showed significant differences between subjects.

(a)	Mean skin-fold thickness — Scapular (mm)							
	Jan.	Mar.	Apr.	Jun.	Sep.	Nov.	Follow-up	Control
	13.1	14.6	16.6	14.9	15.2	16.6	17.9	12.2
(b)	Mean skin fold thickness — Triceps (mm)							
	8.9	6.2		6.5	8.3	8.2	9.4	9.3
(c)	Mean total body mass (kg)							
	76.5	81.0	85.3	81.4	81.5	81.7	82.4	76.1
(d)	Mean arm circumference (mm)							
	29.7	30.4	31.7	31.3	32.2	32.3	32.0	30.1

Table 6. Mean skin-fold thickness (scapular and triceps), total body mass and arm circumference of the cold-stress group

	Jan.	Mar.	Apr.	Jun.	Sep.	Nov.	Follow-up	Control
(a) Onset of shivering (minutes after entry into cold chamber)								
Mean	32	25	40	33	42	56	37	38
(b) Rectal temperature (°C) at the onset of shivering								
Mean	37.4	37.3	37.3	37.3	37.3	37.2	37.2	37.3
(c) Changes in rectal temperature (°C) from basal value to that at the onset of shivering								
Mean	−0.01	−0.01	0.08	0.02	0.06	−0.01	0	−0.04
(d) Chest-skin temperature (°C) at the onset of shivering								
	30.3	31.0	30.1	30.2	29.3	29.2	29.1	29.6

Table 7. Mean chest-skin and rectal temperatures at the onset of shivering

The rectal temperature at the onset of shivering (Table 7b) and the change in rectal temperature from the basal value to that at the onset of shivering (Table 7c) showed no seasonal pattern, but there was pronounced individual variation. The chest-skin temperature at the onset of shivering (Table 7d) was significantly different between individuals, but decreased linearly with a slope of −0.23 in Antarctica.

The follow-up series and the control group gave similar results to those of the January series.

Oxygen consumption

The oxygen consumption in mL/min was greater in the cold phase than in the pre-stress warm phase, but of the main series this increase was significant only in March ($P<0.001$) and November ($P<0.05$). Regression analysis showed no time trend in the cold-phase oxygen consumption, but the change from warm- to cold-phase oxygen consumptions showed a quadratic trend, with all subjects adapting in the same way. The minimal response was in midwinter, followed by a moderate increase towards the end of the year (Figure 26).

3.2.4 Discussion

Skinfold thickness, body weight, and arm circumference

Omitting Subject 2, who deliberately lost weight during the year, the bodyweight and scapular skinfold thicknesses both increased linearly, indicating that the increase in bodyweight was largely due to an increase in adipose tissue. The subcutaneous fat is a measure of the total body fat; in fat people about two-thirds of the excess adiposity is located subcutaneously (Allen et al. 1956). Variations in fat content were thus far more important than variations in muscle or water content in influencing changes in bodyweight.

The decrease in skinfold thickness over triceps in the March series without a corresponding decrease in arm circumference may have been due to lifting and carrying heavy crates while settling-in during the preceding month. The findings suggest some loss of subcutaneous fat over the working muscles, with muscular

hypertrophy compensating for the loss of fat. Nevertheless, the work, which involved much lifting, would have led to use of Latissimus dorsi and Infra-spinatus muscles, yet the overlying subcutaneous fat did not show diminution. In the other series, the skinfold thickness changed in the same way at both sites, except for Subject 19 in whom the skinfold over the triceps became less, while increasing over the scapula. It would therefore be undesirable to measure the skinfold thickness only over the triceps, as Tanner (1959) advocated.

The chest-skin temperature after a 2-hour cold stress tended to vary inversely with the skinfold thickness over the trunk, and in accord with previous observations (Baker and Daniels 1956). The absence of a correlation of rectal and peripheral skin temperatures with the thickness of subcutaneous fat is at variance with the findings of Baker and Daniels, who reported the minimal rectal temperature on cold exposure was proportional to, and minimal calf-skin temperature inversely proportional to, the percentage of body fat. Their observations were made at 15°C and temperatures were plotted against percentage body fat rather than skinfold thickness, which could conceivably give a different response pattern. The cooler skin of women exposed to cold air compared to that of men is deemed to be due to their thicker subcutaneous fat (Wyndham et al. 1964b).

Unlike the subjects studied by Wyndham and Loots (1969), in which only the thin men became fatter, in the present study both thin and fat men showed an increase in fatness. A feature of the scapular skinfold thickness is that in the pre-Antarctic and follow-up series of the Casey group and in the control group, there was a random scatter of skinfold thicknesses (Figure 22). However, in the Antarctic series the individual skinfold values tended to coalesce into a medium-thickness group and a fat group (Figure 23), this polarisation becoming more pronounced in the latter half of the year. This effect was due to the thinner subjects having a relatively greater increase in skinfold thickness, thus coming closer to the medium group. The fat subjects also showed an increase in skinfold thickness in Antarctica, so these subjects formed a small cluster away from the main group. The major exception was Subject 2, who moved from the obese to the medium-fat group.

The decline in rectal temperature varied inversely with skinfold thickness in the April series, the opposite to the findings of Baker and Daniels, who, however, studied only four subjects. The pattern of the scatter was very much influenced by the presence of Subject 11 who, though fat, showed a decrease in rectal temperature, and Subject 16 who, though fairly lean, showed a good rectal temperature maintenance. In the main series, however, there was no relationship between rectal cooling and scapular skinfold thickness. These findings do not support the view that, after a standard cold stress, the lower rectal temperature of Bushmen and Bantu than Caucasians was due to the thinner layer of subcutaneous fat in the first two groups (Wyndham et al. 1964a). Bushmen and Bantu had average skinfold thicknesses of 4.75 and 5.25 mm, respectively, and Caucasians 7.71 mm. Taking the March series for example, the leanest Australian had a skinfold thickness over triceps of 3.0 mm, and over the scapula the thinnest (another subject) was 7.6 mm. Most were considerably fatter, being up to 9.4 mm over triceps and 21.7 mm over scapula. Thus even the relatively lean Australians had thicker subcutaneous fat than Bushman and Bantu subjects. It may be that there is a critical level of skinfold thickness below which the amount of fat is of major importance in insulation. Within the range of thickness of subcutaneous fat possessed by the present group,

Fig. 22

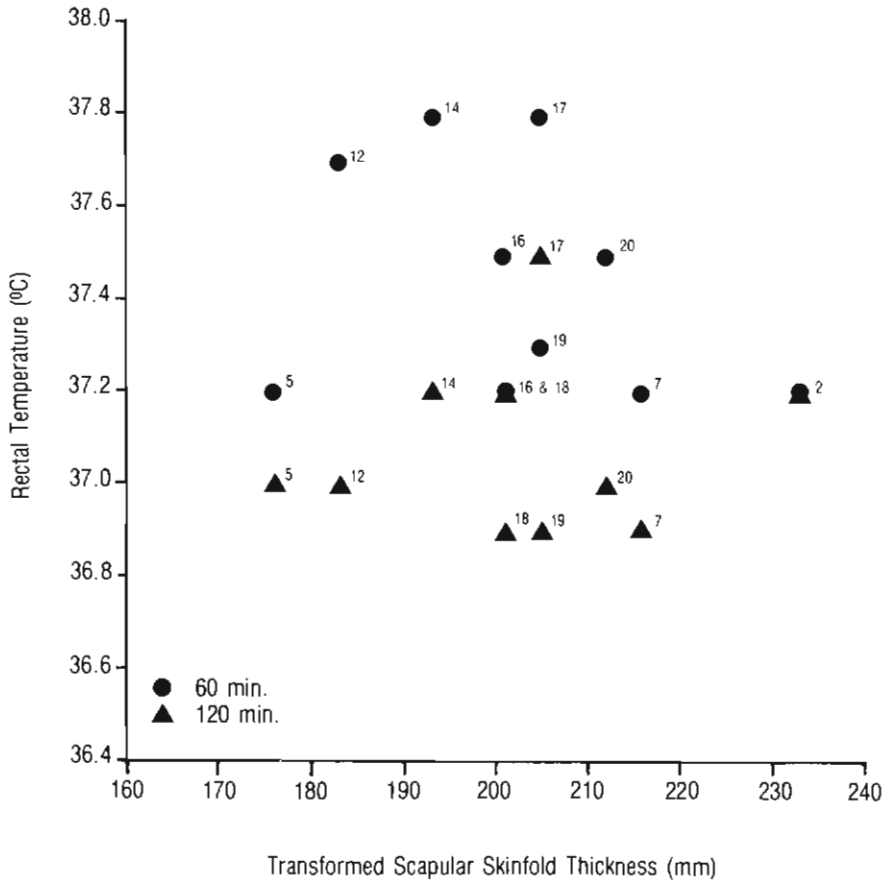


Figure 22. Rectal temperature vs. transformed scapular skinfold thickness at intervals of 60 and 120 minutes, January

Fig. 23

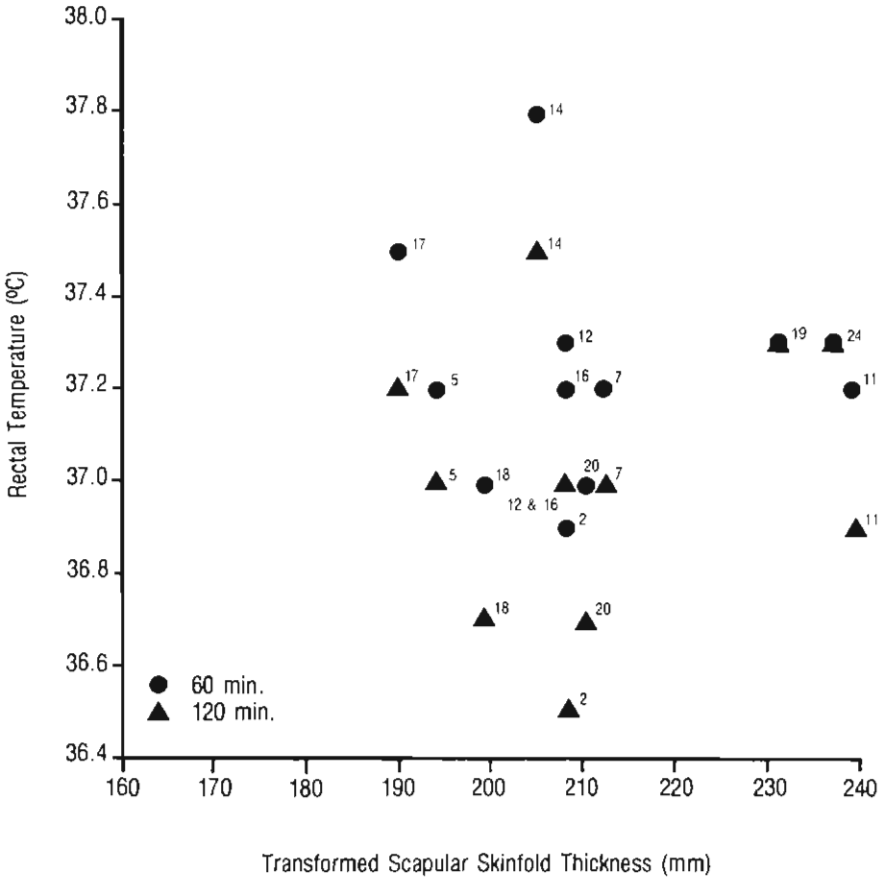


Figure 23. Rectal temperatures vs. transformed scapular skinfold thickness at intervals of 60 and 120 minutes, November

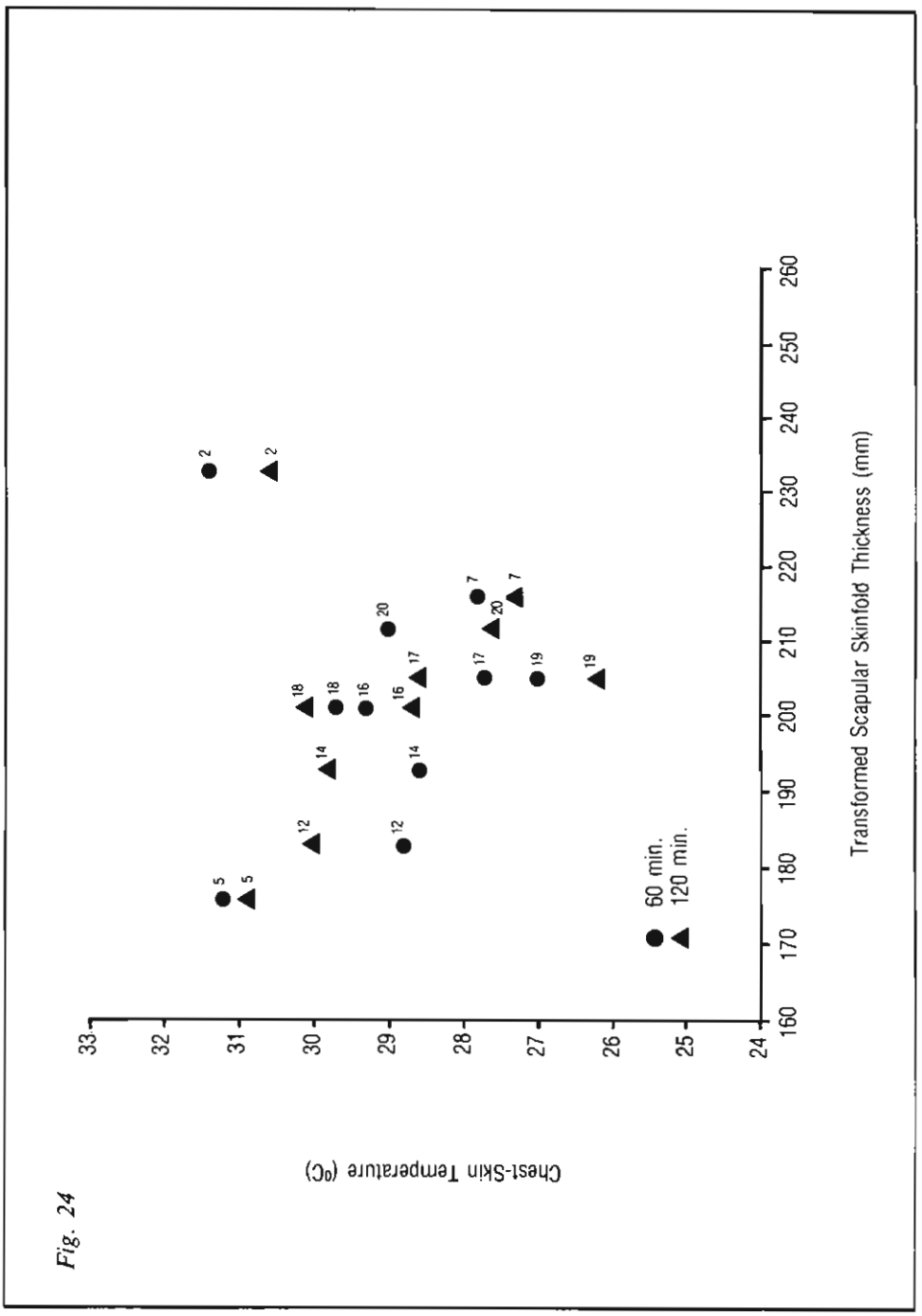


Figure 24. Chest-skin temperatures vs. transformed scapular skinfold thickness at intervals of 60 and 120 minutes, January

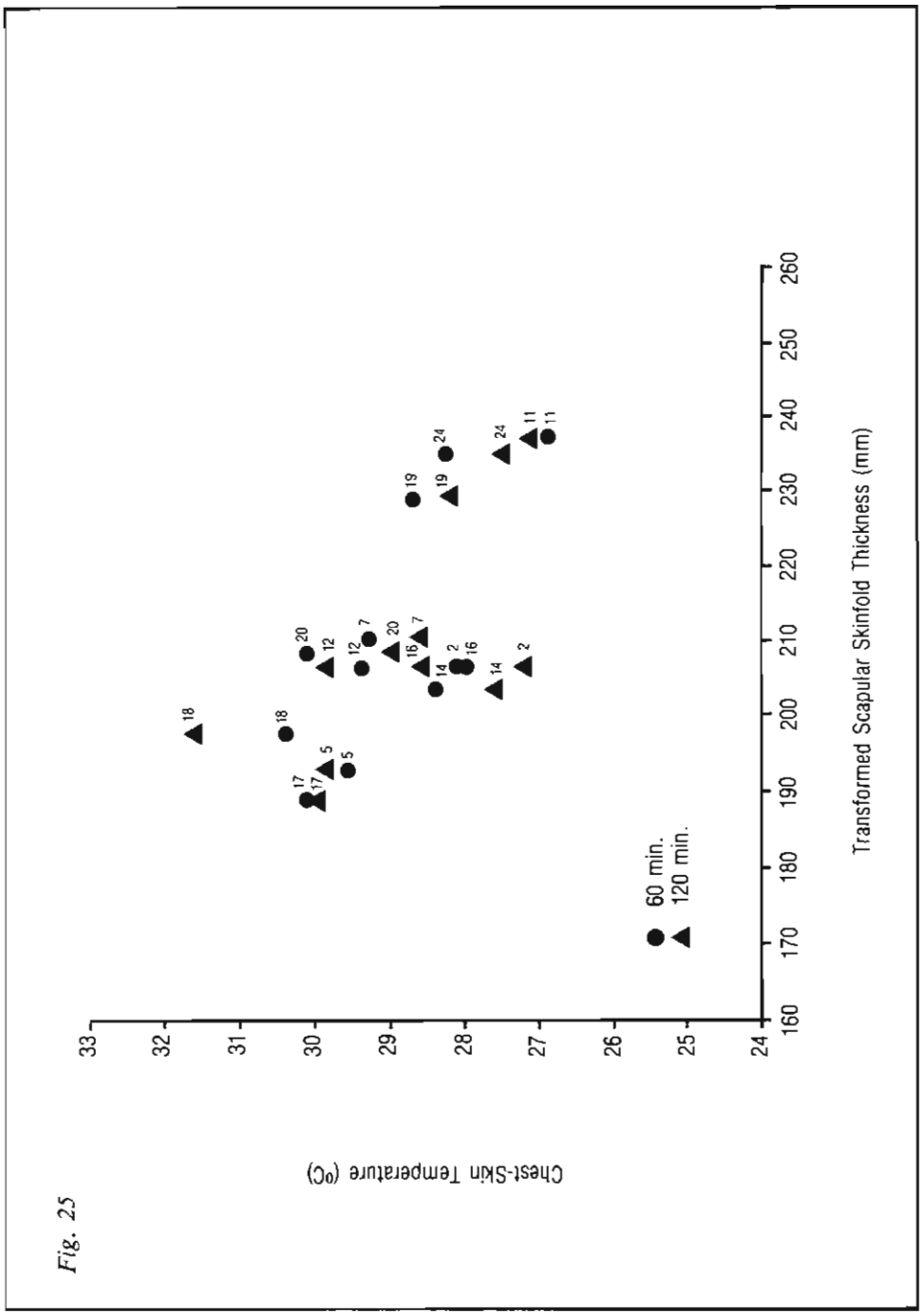


Figure 25. Chest-skin temperatures vs. transformed scapular skinfold thickness at intervals of 60 and 120 minutes, November

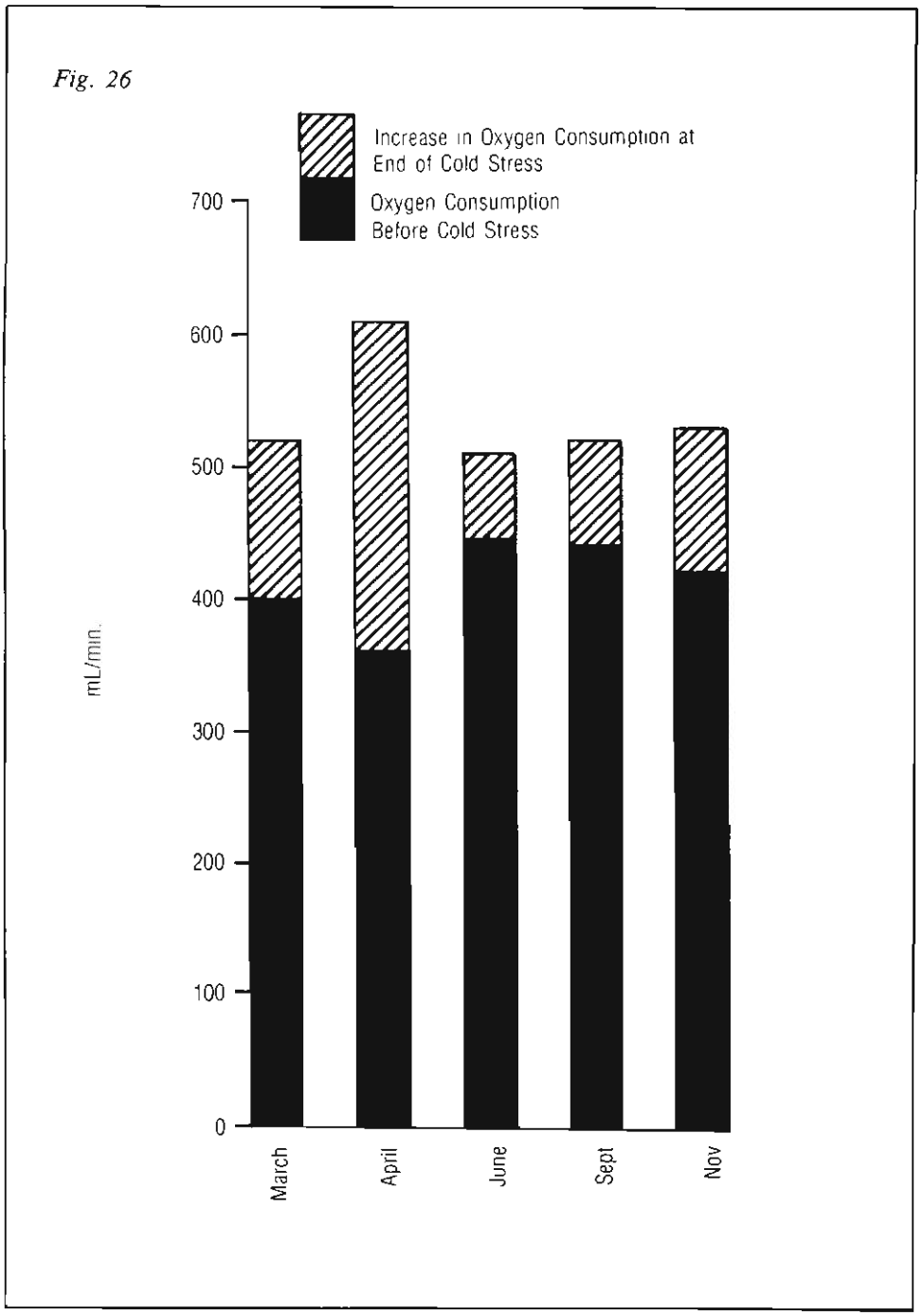


Figure 26. Oxygen consumption (mL/min) before cold stress and at the end of cold stress in the Antarctic series

subcutaneous fat was less important than total tissue mass in determining core temperature maintenance.

The changes in response patterns during the year in Antarctica do not show two separate categories for lean and fat men as observed by Wyndham and Loots (1969). They found that only in lean men was there an improvement in rectal temperature maintenance the longer they stayed in Antarctica. The mean skin temperature became lower after 1 hour at various cold temperatures, including 10°C, but at 20°C the rectal temperature was lower at the end of the Antarctic year, which pattern is in accord with the present findings. The numerical values for the lean men were much lower than in the present work, though they gained an average of 3 kg over the year.

In a study comparing cold-stress reactions of men in wet-cold subantarctic conditions on Heard Island, with previous temperate climate reactions, it was found that the rectal temperature did not decrease as much in the subantarctic series as in the pre-expedition test, even though the skinfold thickness had decreased (Budd 1965). The individual variation of the small number of subjects (4) makes it difficult to reach a firm conclusion.

Weight increases have been observed previously in Antarctica (Wyndham et al. 1964c, Budd 1964), but the most likely explanation for the increase in fat and weight observed in the present work appears to be the ample supply of food with no increase (and probably a decrease) in average energy output compared with pre-Antarctic living conditions. On field trips the use of diesel-powered vehicles caused little increase in energy output compared with that at the station, and was very much less than that required on dog-sledging expeditions. Furthermore, much more food can be carried in the diesel vehicles than on dog-sledges.

The thickness of subcutaneous fat does not appear from the present results to be of much importance in acclimatising to cold air, even though the conductivity of fat is much less than that of muscle (Hatfield and Pugh 1951). Fat seems to greatly enhance the ability to endure immersion in cold water (Pugh and Edholm 1955, personal observations). It may not be of crucial importance if changes can be induced in vascular dynamics, such as an improved counter-current heat exchange between limb arteries and venae comitantes. This appears to be the case in Ama diving women, who are not fatter than other Korean women and are, on average, thinner than American women of comparable age (Hong et al. 1969). However, it is thought that after 2 hours of cold exposure, insulation is proportional to the amount of body fat (Buskirk et al. 1963). This would explain the advantage of fatness to long-distance swimmers, who remain immersed for much longer than do the Ama diving women. Pugh and Edholm (1955) consider the fat of long-distance swimmers is chiefly subcutaneous, so the anatomical location may be more important than the total amount of fat. Most experimental studies have been for less than 2 hours, but in the present investigation the cold stress was of 2 hours' duration exactly. Fat ought, therefore, to have had some influence, but the findings indicate that other factors were more important.

Subject 2 maintained his rectal temperature well when he was obese, but after his skinfold thickness was reduced when he lost weight, his rectal temperature maintenance deteriorated. Other subjects who were leaner, and always had been, showed an improved rectal temperature maintenance in Antarctica. It is postulated that the total insulation is a combination of passive insulation, due to subcutaneous

fat, and the active effect of alteration in vascular dynamics. Possibly the fat person depends chiefly on subcutaneous fat for insulation; if it is lost at a faster rate than the vascular system can adapt to, he will be at a disadvantage compared to the lean person whose vascular dynamics have already acclimatised.

The deterioration of rectal temperature maintenance of Subject 18 in the September series may be related to migraine. The cold-stress test would not have been performed had this been known beforehand. Apart from the unpleasantness to the subject, the alteration in vascular dynamics introduced an additional factor to the experiment.

Onset of shivering

The time of onset of shivering began later in Antarctica for all but five subjects, i.e. 50% of those tested before going to Antarctica showed an earlier onset of shivering in the first Antarctic series than in the pre-Antarctic series, and another two subjects shivered promptly (10 minutes) in both the pre-Antarctic and March series. Subject 20, who shivered after 40 minutes in the pre-Antarctic series and started shivering immediately in the March series, complained that the cold stress felt colder than in the pre-Antarctic series. Other subjects did not notice much subjective difference except Subject 2, who found the cold stresses subsequent to the pre-Antarctic series a little less unpleasant even after considerable loss of subcutaneous fat.

Some degree of suppression of shivering has been observed in cold-climate indigenes (Hammel et al. 1959, Scholander et al. 1958a and b, Andersen et al. 1960, Rennie et al. 1962, Pugh 1963), in cold-chamber acclimatisation (Davis 1961), and in a cold-stressed group in winter compared with their response in summer (Davis and Johnston 1961). In contrast, the shivering response of Caucasians was less than that of Eskimos (Meehan 1955a), but was found to be similar to that of Eskimos (Adams and Covino 1958) and Arctic Indians (Irving et al. 1960). No reduction in shivering response was found in cold-chamber acclimatisation (Keatinge 1961) or after living in Antarctica (Budd 1964). Bushmen and Bantu accustomed to living under fairly cold conditions shivered more than Caucasians in response to a cold stress (Wyndham et al. 1964a).

These conflicting results may be due to differing degrees of acclimatisation. Some cold-climate indigenes may have been more skilful than others in avoiding cold exposure. Suppression of shivering appears to be a slow process, as it was not until the final Antarctic series that the onset was significantly later than in the first Antarctic series. Furthermore, the shivering responses for individual subjects fluctuated considerably from series to series. In some, the onset of shivering began earlier in later Antarctic series. This suggests that the mechanism for suppressing shivering was rather tenuously established and was easily affected by varying amounts of cold exposure of the particular subject. Shivering showed an increased mean delay in the April series in the four subjects who had recently returned from the more severe conditions experienced in the field, but the sample was too small for the difference to be significant.

The delay in shivering did not appear to be related to fatness or size, pronounced suppression of shivering occurring in fairly tall subjects of medium build. Subjects 5 and 17, who were of lean, linear build, showed prompt shivering throughout the investigation. Subject 2 did not conform to the overall pattern, in that he shivered

promptly when fat, but started shivering considerably later in the November series when he had become much thinner. Much individual variation in delay in onset of shivering has been previously observed (Budd 1966).

The onset of shivering was influenced neither by the rectal temperature nor the change in rectal temperature from the basal value to that at the onset of shivering. In some instances the rectal temperature was increasing when shivering started.

The April series shows that delayed onset of shivering was associated with improved rectal temperature maintenance. The subjects had returned from a month in the field just before testing. It appeared that shivering was very largely supplanted by other mechanisms of thermo-regulation. Nevertheless, this association may have been to some extent fortuitous, as only four subjects were involved. Of these, one (Subject 16) had a considerably delayed onset of shivering and his rectal temperature increased over the 2-hour cold stress. Subject 20 showed prompt shivering and a moderate fall in rectal temperature. The other two subjects did not show a remarkably different response pattern from earlier series.

The chest-skin temperature became cooler as the onset of shivering became later. This conflicts with the idea that shivering is activated when the skin temperature drops below a critical level (Horvath et al. 1956).

The later onset of shivering at the end of the year, when the peripheral skin was warmer than earlier in the year, is in contrast to the lack of shivering observed in Australian Aborigines whose peripheral skin showed pronounced cooling (Scholander et al. 1958a, Hammel et al. 1959), and American Negroes, in whom shivering was delayed. The Negroes' core and periphery had a higher cooling rate than that of Eskimos, who shivered vigorously (Adams and Covino 1958). The observed change in the shivering pattern also differs from that of Bushmen and Bantu, who shivered vigorously yet whose core cooled more rapidly than that of Caucasians (Wyndham et al. 1964a).

Le Blanc (1956) suggested that cold-acclimatised people shivered less than non-acclimatised when exposed to a cold stress because the body's thermostat [was reset] at a lower level' (LeBlanc 1956). The lower body temperature of the elderly has been attributed to the same cause (Ward 1975). Experiments with rodents, however, suggest that this reduction of shivering is due to blood warmed by NST keeping the shivering centre located in the cervical spinal cord above the temperature at which it activates shivering (see 6.1 Neural Integration of Thermogenic Mechanisms).

Oxygen consumption

The change in oxygen consumption due to the cold stress showed a biphasic pattern, diminishing at first then increasing towards the end of the year. This change may be due to the cooler warm-phase temperature in the June series than in the other series. The greater oxygen consumption in the cold phase than in that in the warm phase represents the additional energy output to attempt to maintain body heat. Shivering was well established when the cold-phase oxygen consumption was determined; therefore, if NST was established, this component could not be distinguished from the heat derived from shivering. The absence of a time trend in the cold-phase oxygen consumption does not, therefore, exclude the possibility that NST was established. In those series in which Subject 11 did not shiver, his oxygen

consumption in the cold, did not increase, which suggests that NST had not been established. Subject 12, however, showed a pronounced increase in oxygen consumption in the cold not associated with shivering; NST may have been established in him. Subject 11 was much fatter than Subject 12, and probably reliance on passive insulation inhibited the development of NST.

The absence of significant changes in the oxygen consumption at the end of the cold stress while living in Antarctica has been observed previously (Budd 1964). The lack of significant variation in the resting, warm-phase oxygen consumption through the year is in keeping with the stability of the basal metabolic rate found over two years' observations in the Arctic (Lewis et al. 1961).

The metabolic response of both men and women to cold was thought to be inversely related to the percentage of body fat (Buskirk et al. 1963), but individuals paired for age, previous cold exposure and body fatness showed wide differences in metabolic response. Although women are on average fatter than men, the metabolic response of Caucasian females when expressed per square metre of surface area was the same at 5°C as the male response to cold exposure (Wyndham et al. 1964b). There were great differences in metabolic response not only between subjects but in the same subject between series. The response does not appear to be related to fatness.

Others have shown the metabolic response of cold-acclimatised Caucasians to a cold stress to be lower than that of controls (Davis and Johnston 1961, Milan et al. 1961, Wyndham et al. 1964d), and to become lower in thin subjects but on acclimatisation to remain the same in fat subjects (Wyndham and Loots 1969).

The oxygen consumption of cold-climate indigenes in response to various kinds of cold stress is more variable than in Caucasian controls: it can be lower (Andersen et al. 1960, Scholander et al. 1958a, Hammel et al. 1959, Irving et al. 1960), or higher (Wyndham et al. 1964a, Meehan 1955a, Adams and Covino 1958). This variation in response patterns may reflect differences in the experimental methods. Indigenes accustomed to cold may sleep in a sleeping bag without shivering, and hence without the concomitant increase in metabolism; Wyndham et al. (1964a) speculated that they might shiver if they were awake under the same conditions.

Carlson et al. (1953) considered the cold-adapted person kept the extremities warmer but called less on metabolism by the 'core' withdrawing during adaptation; they have a thicker 'shell' of cooler tissue which thus reduces the temperature gradient, and the cooled tissues themselves act as an insulator.

The metabolic response appears to be related to the severity of shivering induced by the cold stress, but an increased oxygen consumption in association with no shivering, or less than that of controls, has been observed in the Nepalese pilgrim (Pugh 1963), Ama diving women (Rennie et al. 1962, Hong et al. 1969), and cold-chamber acclimatised subjects (Davis 1961). This suggests that a level of cold-acclimatisation can be developed in which a mode of thermoregulation other than shivering becomes activated.

3.2.5 Conclusions

The slight increase in the bodyweight and subcutaneous fat of the subjects over the year appeared to be due to the abundance of food rather than a specific form of cold-acclimatisation. At a time of physical exertion, skinfold thickness of the upper

arm became less without reduction in circumference; probably reduction in fat was compensated by muscular hypertrophy.

The findings of this and several other studies do not support the concept that an increase in subcutaneous fat is a specific form of cold-acclimatisation. Improved insulation appears to be achieved by counter-current heat exchange between arteries and their venae comitantes: shunting of cooled blood through muscles reducing the shell temperature, while warmer blood directed to the skin vessels reduces the risk of cold injury to the skin. Total insulation is the combined effect of readjustments in vascular dynamics as well as the passive insulative effect of subcutaneous fat.

Oxygen consumption at the end of the cold stress showed no time trends. The onset of shivering began later in the later series, though there was pronounced individual variation. Shivering was not related to the rectal temperature or the change in rectal temperature. In many instances shivering started after an increase in rectal temperature.

There was a trend for delayed shivering to be associated with a lower chest-skin temperature. This may, as in guineapigs, be due to suppression of shivering during skin cooling by warming the spinal shivering centre with blood, warmed by thermogenesis other than shivering.

3.3 CATECHOLAMINE AND CORTISOL RESPONSES

Adult males, who were not cold-acclimatised, standing in 'light weight summer clothing' for 1 hour at 6.5°C showed increases in urinary excretion of noradrenaline and adrenaline. The adrenaline increase was the more pronounced. The rectal temperature did not decline significantly, but shivering occurred during the exposure period (Arnett and Watts 1960). Under a milder stress of 10–15°C for 1 hour, catecholamine excretion and rectal temperature increased (Suzuki et al. 1967).

A group of nine 'white' men aged 21 to 25 were exposed to an ambient temperature of 5 ± 2°C for 5 weeks, 8 hours per day, Mondays to Fridays. They were seated and wore socks, boots, cotton underpants and cotton boxer trunks. For the first week cotton singlets were also worn.

Two weeks before and 2 weeks after cold exposure, noradrenaline bitartrate was infused at the rate of 0.15 μg/kg/min for 20 minutes in a semidarkened room at 27 ± 2°C. The men lay nude and supine on a mesh cot for 16 hours post-prandial. The rectal temperature was uninfluenced by the noradrenaline, and the rectal temperature did not change following cold exposure. Skin temperature was higher after cold exposure but was not influenced by noradrenaline. Blood pressure, both basal and after noradrenaline, was lower ($P<.025$) after cold exposure. Bradycardia occurred after noradrenaline both before and after cold exposure. Minute and tidal pulmonary volume (but not rate) were increased by noradrenaline, but as much before as after exposure.

There was no significant difference in basal oxygen consumption before and after cold exposure, and infusion of noradrenaline caused no significant change in the basal metabolic rate before cold exposure. After cold exposure, however, noradrenaline infusion caused an increase ($P<.025$) in oxygen consumption, the average rise being 18 cc/min/m^2. All measurements returned to the basal level within 8 to 10 minutes after infusion (Joy et al. 1963).

A decrease in the vasopressor effect of noradrenaline infusion was observed in men wintering in Antarctica (Budd and Warhaft 1966b), though the degree of cutaneous vasoconstriction due to noradrenaline was the same in Antarctica as in Australia.

Noradrenaline was infused at the rates of 0.038, 0.075, 0.15 and 0.3 μg/kg/min. Blood pressure rose and heart rate fell in proportion to the dose of noradrenaline, but this response was much less in Antarctica. The finger temperature fell in proportion to the dose in three subjects. The response was unchanged in some subjects and increased in others, after acclimatisation.

Oxygen consumption was unaffected by noradrenaline infusion before going to Antarctica, but in the Antarctic series it was increased in three subjects in proportion to the dose infused. Leaking face masks due to beards, however, reduced the reliability of these results. The fourth subject had a high control value for oxygen consumption, but his oxygen consumption showed an upward trend with higher doses. Pulmonary ventilation was increased by noradrenaline infusion, this increase being slightly greater in Antarctica.

3.3.1 Aims

The aims of this part of the investigation were to observe changes in the urinary excretion rate of noradrenaline and adrenaline due to a standard cold stress and to see if residence in Antarctica altered the response of these catecholamines.

As cold-adapted rats require less cortisol to withstand a cold stress than do warm-adapted ones (Heroux and Hart 1954), plasma cortisol response to the standard cold stress was used as a marker to indicate objectively whether the subjects were cold-acclimatised.

3.3.2 Methods and materials

Catecholamines

In the case of cold-stress determinations, the entire volume of urine was used and made up to 250 mL with distilled water. The time over which this urine was excreted (2 hours during the cold phase) was used to calculate the excretion rate in μg/h. If the volume exceeded 250 mL then an appropriate fraction, (e.g. one half), was taken and the final result corrected arithmetically. Otherwise the methods were as for 24-hour catecholamines (see 2.3). For plasma collection see 3.1.I.

Determination of plasma cortisol

Two mL of plasma were added to 15 mL of dichloromethane (Matheson, Coleman and Bell, spectrograde quality) and shaken for 15 minutes to extract cortisol. The upper phase (plasma) was aspirated.

Ten mL of the cortisol extract were added to 5 mL of fluorescent reagent:ethanol (Merck spectroscopy grade) and sulphuric acid, 3:7 mixture, the ethanol being added slowly to sulphuric acid cooled by snowdrift (or in Melbourne, crushed ice) in a surrounding basin. Excessive heat will destroy the ability to produce fluorescence. Tubes were shaken for 20 seconds.

Dichloromethane was aspirated and the acid extract transferred to cuvettes.

The fluorimeter was set with excitation wavelength 480 mμ and analysing wavelength 530 mμ. Fluorescence was measured 18 to 30 minutes after mixing the fluorescent reagent with dichloromethane. The instrument was zeroed on a method blank using 2 mL of distilled water instead of plasma. The working standard was 25 μg/dl of cortisol (Ikapharm). Two mL of this was treated in the same way as for plasma. The results for plasma are given in terms of μg/dl. This method was essentially that of Mattingly et al. (1964).

3.3.3 Results

Table 2 summarises the physical characteristics of the subjects who participated in the cold-stress tests.

Noradrenaline excretion

The rate of noradrenaline urinary excretion was greater in the cold stress than in the pre-stress tests, but the cold phase excretion rate showed an overall decrease (Figure 27), being less ($P<0.025$) in the November than in the March series.

Regression analysis showed that while each subject differed in his noradrenaline response to the cold stresses, all their responses decreased linearly over the year (Figure 27) at about the same rate, the regression line having a gradient of -0.98.

Adrenaline excretion

The adrenaline excretion rate during the cold stress varied with individuals, but the rates all tended to increase linearly in Antarctica, with a gradient of 0.96 (Figure 27). The November mean was very significantly greater than that of the March series ($P<0.005$). The change in adrenaline excretion due to the cold stress (Figure 27), however, showed no differences between subjects or between series.

The catecholamine responses of the control group were similar to those of the January and follow-up series.

Plasma cortisol

The plasma concentrations of cortisol at the end of the cold stresses in Antarctica showed a quadratic regression, in which there were differences between individuals, but in all subjects the concentrations rose to a maximum in early August and then decreased (Figure 28). The values of the Antarctic series were, however, lower than in the January or the follow-up series ($P<0.05$).

Regression analysis of the changes in plasma cortisol in response to the cold stress did not indicate between-series or between-subjects differences in Antarctica, but the cold stress caused an increase in plasma cortisol concentration in the January and follow-up series, whereas it slightly decreased or was unchanged in the Antarctic series. Pooled Antarctic values were significantly ($P<0.01$) lower than those obtained in Melbourne.

Nine subjects of the control group showed a decrease in the mean plasma cortisol concentration while sitting for 2 hours in warm conditions, but the same subjects showed an increase during the cold stress. There was no significant difference in

Fig. 27

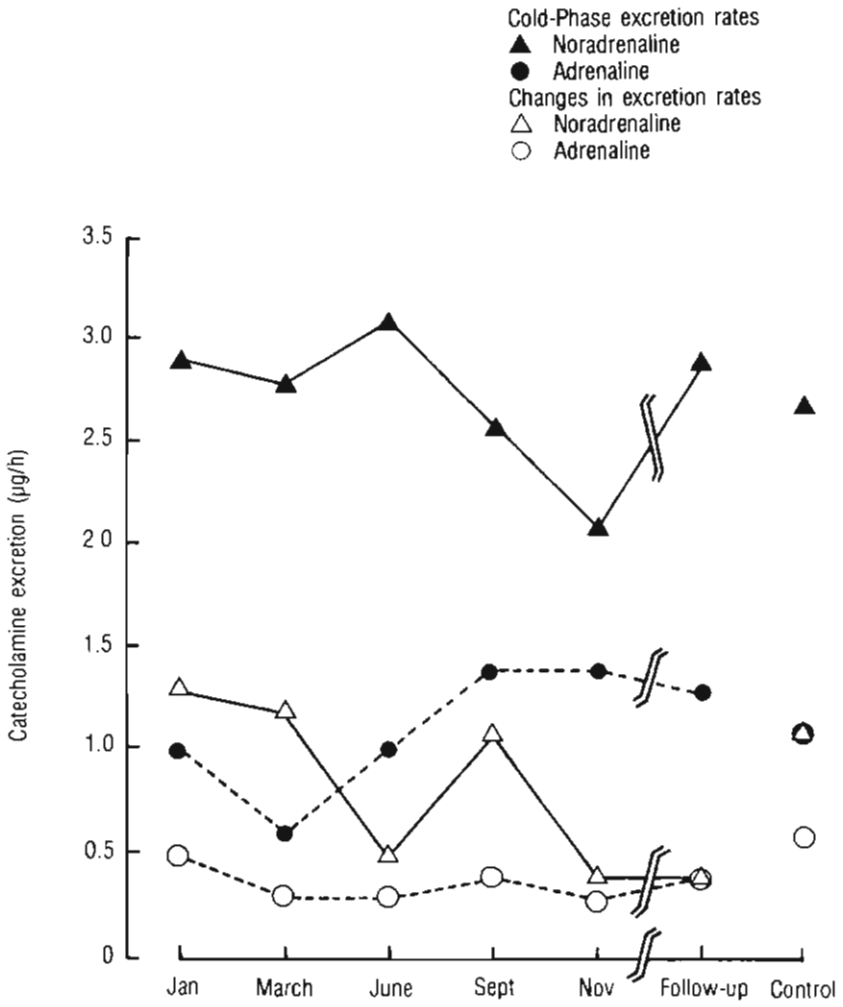


Figure 27. Catecholamine excretion rates during cold phase of pre-Antarctic, Antarctic and post-Antarctic series, and change in excretion rates caused by cold stress

Fig. 28

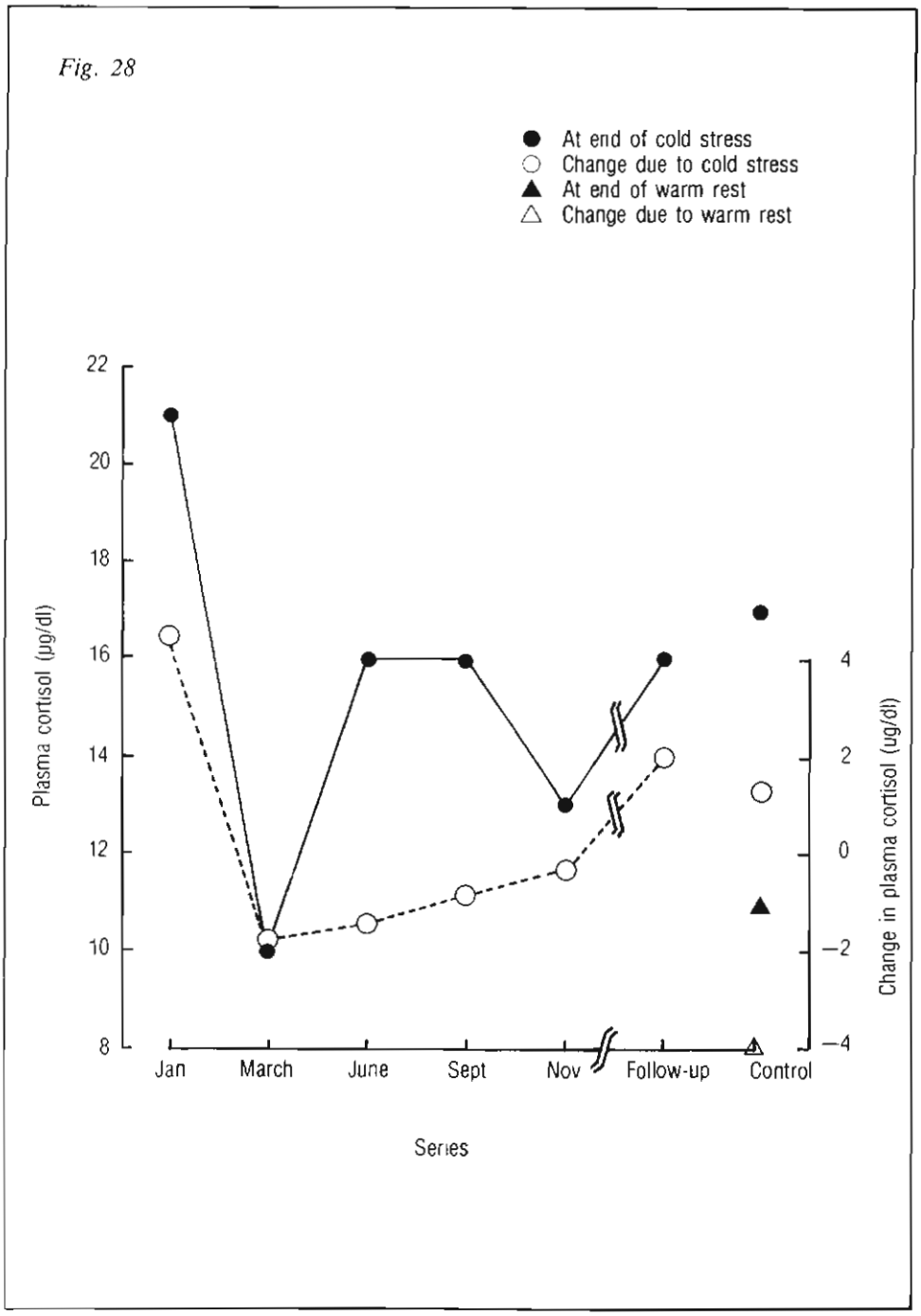


Figure 28. Plasma cortisol concentration at end of cold stress and change in plasma cortisol concentration caused by cold stress in pre-Antarctic, Antarctic and post-Antarctic series

the cortisol response in the January series between the Antarctic and the control groups.

3.3.4 Discussion

Catecholamine excretion

Catecholamine excretion has been found to increase in unacclimatised men exposed to a cold stress while standing (Arnett and Watts 1960, Suzuki et al. 1967). Suzuki's subjects were tested in summer, but three subjects retested in winter showed no change in catecholamine response. The absolute values were respectively lower and higher than those obtained in this work. The cold stress was similar to that used in this investigation, but while the pre-stress values were comparable, the responses were greater. The results in Antarctica were close to those obtained in Melbourne. The Antarctic samples, which were chilled, not frozen, were returned to Australia for analysis (Budd and Warhaft 1970). The Antarctic test was performed 24 weeks after the subjects arrived in Antarctica, which was probably too early to achieve significant changes. Furthermore, catecholamines are not stable in unfrozen urine (see 2.3). The absolute values in this investigation were similar to those obtained previously (Kärki 1956; Hale, Williams and Ellis 1963). However, the response patterns of catecholamines to a standard cold stress applied at regular intervals to men dwelling in a cold environment have not, apparently, been studied.

The reduction in both the cold-phase noradrenaline excretion and the response (i.e. the change from warm to cold-phase values) resembles the findings in rats: the longer they were kept in the cold, the smaller was the noradrenaline response on re-exposing them to the cold (LeDuc 1961). The June series was exceptional in that the defective station heating at that time gave a temperature of 16°C instead of 21°C during the pre-stress warm phase. The warm-phase noradrenaline excretion was therefore unduly high, and some of the noradrenaline secreted at that time would have been carried over, to be excreted in the urine produced during the cold phase; hence the high cold-phase noradrenaline excretion, but very low nett response. Also, in the follow-up series one subject (20) drove a car 120 km to the laboratory and another (24) flew as a passenger. It has been shown that flying even as a passenger increases catecholamine excretion (von Euler and Lundberg 1954). These two subjects had high noradrenaline excretions in the warm phase samples, but there was time for the sympathetic nervous activity to settle down before the cold stress, so there was no carry-over. Thus, their follow-up noradrenaline excretion rates in the cold phase were similar to their pre-Antarctic values, but their cold-stress responses were greatly reduced by the spuriously high warm-phase values. The noradrenaline response appears to be a more suitable variable to consider than the absolute cold-phase value, provided that cognisance is taken of possible nonclimatic factors that may lead to unduly high warm-phase values. The response showed a linear decrease in Antarctica.

The level of adrenaline excretion correlates with stressful activities better than does that of noradrenaline, but in certain personality types the reverse is true (Bloom et al. 1963). Subject 20 was of a personality type that has a high noradrenaline response. He was found to have labile hypertension during the routine monthly blood-pressure measurements. Patients with essential hypertension show a close relationship between the diastolic blood pressure and plasma noradrenaline

level (Louis et al. 1973). This subject's diastolic blood pressure was not as high at follow-up, but blood pressure measured in the laboratory would not necessarily reflect the value when the urine was being produced while he was driving. Some subjects showed a reduction in noradrenaline excretion during the cold phase because recumbency (Kärki 1956) more than compensated for the cold stimulation.

The rise in adrenaline excretion in both the warm and cold phases in the latter half of the year is in accord with the rise in 24-hour excretions that were observed at that time. As noted in 2.3, the rise does not appear to be climatic in origin, but is more likely related to anxiety. The cold stress caused an increment in the excretion rate, which remained fairly constant over all series. The follow-up series showed a high pre-stress excretion rate, which is probably due to the same causes as discussed for noradrenaline.

Plasma cortisol response to cold stress

Suzuki et al. (1967) found that after a 1-hour cold stress, plasma cortisol increased, the pre- and post-cold levels being similar to those obtained in the pre-Antarctic series of the present investigation. The cortisol concentration returned to approximately basal level 6 hours later.

In the Antarctic series, the plasma cortisol concentration did not merely show a reduced increase, but decreased over the 2-hour cold stress. This is explained by the decrease in plasma cortisol in the control group after the subject sat quietly, fully clothed, for 2 hours at 20°C. As with the excretion of noradrenaline, the concentration of plasma cortisol, under the conditions of the experiment, was determined by two opposing factors. Lying still tends to cause a reduction in plasma cortisol concentration, whilst cold stress tends to increase it. In the unacclimatised men, the demand for cortisol due to the cold stress outweighed the influence of physical rest. In the acclimatised person, rest had more influence than the now much-diminished cortisol requirements in the cold; hence the negative response. These results thus accord well with the finding that cold-acclimatised rats had reduced adrenal corticosteroid requirements (Heroux and Hart 1954), but are in conflict with Budd and Warhaft's (1970) findings on adrenal cortical activity as measured by urinary hormonal excretions: they reported that the urinary excretion of 17-hydroxycorticosteroids and 17-ketosteroids increased in response to a cold stress, and the increase was significantly greater in Antarctica than in the pre-Antarctic series. Cortisol is derived from the hydroxycorticosteroid metabolic pathway, so measurement of either substance gives an index of adrenal cortical activity. Russian scientists also found an increase in the excretion of 17-ketosteroids in Antarctica, accompanied by a decrease in the number of negative Thorn tests, which indicates an increase in hydroxycorticosteroids. In earlier Belgian work, though, steroid metabolism appeared to be depressed in the cold (Podolyan 1969).

Prolonged exposure of rats to cold causes an initial rise, followed by a reduction, in corticosteroid activity. Corticosteroids appear to be necessary for induction of adaptational processes, but their role in maintaining acclimatisation to cold is relatively small (Jansky et al. 1975).

The minimal cortisol requirement was in the first Antarctic series. The relative increase in response (the regression curve shows this occurred in August) suggests some de-acclimatisation, at least so far as the adrenal corticosteroid system is concerned, which may be related to the increased time spent indoors in the winter.

It seems to vary much more rapidly with degrees of exposure than does the catecholamine system.

3.3.5 Conclusions

The changes in plasma cortisol levels in response to cold stresses verify that cold acclimatisation had taken place, but how this is related to adaptive processes is uncertain. It probably indicates that, as a result of cold adaptation, the organism had come to 'recognise' the cold stress as less severe.

The reduction in noradrenaline response suggests an enhanced efficiency of the cold-acclimatisation processes mediated by this hormone. The reduced response to a cold stress resembles that of cold-adapted rats on re-exposure to cold. This alteration occurred coincidentally with the altered patterns of response of core and shell temperatures noted in 3.1. No change was found in adrenaline response to cold stress.

3.4 PHYSICAL FITNESS

A program of physical training results in a warmer periphery, even when the subject is at rest. Persons who have been under training for some vigorous sport usually do not suffer as much from cold feet when they go into snow country as do others of similar age and bodily habits who have been leading a sedentary life. Subsequently, the peripheral temperature of the formerly sedentary people improves (unpublished observations). As the recreational trips into snow country were associated with a high degree of physical exertion, the improved peripheral warmth whilst resting in sleeping bags could quite probably have been due to the effects of exercise rather than, or as well as, the cold environment.

Experiments have been conducted by Keatinge (1961) to elucidate this point. Three groups of sailors were compared: one group did exercises in thermally comfortable surroundings; the second group relaxed comfortably; the third group, lightly clad, went into a cold-chamber at 6°C for 7½ hours a day for 19 out of 21 days. All groups were given a cold stress test before and after the test period. The subjects were all Fleet Air Arm ground staff whose occupations before the experiment did not subject them to much cold exposure.

The exercised group were deemed to have improved physical fitness. They showed a later metabolic response to the cold stress and maintained higher forearm temperatures than the other groups, but the finger temperatures were not significantly higher.

In another experiment, five Caucasian men were exposed nude for 1 hour to a temperature of 10°C before and after a period of physical training, and later after a bivouac of 6 weeks in the interior of Alaska. Training was by callisthenics and later, volleyball. A treadmill test showed that a plateau of physical fitness had been reached. Fitness scores were sustained after the bivouac, though training had stopped. At room temperature, skin temperatures were significantly higher after physical training, and there was a slight, non-significant, further increase after the bivouac. Rectal temperatures were not significantly different in any of the series, but the post-bivouac value was the highest. The metabolic rate fluctuated during the cold stress, but after 1 hour of cold stress, there was little difference between series, and the patterns over the hour were somewhat similar for all three series.

However, the core temperature was much the same in every series, which was an apparent paradox. The periphery was warmer after exercise and bivouac. The changes observed in these subjects were believed to be due to improved physical fitness (Heberling and Adams 1961).

A group of men subjected to a standard cold stress in Antarctica showed cooler periphery and better maintained rectal temperature. These men considered their physical fitness had improved; a numerical value was assigned to their subjectively assessed levels of physical fitness (Budd 1964). Another Antarctic group showed similar changes in rectal and skin temperature responses to a standard cold stress; an improvement in their physical fitness had 'almost certainly developed' in the Antarctic (Budd and Warhaft 1966a).

A group of six men on Heard Island in the subantarctic had a higher fitness score on the Harvard Step Test than they had before the expedition (Budd 1965), but finger temperature fell more rapidly during a standard cold stress and rectal temperature response was not significantly different in the Heard Island series.

To determine to what extent physical fitness affects physiological responses, an objective and quantitative assessment of cardio-pulmonary fitness was required. Ideally this should be a true test of the subject's cardiac and pulmonary function, and not give a falsely high reading for an individual who happened to be trained for a particular test. The Harvard Step met these requirements.

3.4.1 Aim

1. To obtain an objective, quantified assessment of physical fitness.
2. To observe whether this altered over the year.
3. To see whether any changes in responses to 3-monthly standard cold stresses might be related to the level of physical fitness.

3.4.2 Methods and materials

The entire party was included in this phase of the study.

The determinations were made between the 3-monthly cold stress tests: in May, July and October.

The technique used was the Harvard Step Test, as described by Keen and Sloan (1958). The subject steps onto and down from a chair approximately 50 cm high. The subject does this thirty times a minute for 5 minutes (or until unable to continue). The subject can change step if desired. The centre of mass of the body must be lifted the full distance each step.

Keen and Sloan advocated the use of a metronome for timing. As none was available, the second hand of a large-faced electric clock was used. The conspicuous red second hand was observed by both the experimenter and the subject, the former calling 'up' as required to ensure that the subject performed the test at the right speed.

At the conclusion of the test, the subject sat down and his pulse rate was measured, using the second hand of a wrist watch that was gaining 3 seconds in 24 hours, as checked by an electrical standarised clock, used as the standard for all station time-observations.

The pulse rate was counted over three 30-second periods: from 1–1½ minutes; 2–2½ minutes; and 3–3½ minutes after the exercise.
An arbitrary fitness index was calculated from the following formula:

$$\frac{\text{duration of exercise in seconds x 100}}{\text{2 x sum of three 30-second pulse counts}}$$

The indices obtained are graded as follows:

Poor	Less than 55
Low average	55 — 64
High average	65 — 79
Good	80 — 89
Excellent	Over 90

3.4.3 Results

The mean index declined from 86 in May to 84 in July and 83 in October. The mean indices for the cold stress group were 84, 84 and 81 respectively. These slight variations were not significant. The highest score was 108 and the lowest 72. The subjects were all in the high average to excellent ranges.

3.4.4 Discussion

Any discussion on physical fitness immediately raises the question 'fitness for what?' Training is highly specific, and an animal trained for level walking performs no better in grade walking than another animal that has not been trained at all. Humans train specifically for a particular sport. This brings about conditioned reflexes leading to quicker muscular responses and greater efficiency, as unnecessary muscular activity is eliminated. In endurance contests the ability to tolerate higher levels of lactic acid appears to be developed.

In team games, players co-ordinate their movements with their team-mates, but this has nothing to do with enhancing physiological capacity. Team games tend to require short bursts of activity interspersed with quite long periods of inactivity, so do not provide the stimulus for increased physiological powers. Thus an expert at a certain team game may have a high fitness for that activity, yet achieve a low score in the Harvard Step Test.

This test has an inverse correlation with the resting pulse. During exertion the pulse rate increases; a maximum effort by a trained athlete will result in much the same pulse rate as a maximum effort by an untrained man, though of course the trained man will produce a vastly better performance. A pulse of 180 beats per minute is associated with a maximum effort, but rates of up to 240 beats per minute are common. After exertion the fit person's pulse slows down more rapidly than in the unfit.

How efficiently tissues may be oxygenated and carbon dioxide be eliminated are the criteria for true physiological fitness for exercise.

Cardio-pulmonary fitness depends on the lungs permitting efficient gaseous exchange between the inspired air and the blood, and a strong heart muscle

propelling blood rapidly through the pulmonary and systemic vasculature, promoting rapid gaseous exchange between blood and tissues. Oxygen debt incurred during severe exercise is paid off quickly, so that the heart rate rapidly returns to the resting level. After very strenuous exercise, it may take several hours for the pulse rate to return completely to resting level.

Enhanced physical fitness also increases vagal activity, causing a slow resting pulse. This slow resting pulse is associated with a larger stroke volume of each ventricle so the tissue requirements are adequately met with this slow heart beat. This situation gives a wider margin for increasing the pulse rate to the maximum value, and hence the fitter man achieves a better performance, as well as gaining improved locomotor skills. This permits a higher steady-state power output, the heart continuing to supply sufficient oxygenated blood to the active muscles for their requirements without incurring an oxygen deficit. The slow resting pulse is thus seen chiefly in those who have trained for endurance sports.

The Harvard Step Test is, therefore, intended to measure cardio-pulmonary fitness. The standard exertion could be achieved by any means permitting an accurately measurable rate of energy output, such as a bicycle dynamometer (often called an 'ergometer') driven against a brake of known resistance (brake dynamometer — the power output or brake horsepower is measured in watts) or the bicycle can be used to generate electricity, the measured wattage showing the power the subject develops. Power is the time rate of energy (or work) output.

The bicycle tests give an unfair advantage to cyclists, due to the specific training effect. The Harvard Step Test has the advantages that the apparatus is extremely simple, and very largely eliminates spuriously high results due to specific training, as very few subjects are likely to be trained in stepping onto chairs.

Australian Antarctic personnel underwent a course of callisthenics for about half a day for 5 days, 2 months before sailing. If this had any effect at all on fitness it would have worn off by the time the subjects were tested. Such exercise programs are of dubious value.

Budd (1964) assessed physical fitness by each person's subjective impression of his own physical fitness, allotting numerical gradings from which graphs were plotted that suggested physical fitness had improved. The fallacy of a subjective technique can be illustrated from Subject 10, who was a competitive cyclist. He stated that he felt very 'unfit' before the October test, at which he scored an index of 100. Cycling may have stood him in good stead for the step test, as the cycling action has similarities with stepping up, but it is unlikely that this test reflected any specialised training skills, because, as mentioned above, physical training is highly specific: one form of training gives little or no benefit for some other, even quite similar, activity. He retained good cardio-pulmonary fitness, though it was over a year since he had been in training. He undertook no special exercise in Antarctica, but was very active in general work about the station. It seems probable that his good cardio-pulmonary fitness was at least partially induced by his previous training.

Subject 23 was a physical fitness enthusiast and maintained a high index of over 90 throughout the year. Subject 20, however, had a relatively low index. He was a strongly built man who had been a competitive sprinter and professional runner. He had a fairly high resting pulse rate, which would have tended to lower his score. A sprinter does not require the same degree of cardio-pulmonary fitness as the endurance man, and tends to have a lower vagal tone than does the latter.

The improved fitness during an expedition on Heard Island (Budd 1965) was associated with a decreased skinfold thickness which suggests energy output was increased on this expedition. The wet-cold conditions of Heard Island, where field parties move on foot, are thus much different from field conditions on the Antarctic continent. The scores were much lower than in the present study: the pre-expedition and Heard Island indices were 52.5 and 67.5 respectively, the latter representing a significant increase.

In the present study, physical fitness did not improve, probably because of the general lack of exercise on an enclosed station. Whilst there were occasional episodes of high physical activity, particularly during 'change-over', most of the ordinary forms of exercise associated with urban life were absent. The field traverses were motorised, so physical exertion on these was scarcely greater than at the station. Expeditions that depended greatly on dog-sledging for transport probably resulted in improved physical fitness. When manhauling of sledges was common, the level of sustained physical exertion was great and concomitant improvement in physical fitness was likely to be considerable.

The fact that some of the changes observed in relation to cold exposure, such as warmer periphery, are also observed in relation to increased physical fitness appears to be an example of different types of stress or stimulus resulting in a similar physiological response. The implications drawn by Keatinge (1961) from reduced metabolic response to cold, and Heberling and Adams (1961) from warmer skin temperatures after physical training, are that changes observed in people who have gone to a cold climate are due to improved physical fitness. Heberling and Adams (1961), using the same subjects after physical training, found no significant changes after a subsequent period living in a cold environment. Presumably the physical training had caused near-maximal changes in vascular dynamics, and the subsequent cold exposure was not sufficient to significantly increase the changes already induced by physical exercise. This aspect must be considered when interpreting results obtained from subjects on a cold-climate expedition that entails increased physical exertion.

The step test to assess physical fitness was the only investigation in the present study in which any declined to continue with the test. The missing values are chiefly due to some physical cause that precluded an accurate performance of the test. Nearly everyone disliked the test; only the competitive, athletic subjects looked forward to it. Of those who declined, one did so because he disliked it intensely. Another declined ostensibly because of pain in the thigh, but it was more probably because his second test gave a much lower index than the first, in which he had top score, and he did not want to be beaten. Another person objected to most forms of competitive sport; when he found many of the others were enthusiastically comparing their scores, he deemed it to be a competitive sport and withdrew from the subsequent series.

3.4.5 Conclusions

The Harvard Step Test applied at three, evenly spaced, intervals during the year in Antarctica excluded improved physical fitness as a factor in the biochemical and physiological changes that occurred; it is suggested that these changes were due to the cold climate. The fitness indices were high, declining slightly, though not significantly, over the three series.

3.5 EFFECT OF COLD STRESSES ON BLOOD PRESSURE AND PULSE RATE

Increased noradrenaline levels would be expected to raise blood pressure, though Budd and Warhaft (1966b) showed that the pressor effect of noradrenaline infusions was abolished in Antarctica. Noradrenaline does not accelerate the heart rate, but the pressor effect secondarily causes a slowing of heart rate. Adrenaline, on the other hand, accelerates the heart rate.

Peripheral vasoconstriction would tend to increase blood pressure. A warmer periphery ought therefore to be associated with a lower blood pressure if the condition of other parts of the vascular tree remain constant.

3.5.1 Aim

Blood pressures and pulse rates were measured in conjunction with the standard cold stresses to see if catecholamine and vascular responses altered these parameters in any consistent way.

3.5.2 Methods

Blood pressure and pulse rates were determined after the subject lay supine for 15 minutes in the warm phase. The measurements were repeated within 10 minutes of entering the cold chamber, after 110 minutes of cold stress, and after 30 minutes in the rewarming phase.

Blood pressure was determined by auscultation over the right brachial artery, using an inflatable cuff attached to a mercury-in-glass manometer. Blood pressures were determined in accordance with the requirements of the standard protocol for Australian Antarctic stations (Section 4.1), in which the systolic blood pressure is that at which the Korotkow sounds become audible, and the diastolic blood pressure is that at which they become muffled. The pressures are given in terms of the Torr (mm of mercury).

The pulse rate was determined by palpation of the radial artery and counting the number of pulsations per minute.

3.5.3 Results

The blood pressures and pulse rates at various phases of each series are illustrated in Figures 29 and 30, and their variation between series at a given phase are shown in Figures 31 and 32.

The pre-stress, warm-phase systolic blood pressure was lower in the later Antarctic ($P<0.01$) and follow-up series ($P<0.02$) than in January. The initial cold-phase pressure at follow-up was lower ($P<0.02$) than in January, series and lower in September than in June ($P<0.05$). There was a highly significant increase on entering the cold chamber in the June series ($P<0.001$). In the November series the systolic blood pressure rose on entering ($P<0.01$) and decreased after leaving ($P<0.02$) the cold chamber.

The diastolic blood pressure was lower than the January value in the June pre-stress warm phase ($P<0.005$), in the September initial cold phase ($P<0.05$), and

Figure 29. Systolic and diastolic blood pressures within each series

Figure 30. Pulse rate (beats/minute) within each series

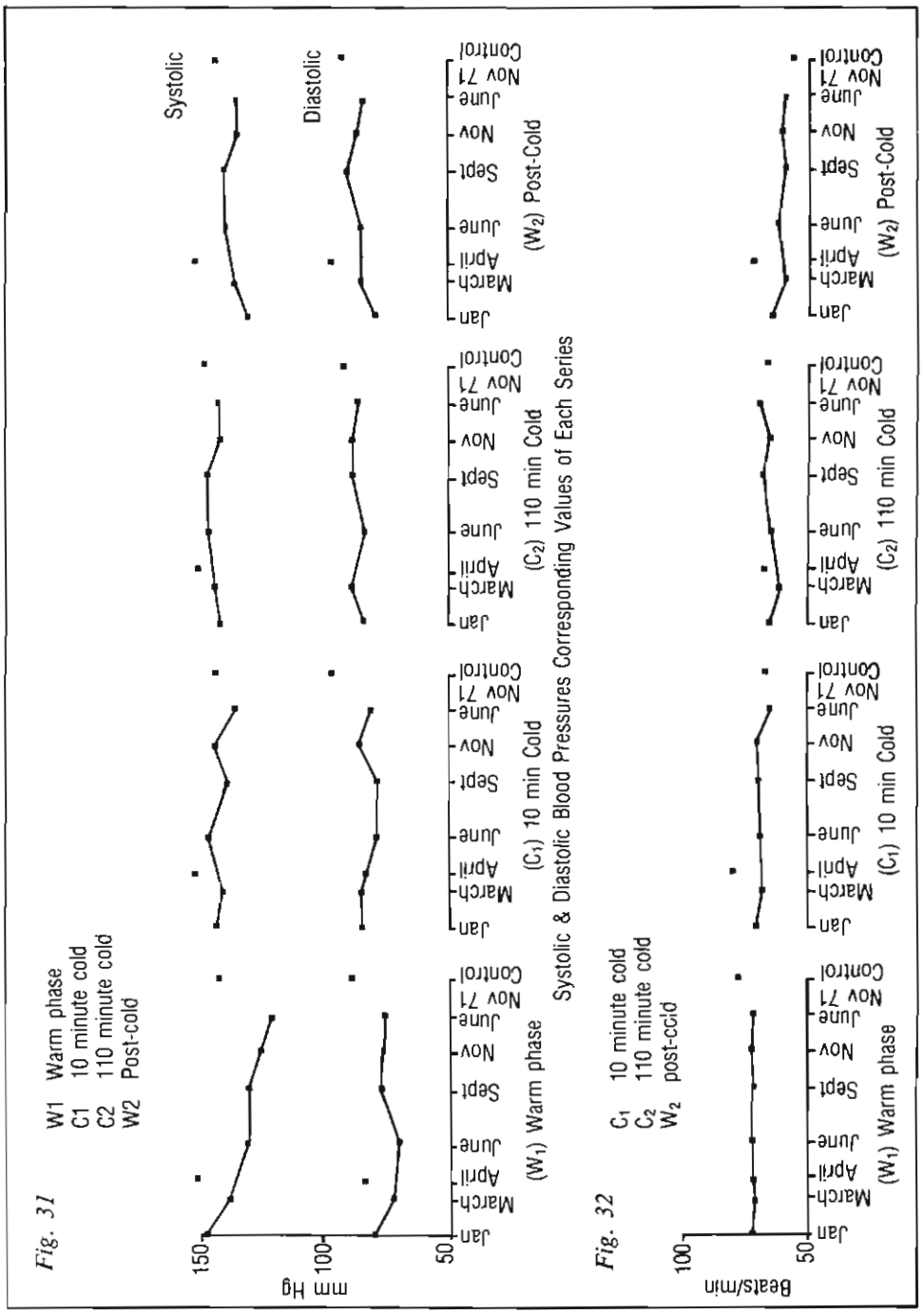


Figure 31. Systolic and diastolic blood pressures: corresponding values of each test

Figure 32. Pulse rates (beats/minute): corresponding values of each test

in the control group rewarming phase ($P<0.05$). The September pre-stress warm phase value was higher than in June ($P<0.02$). The initial cold-phase value in November was higher than in the September ($P<0.01$) and follow-up series ($P<0.025$). On entering the cold chamber, the diastolic blood pressure increased in the January ($P<0.05$), June ($P<0.02$) and November ($P<0.005$) series, as well as in the control group ($P<0.005$). A highly significant rise in the diastolic blood pressure occurred in the September series ($P<0.005$) during the cold stress. The control group showed a highly significant decrease in diastolic blood pressure during the rewarming phase ($P<0.001$).

The pulse rates at the various phases of each series did not differ significantly from those of the January series, but the values at the end of the cold stress and in the rewarming phase were higher in June than in March ($P<0.02$). Significant decreases in the pulse rate occurred during the cold stress in the March series ($P<0.05$) and during the rewarming phase in the September ($P<0.001$) and November ($P<0.005$) series, at follow-up ($P<0.025$), and in the control group ($P<0.001$).

3.5.4 Discussion

In the pre-stress warm-phase, systolic blood pressure and pulse rate were reduced during the rewarming phase in the latter half of the year. Otherwise, apart from occasional sporadic exceptions, there were no significant differences between or within series. There were, however, trends for blood pressures to increase (Figure 29) and pulse rates to decrease (Figure 30) during each cold stress, resembling the patterns observed by Arnett and Watts (1960) in unacclimatised men.

In comparing systolic blood pressures at different phases of each cold stress, only the series in the latter half of the year and at follow-up showed a significant increase immediately after cold exposure. This, probably, was due to the reduction in warm-phase systolic blood pressure, for the initial cold-phase systolic blood pressures showed no significant differences between series (except for follow-up). The absence of other changes in the response of blood pressure to the cold stress had been found previously by Budd and Warhaft (1966a), though, in contrast to the present study, they found a more pronounced slowing of the pulse rate in Antarctica than in Melbourne. Although the reduction in pre-stress, warm-phase systolic blood pressure in the later Antarctic series is suggestive, there is no clear evidence of a loss of noradrenaline pressor effect in cold-acclimatised men (Budd and Warhaft 1966b). The virtual absence of a consistent and significant within-series variation of blood pressure and pulse rate suggests that they were not affected by catecholamine responses to any of the cold stresses. Likewise, the variations in peripheral temperatures observed in different series (see 3.1), which probably reflect differences in the degree of peripheral vasomotor tone, were not reflected in the blood pressure and pulse rate. The warmer periphery was probably achieved by shunting blood to the skin rather than through deeper vessels; hence the nett peripheral resistance did not alter consistently.

3.5.5 Conclusion

The responses of blood pressure and pulse rate to standard cold stresses did not show changes that could be ascribed ro cold-acclimatisation.

3.6 EFFECT OF THE STANDARD COLD STRESS ON URINE FLOW RATE

Exposure to cold causes an increased rate of urine flow termed 'cold diuresis'. There is little evidence on the effect of prolonged exposure to cold on the diuretic mechanism.

Budd and Warhaft (1966a) found that the urine flow rate was two to three times greater during exposure to 10°C than during the pre-exposure period. They found no consistent differences between series. Cold diuresis was greater in the Antarctic than in the pre-Antarctic series in 2 subjects, less in one and unchanged in another. Suzuki et al. (1967) found a cold diuresis occurred during a cold stress of 10°C–15°C for 1 hour during the summer. On repeating the test in winter, this time on only 3 subjects, there was no significant increase in the urine flow rate during cold stress. An absence of cold diuresis was observed in the Nepalese pilgrim, who appeared to be well cold-acclimatised (Pugh 1963).

3.6.1 Aim

To see whether the diuretic effect of a standard cold stress in Antarctica was different from that observed in Melbourne.

3.6.2 Methods

Before coming for the cold stress, the subjects were asked to note when they last urinated. Just before going into the cold room, each subject urinated and the time was noted so that the pre-cold stress rate of flow could be determined. At the conclusion of the cold stress, each subject again emptied his bladder.

Urine volumes were measured at the same time as urine samples were collected for assaying the catecholamine content.

3.6.3 Results

The urine flow rates in mL/min before and during the standard cold stress, and including the results for a separate control group of twelve different subjects, are shown in Figure 33.

There was a rise ($P<0.05$ to <0.001) in urine flow rates in response to the cold stress in all series, especially in June.

There was no significant difference between the pre-cold phase urine flow rate of the pooled Melbourne series and the pooled Antarctic series. Excretion during the cold stress, however, was greater ($P<0.05$) in the latter than in the former.

The changes in the urine excretion rate from the pre-stress to the cold stress level were not significantly higher in the Antarctic than in the pre-Antarctic series, nor were there significant differences between one series and the next. Pre-Antarctic, follow-up, and control group values were all similar. The pooled Antarctic values, however, showed a greater increase in the urine flow rate due to the cold stress than in the pooled Melbourne series.

Fig. 33

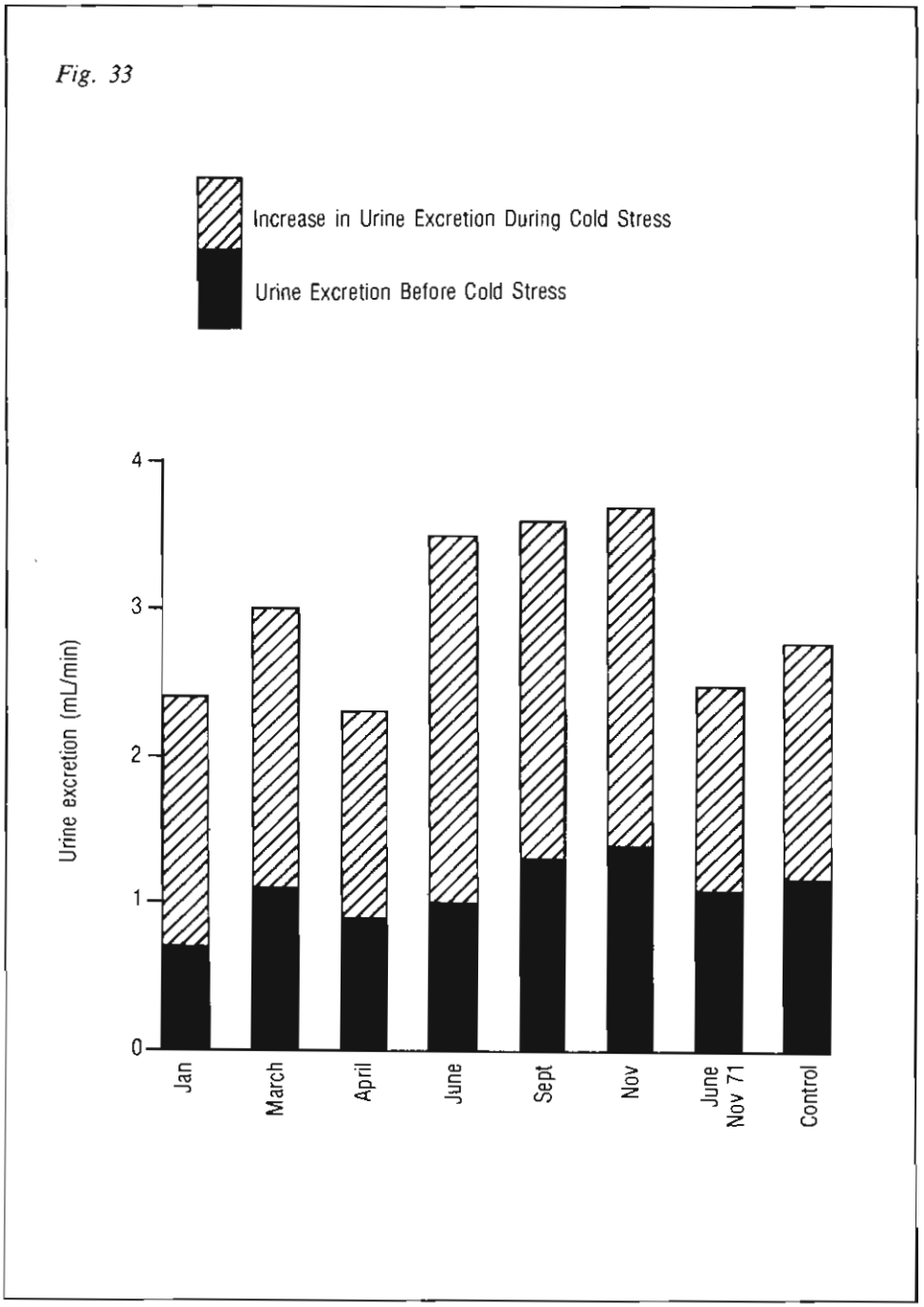


Figure 33. Urine excretion (mL/minute) before and during cold stress

3.6.4 Discussion

Cold diuresis is a fact apparent to everyone. The urine flow rate at the end of the cold stress was significantly higher than before the cold stress, the increases in the pre-Antarctic series and the first Antarctic series (March) being highly significant ($P<0.001$).

The degree of this cold diuresis was not significantly different between series, though it tended to be somewhat greater in the Antarctic than in the Melbourne series, much the same as Budd and Warhaft (1966a) found in two out of four subjects in a previous investigation. Wyatt (1963), however, found no diuretic response to a winter cold stress in two subjects in Antarctica, no difference between seasons in another subject, and a marked diuresis in summer and only a small diuresis in winter in the fourth subject. A very marked urine flow was observed on sledge journeys. Subsequently it has been reported that cold diuresis was reduced or absent in winter (Suzuki et al. 1967), in Antarctica (Ward 1975), and in the Nepalese pilgrim (Pugh 1963).

The 24-hour volumes in the latter half of the year (see 2.4) were less than in earlier series, but the slightly greater increase in urine flow rate in Antarctica than in Melbourne in response to the standard cold stress remains puzzling.

A possible explanation is the one put forward to explain the reduction in the 24-hour flow rate in the Antarctic compared with pre-Antarctic: that rehydration occurred up to June, as the subjects spent more time indoors in the winter, leading to a reversal of body water-content. Then, when the body was challenged with the standard cold stress, an enhanced diuresis occurred. Alternatively, or perhaps as well, the cold diuresis mechanism may have become more sensitive as part of the acclimatisation process. If this were so, the water volume of the body adjusts more quickly to changing levels of cold stress than do other variables. The reduction in body-water volume would therefore represent a rapid adjustment to cold rather than a prolonged form of acclimatisation. The fact that a cold diuresis persisted suggests that the degree of acclimatisation was not as great as that of the Nepalese pilgrim (Pugh 1963), who showed no cold diuresis. This is not surprising, as personnel were subjected only to relatively short periods of cold, whereas the pilgrim stayed out in severe weather conditions and was poorly clad.

3.6.5 Conclusions

Less urine was excreted over a 24-hour period in the latter half of the Antarctic year than earlier in the year or in Melbourne. By contrast, cold diuresis in response to a standard cold stress was slightly greater in the Antarctic, possibly due to a more sensitive cold diuresis mechanism, or as a result of some rehydration during winter when the short days reduced the amount of outdoor activity.

It seems that reduction in body water-content is part of the cold-acclimatisation process, but the responses to environmental changes appear to be more rapid than in other mechanisms. The different levels of diuresis in response to a cold stress that have been reported by various investigators may be at least partly due to the small number of subjects having different levels of acclimatisation. However, cold diuresis may be merely a de-acclimatisation: a person going into a hot environment will have an enhanced survival potential by increasing body water-content.

4. Other Observations

4.1 MONTHLY PHYSIOLOGICAL MEASUREMENTS

A program of physiological measurements has been carried out on many Australian Antarctic and subantarctic expeditions. A standardised protocol is followed so that the measurements can be compared. Furthermore, such measurements done in conjunction with the present investigation would show whether the subjects responded to the Antarctic environment in a manner similar to that of subjects on other expeditions.

4.1.1 Methods

The measurements were made each month from April to December, as far as possible on the same date each month. The measurements were:

- Basal readings, before subject arose:
  - blood pressure
  - pulse rate
  - oral temperature
- Casual readings, later the same day:
  - blood pressure
  - pulse rate
  - oral temperature
  - weight
  - skinfold thickness:
    - over angle of the scapula
    - over triceps
  - arm circumference
- Barefoot height at beginning of the year
- Length and breadth of the sphygmomanometer cuff

The basal readings were made before the subject arose; he was often asleep until measurements were begun. At some convenient time later that day he would come to the medical block for the corresponding casual measurements, weighing, and measuring his skinfold thicknesses and arm circumference.

Body weight

This was measured on a Wedderburn beam balance zeroed before use. Subjects wore only light underpants during weighing.

Skinfold thickness and arm circumference

Skinfolds were measured just beneath the angle of the left scapula and over the left triceps midway between the acromion and the top of the olecranon.

The measurements were made three times at each site, relocating the site each time, using the Harpenden skinfold calipers. The left side was always used.

The circumference of the arm was measured while the arm was relaxed, using the same flexible non-extensible tape measure each time. Measurement was made at the position corresponding to midway along the sphygmomanometer cuff.

Blood pressure

This was always measured on one arm, the one accessible from the direction the subject lay in his bunk. It was determined by a mercury sphygmomanometer and using the bell of the stethoscope. The arm was bared to the shoulder, avoiding constriction; the lower edge of the cuff was 2 to 3 cm above the elbow; the middle of the rubber bag lay over the brachial artery. Systolic pressure was read as the point at which sound first appeared. Diastolic pressure was read as the point at which the sounds suddenly became muffled (phase 4 of Korotkow).

The observations were read to the nearest 2 mm of mercury (Torr) for the purpose of this investigation, though the program requires results recorded only to the 5 or 0 below the point at which sounds appear. The more precise recordings were made to be in accord with the measurements made during the standard cold stresses.

Pulse rate

The radial pulse was timed for a minute, using the second hand of a wrist watch with an error of +3 seconds per 24 hours.

Oral temperature

This was measured by clinical thermometers calibrated in degrees Celsius. These were checked against the standard mercury in glass thermometer used in the standard cold stress tests (Section 3.2). Subjects were kept in a warm room (20°C–21°C) for 15 minutes before measurement of the oral temperature. The thermometer was left in the mouth for 3 minutes, it was then read, replaced for a further minute and read again. This procedure was repeated if the two readings were not the same.

Meteorology

Monthly mean maximum, mean mimimum and extreme air temperatures as well as mean and extreme windspeeds were obtained from the Meteorology Section on the station (Figures 1 and 2).

Analysis

Regression analysis was applied to these variables. The time in months t was taken from April to December.

4.1.2 Results

Body weight

There were no consistent patterns. The changes were non-linear: some subjects increased, some decreased and some fluctuated. Overall, however, there tended

to be a slight increase until October, followed by a decrease to December (Figure 34).

Skinfold thicknesses over the scapula and triceps

There was no consistent pattern of change. Overall, there were slight non-linear increases. The triceps skinfold thickness increased to October, then decreased (Figure 34).

Arm circumference

There was no consistent pattern among the subjects, but overall the arm circumference increased (Figure 34).

Systolic blood pressure

The basal systolic blood pressure showed no significant changes with the time spent in Antarctica. The linear regression model showed a decrease that was not quite significantly different at the 5% level from a constant relationship with time (Figure 35).

The casual systolic blood pressure differed for each subject, but there was a quadratic regression on time (Figure 35): the blood pressure decreased from April to September, then increased to December, the rates of change being similar for each subject.

Diastolic blood pressure

The basal diastolic blood pressure differed among individuals, but it increased linearly in all subjects over time at the same rate (Figure 35).

There were no consistent trends in the casual diastolic blood pressure, but it tended to decrease until July, then increase to December (Figure 35).

Pulse rate

The subjects showed different basal pulse rates, but had common linear increases over time (Figure 35). The casual pulse-rate regression, though not quite significant at the 5% level, suggests an approximately linear increase. However, the rates varied from subject to subject (Figure 35).

Oral temperature

The subjects varied with time in a quadratic mode in which the basal and casual oral temperatures both tended to increase from April to September-October, and then decrease (Figure 35).

Age and height

The means and ranges were: age 31.5 years (22–49); height 175.2 cm (164.5–187.0); weight 75.0 kg (62.3–103.6).

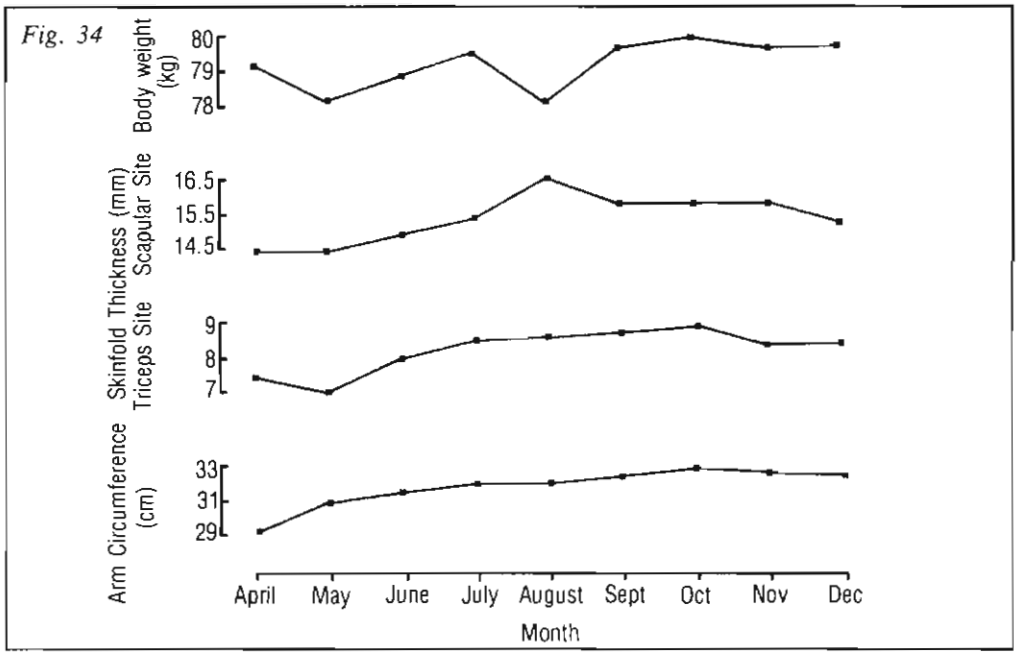


Figure 34. Arm circumference, skinfold thickness at triceps and scapular sites, and body weight during Antarctic phase

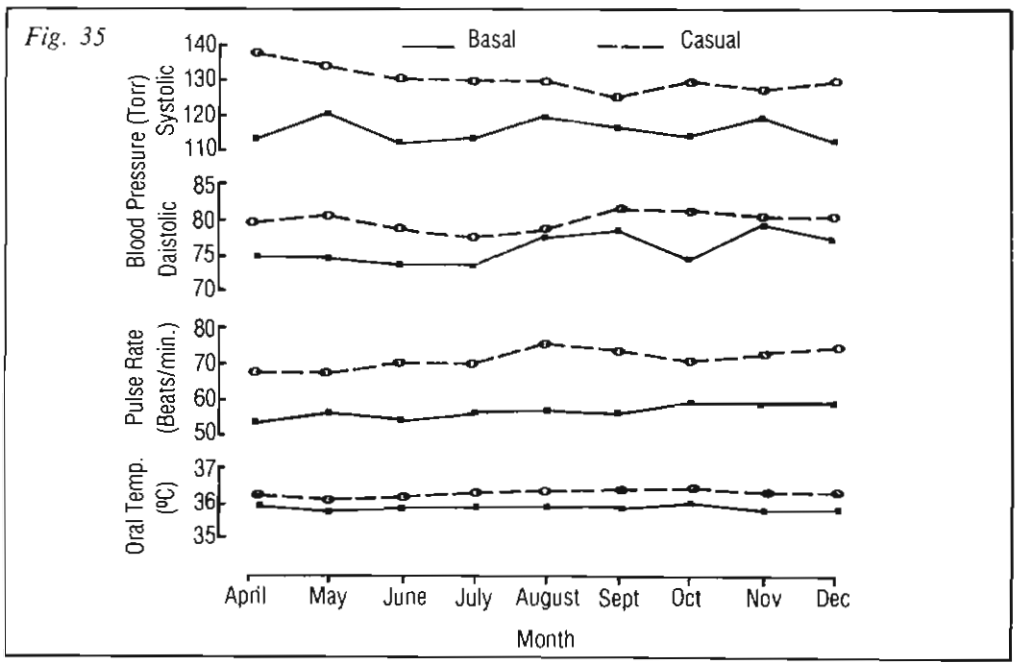


Figure 35. Oral temperature, pulse rate and blood pressure during Antarctic phase

4.1.3 Discussion

Body weight, skinfold thicknesses and arm circumferences showed no consistent patterns. Nevertheless, overall body weight and skinfold thickness tended to increase to October, and then decrease to December. This may be related to the cook being unable to continue his duties after October, the cooking being done by all the others in turn. Although the quality and quantity of the food remained high and no one complained of feeling hungry, there was somewhat less variety.

Changes in weight appear to be chiefly due to changes in the amount of fat. It is thought that dehydration may be part of the cold-acclimatisation process (K.E. Hicks, personal communication); if so, deposition of subcutaneous fat more than compensated for water loss, though the opposite trends in weight and scapular skinfold thickness in August may be due to reduction in water content. Muscle involution could also account for loss of body weight, but as the physical fitness index was virtually constant (see 3.4) it is unlikely this played any appreciable part in the variations. Other studies have reported a similar reduction in weight in or around August (Palmai 1962b, Hicks 1966, Lugg 1973).

The mean weight of 75 kg at the beginning of the year was close to that found in two of the other investigations (Palmai 1962b, Hicks 1966), but the mean height was less, (175 cm) which is the same as Lugg's (1973) group, whose mean weight was 70.8 kg. The present subjects were therefore more thickset than the others. Although the skinfolds were much thicker, their patterns of change over the year were similar to those observed by Lugg (1973).

Alterations in the body weight and fat do not appear to be a specific form of cold acclimatisation, being essentially related to the balance between food intake and energy output.

The increase in the basal diastolic blood pressure during the time spent in Antarctica corresponds approximately to the increase in adrenaline excretion (see 2.3). This hormonal relationship is not so pronounced with the systolic blood pressure: it just fails to give a significantly linear regression. The basal diastolic pressure might be expected to be strongly influenced by the pressor amine status of the subject, whereas the basal systolic blood pressure may have been appreciably affected by other factors. Likewise, casual blood pressures are more likely to be affected by the subjects' recent activities and thus obscure the effects of environmental background on pressor amines. Nevertheless, the casual systolic pressure rose during the latter months when adrenaline excretion was increasing. Palmai (1962a) found blood pressures varied in the same manner as skinfold thickness, both reaching a maximum in August, and Hicks (1966) found they decreased throughout the year. Lugg (1973) found significant changes only in the casual systolic and basal diastolic blood pressures, the former decreasing and the latter increasing from July to December, with magnitudes as well as pattern being similar to those presented here. The basal diastolic blood pressure in the present investigation was unrelated to body weight or skinfold thickness, these variables tending to decrease in the latter months when the basal diastolic blood pressure was increasing significantly. Previous investigations in Antarctica showed no relationship between weight and blood pressure (Hicks 1966, Lugg 1973), though they found that the blood pressure showed a downward trend while the weight was increasing. In the subantarctic, both weight and blood pressure increased (Palmai 1962a).

The increase in the basal pulse rate, as with the basal diastolic blood pressure, is probably related to increased adrenaline concentration, as indicated by the rising adrenaline excretion in the latter half of the year (2.3). The casual pulse rate just falls short of a significant regression on time. As with blood pressure, the casual pulse rate seems to depend on many variables associated with each subject's activities before the measurement was made. The magnitude of the basal pulse rates was similar to that observed previously (Hicks 1966, Lugg 1973). Hicks (1966), however, observed a progressive decrease of the casual pulse rate in Antarctica, whereas Lugg (1973) found that it increased from September to December in a similar fashion to that found in the present investigation.

The basal and casual oral temperatures reached a maximum in September-October, when rectal temperature of the cold stress subgroup in the pre-stress warm phase had decreased and the extremities had become warmer (see 3.1). This pattern had not been observed previously (Palmai 1962b, Hicks 1966, Lugg 1973). Therefore the oral temperature, though somewhat higher than the skin temperature, reflects the effects of cold-acclimatisation on the peripheral rather than the core temperature.

Overall, the present findings are more in accord with those Hicks (1966) and Lugg (1973) obtained in Antarctica than those of Palmai (1962a, 1962b) in the subantarctic. The uniformly wet-cold conditions in the latter location may account for these differences in response patterns. The number of subjects, however, was relatively small in all cases (12, 9, 23 and 24), so it is possible that a particular expedition might not represent a random sample of subjects.

4.1.4 Conclusions

There were no consistent patterns of change in the body weight, thickness of skinfolds, or arm circumference. The casual systolic blood pressure showed a quadratic regression in which it decreased and then increased. The basal diastolic blood pressure and the basal pulse rate increased linearly with time spent in Antarctica. These increases were probably related to increased adrenaline levels. The basal and casual oral temperatures showed quadratic regressions, increasing at first, followed by a decrease over the last 2 months.

The patterns are somewhat similar to those found previously in Antarctica, but differ from those obtained in the subantarctic.

5. Skin studies

5.1 STRUCTURAL CHANGES IN THE SKIN

The skin, in forming the interface between the individual and his environment, might be expected to show acclimatisation effects. The sweat glands show functional changes in the tropics (Adam et al. 1953). Solar irradiation causes thickening of the epidermis, melanin deposition, altered dermal collagen and elastosis (Lever and Schaumberg-Lever 1975a).

In Antarctica it has been found that two-point discrimination of the skin at low temperature improves (Massey 1959), sebaceous gland activity on the forehead and back is reduced (Corner 1966), and the proportion of linoleic acid in the subcutaneous fat increases (Easty et al. 1967). Racial and anatomical site differences have been found. Negroes were found to have proportionately more stearic acid and palmitic acids (which are saturated), and proportionately less unsaturated palmitoleic acid than Caucasians. Myristic acid, a saturated fatty acid, decreased in amount from white to Cape Coloured to Bantu. The adipose tissue of the extremities had proportionately less palmitic, stearic and myristic acids and proportionately more palmitoleic than the trunk (Itoh et al. 1969).

The effects of cold injury on the skin have been extensively studied, but structural alterations in the skin that could be associated with acclimatisation to a cold environment have received little attention.

5.1.1 Aim

The aim was to see whether living in Antarctica affected the thickness of the epidermis and, if it did, whether it was a localised effect of chronic cold exposure or was systemic. Alterations in dermal components were also sought.

5.1.2 Methods

Ten members of the twenty-four-man expedition volunteered for the skin biopsy program. Their mean age was 31 years (range 23–45), mean height 177 cm (167–185) and mean weight 78.5 kg (65–98).

The subjects, before going to Antarctica, lived in temperate to tropical zones in Australia where solar irradiation is intense. All subjects received high exposures, either due to work or recreation. In Antarctica they were fairly uniformly exposed to cold, for the same reasons. As in other Australian expeditions (Lugg 1977), the subjects spent 3 or 4 hours outdoors each day. The only cold injuries were occasional frostnip to the pulps of the index finger and thumb of mechanics when putting on nuts with bare fingers. No cold or other injury affected the areas of skin from which the biopsies were taken. The biopsy sites healed uneventfully. The usual hand covering was soft woollen mitts with windproof over-mitts.

During the Melbourne series, the temperature range was 9.5°C to 31.7°C, with windspeed up to 10 kt. In Antarctica, the lowest station temperature (−33.3°C) occurred in August; the maximum windspeed in that month was 114 kt. Minimum temperature in the field was −40°C.

In January, while the subjects were in Melbourne, an ellipse of skin about 10 mm long and 3 mm wide was excised, under local anaesthesia, from the dorsum of the left hand and from the lower abdomen. The biopsies were repeated on eight of the subjects during August, after 6 months in Antarctica; the remaining two were done in November and the following January, respectively, when the subjects' work commitments permitted operative interference.

The dorsum of the hand was chosen as a convenient site to observe changes that might be due to local cold effects. The lower abdominal biopsies were to see whether changes occurred in the skin that might be attributable to generalised cold-acclimatisation.

The specimens were placed in 10% formol saline, sealed and labelled. In an Australian laboratory, each ellipse was hemisected at right angles to the epidermal surface, care being taken to block the tissue so that the epidermis would be vertical to the microtome knife. Sections were cut from all specimens at 4μ thickness. They were stained for elastica by Verhoeff's method combined with Masson's trichrome stain. The number of epidermal cells within an eyepiece graticule were counted under high power (magnification 400X). Ten such fields were counted for each specimen. Fields were chosen to exclude hair follicles but rete ridges were included. The number of layers of granular cells was also counted. The results were analysed using the two-tailed paired t test. The amount of elastica, and the appearances of the collagen fibres and sweat glands were observed in the dermis.

The other halves of the skin ellipses were processed over 2 years later to see whether the Melbourne specimens had been affected by their longer stay in formalin.

5.1.3 Results

The mean number of epidermal cells per graticule are shown in Table 8. The overall mean in Antarctica showed a highly significant ($P<0.001$) increase compared with the Melbourne mean, with all subjects showing an increase. Typical examples are shown in photomicrographs (Figures 36 and 37). The abdominal epidermis showed very little change in cellularity, individual subjects showing slight increases or decreases in Antarctica. Typical examples are shown in photomicrographs (Figures 38 and 39). The number of layers of granular cells of the hand skin increased in Antarctica in all subjects, but there was no change in the abdominal skin (Table 9). Cell counts of the second batch of sections were similar to those of the first, indicating that the length of stay in formalin did not affect the cell counts; it also confirmed that the specimens had been blocked accurately.

The dermis of the hand skin showed a decrease in elastica in six subjects and no change in one. The abdominal skin elastica showed no change in five subjects and a decrease in one. In the specimens taken in Melbourne, most of the hand skin biopsies had less elastica than had the abdominal skin; in Antarctica they all had

	Melbourne	*Antarctica*	*P*
Dorsum of hand	293	403	<0.001
Abdomen	213	228	>0.10

Table 8. Overall mean number of epidermal cells per high power field graticule

less than the abdominal skin (Table 9). These changes are exemplified by one subject in Figures 40 to 43. No between-series changes were observed in collagen, sweat glands or vascularity at either site.

5.1.4 Discussion

The abdominal skin had long, slender, rete ridges; rete ridges were less conspicuous over the hand, probably because the epidermis between the rete ridges is thicker over the hand than over the abdomen (Figures 36 and 38).

The differences in the skin between the two sites became more pronounced after the subjects had been living in Antarctica for at least 6 months. The increase in thickness of the epidermis in Antarctica almost obliterated the rete ridges by the proliferation of epidermal cells between them (Figures 36 and 37). No change in cellularity of the abdominal epidermis occurred as a result of living in the Antarctic (Figures 38 and 39).

The thickness of keratin could not be compared because of variable separation of laminae during preparation. The thickness of the horny layer is thought to be proportional to the thickness of the granular layer (Lever and Schaumberg-Lever 1975b). If so, the increase in the number of layers of granular cells in the epidermis of the hand indicates that one of the responses of skin exposed to cold air is to increase the thickness of the horny layer.

The generally smaller amount of elastica in the hand (Figures 40 and 41) than in the abdominal dermis (Figures 42 and 43) observed in the Melbourne series was probably due to inherent differences in the skin at these sites, though previous prolonged exposure may account, at least in part, for these differences. There was a reduction in hand skin elastica (typically shown in Figures 40 and 41), but that of the abdominal dermis showed no consistent changes. In Antarctica, all subjects showed less elastica in the hand skin than in the abdominal skin.

The hands were frequently exposed to the cold environment in Antarctica, as handwear was removed to enable fine work to be performed. The abdominal skin was almost always well protected. It is unlikely that the soft woollen inner mitts

	Granular Layers			
	Hand		*Abdomen*	
Subject	*Melbourne*	*Antarctic*	*Melbourne*	*Antarctic*
2	3	3	1	1
5	2	4	1	1
7	2	3	1	1
12	2	5	1	1
14	2	3	1	1
16	1	4	1	1
18	2	4	1	1
19	2	4	1	1
20	3	3	1	1
21	4	6	1	1

Table 9. The number of granular layers in the epidermis of the hand and abdomen in ten subjects in Melbourne and Antarctica

would have stimulated epidermal proliferation by friction; indeed the handwear was more likely to have protected the skin from mechanical forces. The mechanics, of necessity, spent much time outdoors servicing the vehicles, but the others spent as much time in outdoor recreation. The greatest exposure was that of Subject 5, a scientist, who spent as much time in the field as on the station. The occupation of the subjects did not affect the response pattern.

The direct effect of cold appears to be the cause of the histological changes occurring in the skin of the dorsum of the hand. The intensity of solar radiation was much less than the subjects had been used to in Australia, where the initial biopsies were taken, and there was no evidence of sunburn or dermal solar degeneration. The increase in epidermal thickness appears to be a local form of acclimatisation. It is not clear how the loss of elastica may enhance cold resistance; it may be loss of solar elastosis. Cold reduces the rate of aging in mouse-tail collagen (Hrůza and Hlaváčkova 1969) and dermal collagen density becomes less with age (Shuster et al. 1975), but no changes were detected in dermal collagen during this investigation. Although there was evidence of general acclimatisation to cold (Sections 2 and 3), the absence of changes in the abdominal skin excludes a systemic cold-acclimatisation effect on skin structure.

5.1.5 Conclusions

It is concluded that the skin undergoes local, but not generalised, acclimatisation to cold by increasing the thickness of the epidermis. The number of granular layers increased suggesting the surface keratin thickens. The exposed skin showed a reduction in dermal elastica. The relationship to acclimatisation is not clear; it may be merely a reduction in previously induced solar elastosis. The negligible changes in the skin over the abdomen excludes the possibility of cold acclimatisation having a systemic effect on skin structure.

	Elastica			
	Antarctic series vs. Melbourne		*Hand vs. abdomen*	
Subject	*Hand*	*Abdomen*	*Melbourne*	*Antarctica*
2	No change	No change	Less	Less
5	Decrease	No change	Equal	Less
7	Slight decrease	Increase	Less	Less
12	Increase	Increase	Less	Less
14	Decrease	Slight increase	Less	Less
16	Slight decrease	No change	Less	Less
18	Decrease	Slight decrease	Equal	Less
19	Slight increase	No change	Less	Less
20	Slight increase	No change	Less	Less
21	Decrease	Decrease	Greater	Less

Table 10. Differences in the dermal elastica of the hand and abdomen of ten subjects in Melbourne and Antarctica

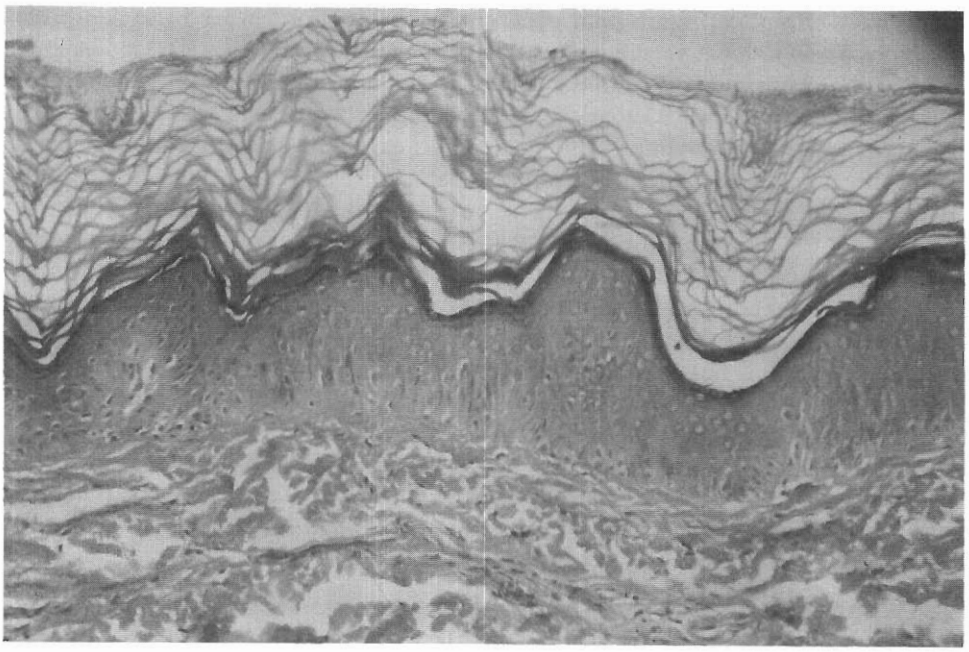


Figure 36. Skin from dorsum of hand, Melbourne biopsy (H & E, x 100)

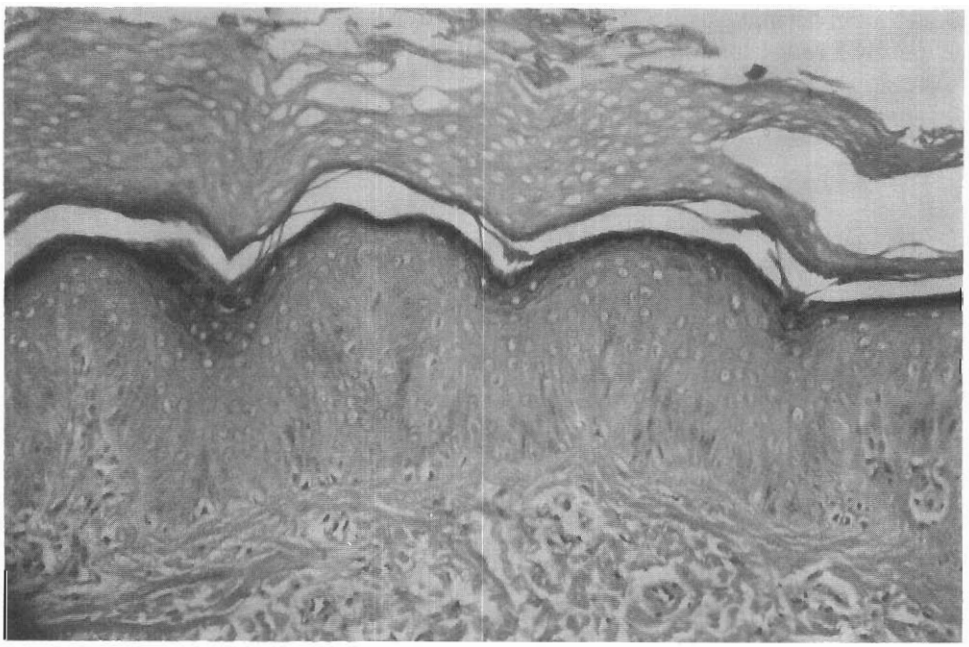


Figure 37. Skin from dorsum of hand, Antarctic biopsy (H & E, x 100). Note the increased thickness of epidermis compared with Figure 36.

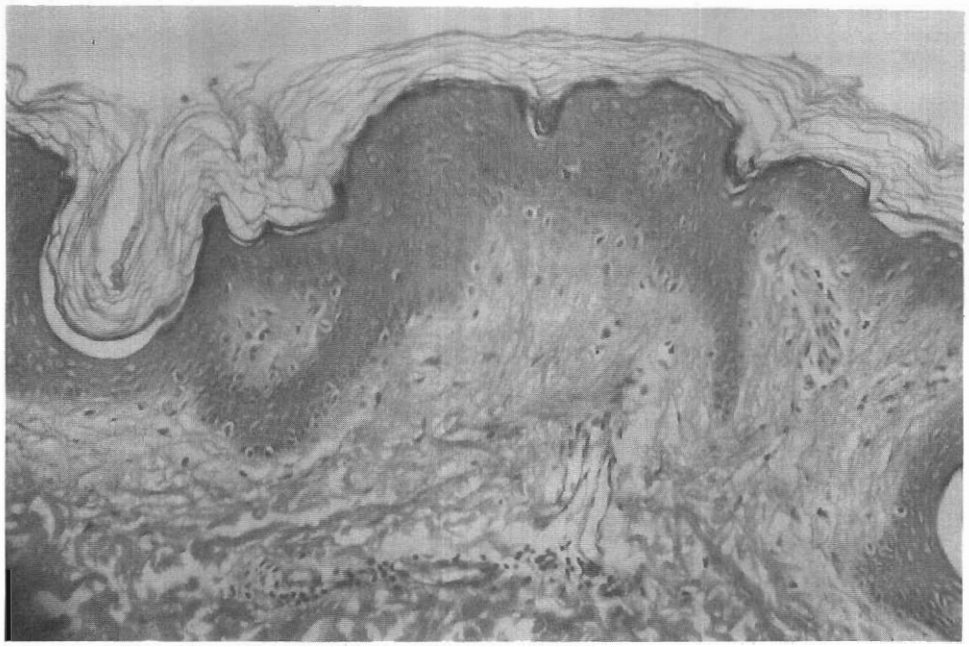


Figure 38. Skin from abdomen, Melbourne biopsy (H & E, x 100)

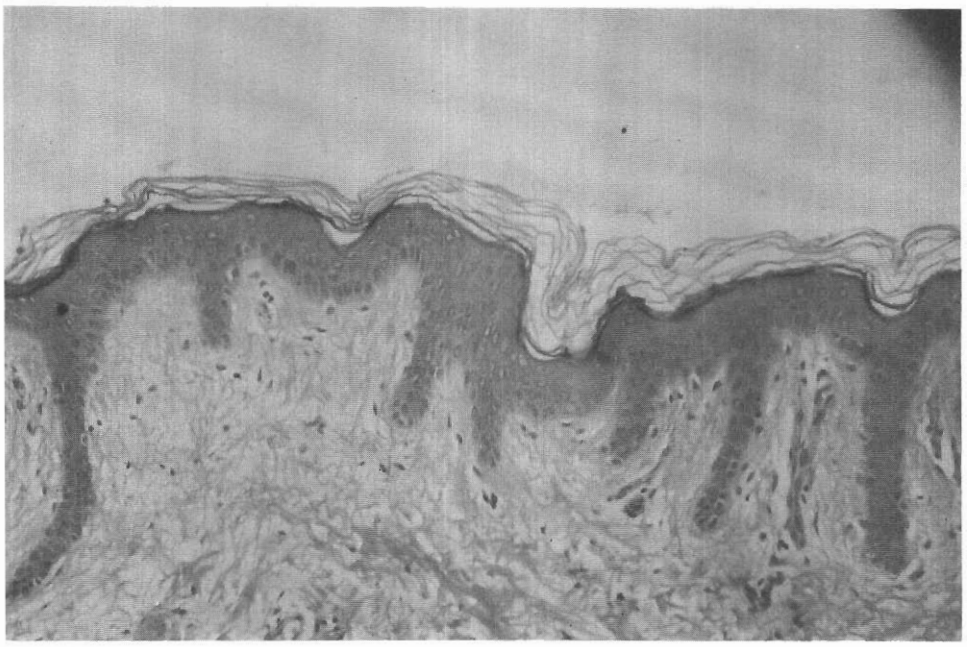


Figure 39. Skin from abdomen, Antarctic biopsy (H & E, x 100). There is scarcely any difference in epidermal thickness between the specimens in Figures 38 and 39.

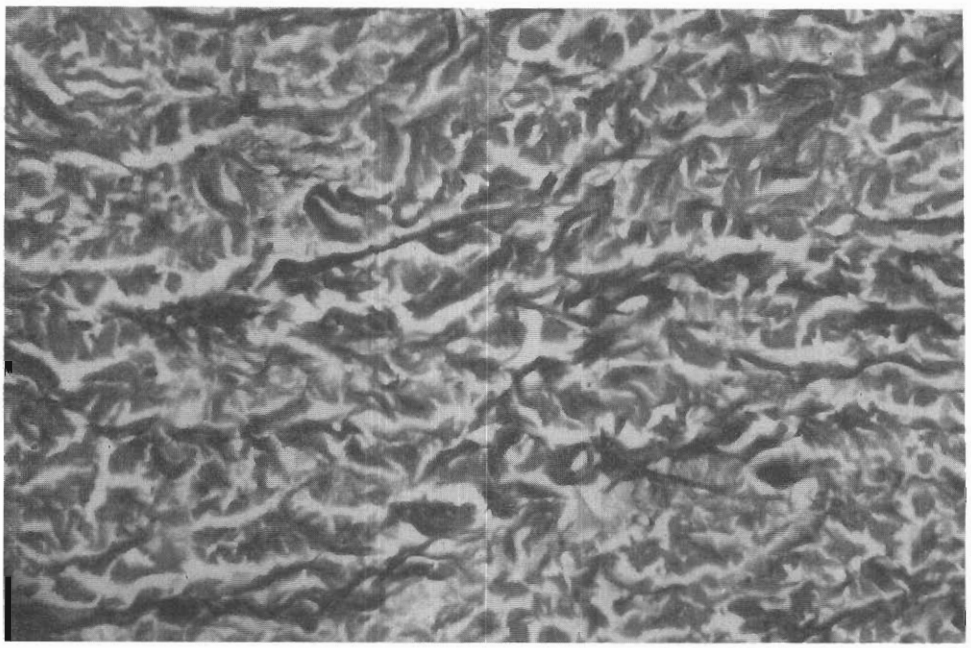


Figure 40. Dermis of skin from dorsum of hand, Melbourne biopsy (Masson and Verhoeff, x 100)

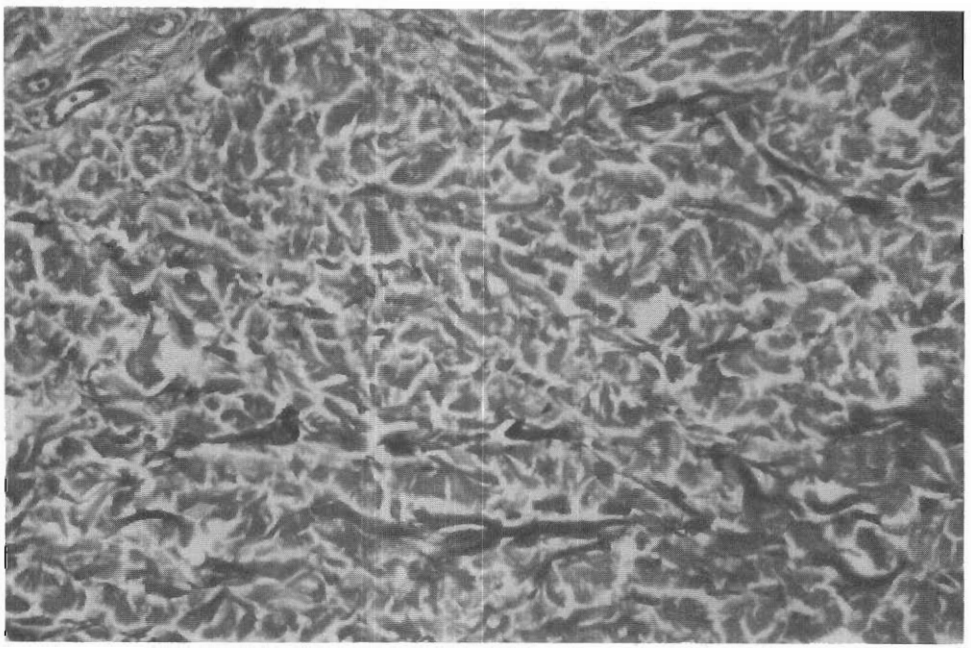


Figure 41. Dermis of skin from dorsum of hand, Antarctic biospy (Masson and Verhoeff, x 100). Note reduction in elastica compared with Figure 40.

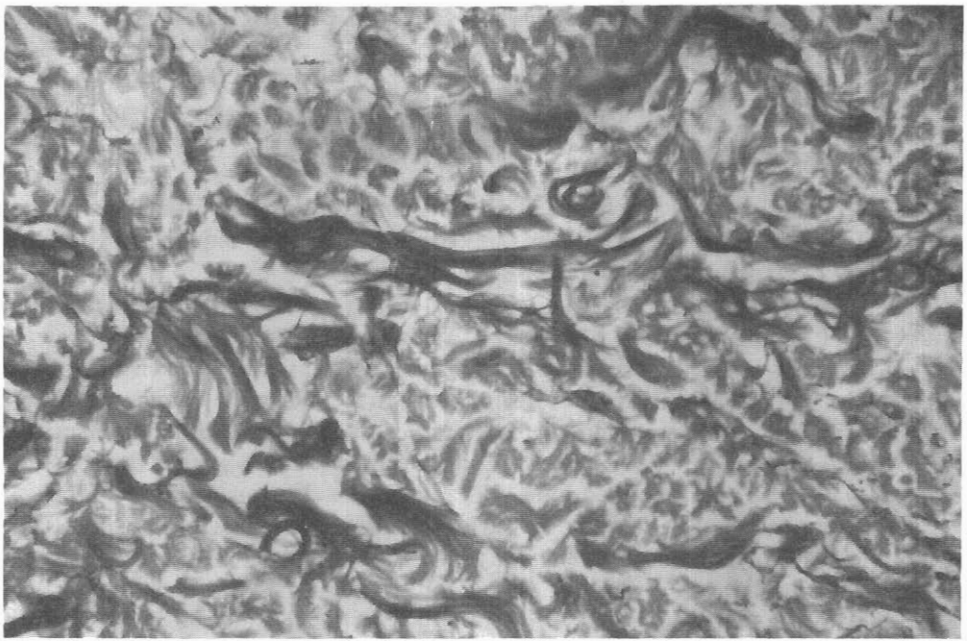


Figure 42. Dermis of abdomen skin, Melbourne biopsy (Masson and Verhoeff, x 100)

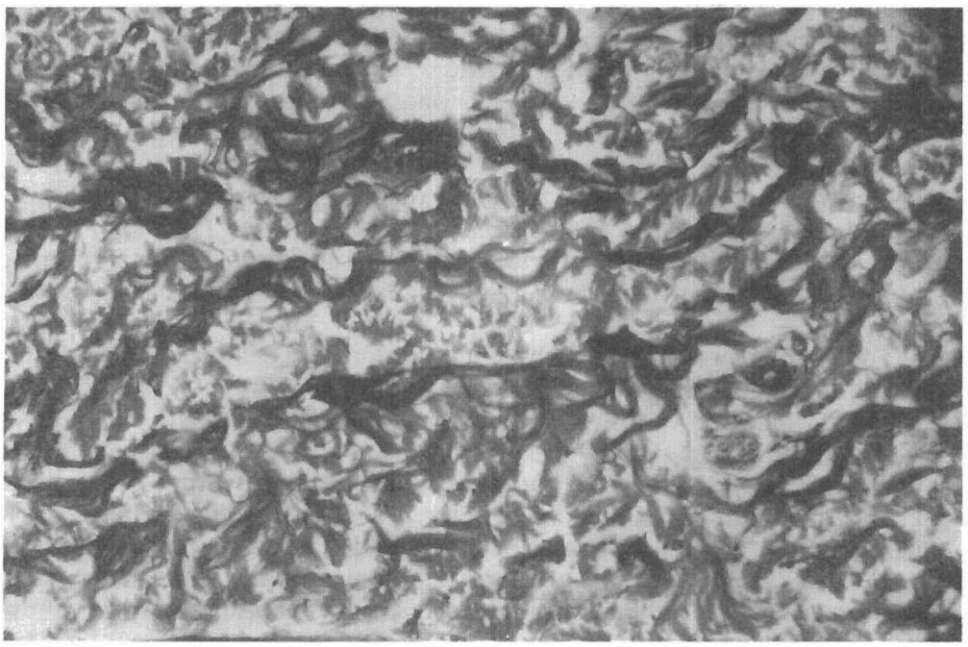


Figure 43. Dermis of abdomen skin, Antarctic biopsy (Masson and Verhoeff, x 100). The amount of elastica is slightly greater than in Figure 42.

6. Mechanisms of Responses and Acclimatisation to Cold

6.1 REVIEW OF CURRENT CONCEPTS OF REGULATORY THERMOGENESIS

Lavoissier demonstrated that heat was derived from combustion of fuels with oxygen in inanimate systems. He extended this concept to include animals, whose consumption of oxygen he considered produced heat.

There are four main biochemical heat-producing reactions: adenosine triphosphate (ATP) degradation, in which oxidation of substrate such as fatty acids leads to the synthesis of ATP; oligomycin insensitive respiration, in which oligomycin (which blocks synthesis and breakdown of ATP in isolated mitochondria) does not inhibit oxidation; loose coupling, which leads to non-thyroid hypermetabolism; and uncoupling, in which oxidative phosphorylation is uncoupled so that the substrate can be oxidised without concomitant ATP formation (Prusiner and Poe 1968).

The heat produced incidentally by the metabolism of organs is termed 'obligatory thermogenesis'. Heat output that can be controlled so that a homeotherm can maintain its temperature within the normal limits for the species is called 'regulatory thermogenesis'. Shivering is one form of regulatory thermogenesis, but a form that does not require shivering is termed 'non-shivering thermogenesis' (NST).

In terms of chemical physics 'oxidation' is regarded as the removal of an orbital electron from the atom undergoing oxidation. Hence the importance of an electron transport system in oxidation reactions.

The rate at which the transport system operates is determined primarily by the supply of substrates (adenosine diphosphate and inorganic phosphorus ion), available for oxidative phosphorylation reactions, and is normally restricted by this supply (Himms-Hagen 1965). In oxidative phosphorylation, part of the energy of oxidation goes to form a high-energy phosphate bond between adenosine diphosphate and phosphorus ion, resulting in production of adenosine triphosphate (ATP). ATP's energy can then be utilised in many reactions, such as activation of fatty acids by acetyl co-enzyme A (acetyl CoA) to form acyl-CoA. These 'active' fatty acids can then be utilised for triglyceride synthesis or can be oxidised. Such oxidation produces more heat in the Krebs cycle than would be obtained from glucose oxidation.

Abdominal viscera produce much of the obligatory heat output, but cold-adapted eviscerated rats responded to a cold stress (Depocas 1958) or to noradrenaline infusion (Depocas 1960b) with an increased metabolic rate similar to that of intact cold-adapted rats which suggests that skeletal muscle is a site of NST. The increased oxygen uptake of leg muscle of curarised cold-adapted rats exposed to cold or infused with noradrenaline could account for 57% of the total metabolic response to cold. The rest of the regulatory NST was considered to be produced by brown adipose tissue (Jansky 1966). Subsequently, noradrenaline was found to depolarise the skeletal muscle membrane activating the sodium/potassium pump, thus producing heat without shivering (Jansky et al. 1975).

6.1.1 Brown adipose tissue

Brown adipose tissue (BAT) was first described, in the marmot, in 1557 by Gesner (Smith and Roberts 1964). Bonnot (1908) made a detailed study of BAT in humans, and considered it to be the homologue of the hibernating gland in rodents. Dawkins and Hull 1964 suggested it was involved in thermoregulation of newborn rabbits. Loss of BAT in the ground hog, occurring naturally in the mating season or by surgical removal, greatly reduces its cold tolerance (Masoro 1966). Lambs up to 5 days old exhibited NST, but this had virtually disappeared by the fourteenth day, by which time BAT had also disappeared (Thomson and Jenkinson 1969). BAT represents 5% of total body weight of newborn rabbits. No significant differences were found in the activities of cellular oxidative enzymes in cold- and heat-adapted monkeys (Chaffee et al. 1966). Primates, however, vary in their BAT content: the tropical *Macaca cynomologus* has none, but it is present in *Macaca fussata*, which lives in northern Japan. In young *Macaca mulatta* exposed to cold, BAT increased, but in adults it caused little, if any, change in BAT mass, although they stopped shivering. No change was observed in the levels of oxidative enzymes (Chaffee et al. 1969). Dawkins and Scopes (1965) have proposed that NST in the human newborn infant is derived from BAT .

Studies of the newborn human infant showed that BAT is distributed in a thin, interscapular, diamond-shaped sheet separated from the overlying white adipose tissue by a discontinuous fibrous layer. Elsewhere it is present in small nodules around muscles and blood vessels of the neck, mainly along the internal jugular vein and common carotid artery, extending under the clavicles to the axillae, with further extensions surrounding the great vessels entering the thoracic inlet. Fine fingers of BAT extend from the midline with each intercostal artery. Similar deposits lie among the internal mammary vessels. Many discrete, moderately large, masses lie in the mediastinum between the oesophagus and the trachea. In the abdomen, discrete masses of BAT accompany the aorta and lie in relation to structures on the posterior abdominal wall. The largest abdominal mass envelops the kidneys and adrenals. Mediastinal and para-aortic deposits consist entirely of BAT, but at other sites it is mixed with some white adipose tissue (WAT). The amount of BAT is proportionately much less in premature than in full-term infants (Aherne and Hull 1966). Many other mammalian species have BAT rather similarly located to that of the human infant (Afzelius 1970).

Histologically WAT consists of cells containing a single, large, intra-cytoplasmic vacuole around which is a thin rim of cytoplasm. The nucleus stains darkly with haematoxylin and eosin. It is flattened, elongated and pushed to one side by the fatty vacuole. Brown fat has a round nucleus, more or less centrally placed. The cytoplasm is granular and may contain many small fatty vacuoles. The cells are pushed together so that the cellular shape is polygonal.

The colour of BAT appears to be partly due to its great vascularity, but individual cells are also somewhat brown because their composition is different from that of WAT. Furthermore there is a greater proportion of non-lipid components. BAT may become fairly light yellow and the cells may be unilocular, resembling white adipose tissue, when it has accumulated much fat. On the other hand, developing WAT may be dark yellow and its cells may be multilocular under conditions of fat depletion.

Electron microscopic examination of white adipocytes shows only a few small mitochondria, located away from the vacuoles. The brown adipocyte has an abundance of mitochondria, which are larger than those of the white adipocyte, are tightly packed and have prominent cristae. They lie close to the vacuoles (Napolitano and Fawcett 1958). In other locations where fat is being inactively stored, as with vacuoles in hepatocytes, the mitochondria are well away from the fatty vacuole. Where fat is being actively metabolised, as in muscle cells, mitochondria lie closely to the fatty vacuoles. Mitochondria of BAT increase in size and number upon cold adaptation.

White adipocytes may be scattered amongst BAT, which is regarded as a quite different tissue from WAT. It does not change into the latter during post-natal development, but is largely replaced by it.

Blood supply of brown adipose tissue

Venous drainage is into the azygos vein by a single vein with mid-dorsal recurrent loops connecting with the vertebral and intercostal veins. Antero-laterally there is a drainage via the left subclavian vein capable of countercurrent heat exchange with its corresponding artery (Smith 1962).

The direct venous connection between the interscapular BAT and the azygos vein was discovered by Sulzer in 1774. Smith and Roberts (1964) therefore refer to it as Sulzer's vein. In addition to the special vascular arrangements of the interscapular gland, these authors observed that in the rat other collections of BAT surround the peripheral vascular routes into the thorax in such a way as to provide direct thermal jacketing of all major returns from areas normally subject to environmental surface cooling.

In the human infant, venous drainage of the interscapular BAT is by veins piercing the trapezoid aponeurosis. There are also veins draining muscles of the back from the posterior vertebral veins. Anastomotic veins pass to the other side. This venous plexus drains to the venous plexuses that surround the spinal cord, which in turn drain to the jugular or azygos veins, depending on the segmental level (Aherne and Hull 1966).

Nerve supply to brown adipose tissue

Stannius in 1853 argued that the entire sympathetic nervous system developed from the interior of BAT. Even though this has been disproved, it points to the important relationship between BAT and the sympathetic nervous system (Steiner et al. 1969).

There are two kinds of postganglionic sympathetic nerve fibres supplying BAT. The conventional 'long' neurons synapse with preganglionic neurons in ganglia of the sympathetic paravertebral chain as well as with certain other specialised structures, such as the cervical or stellate ganglia. More recently, 'short' sympathetic neurons have been identified which synapse with their preganglionic fibres either within or in the immediate vicinity of their effector organs. They are especially prominent in the uterus and vas deferens as well as in BAT. The long postganglionic neurons supply arteries and arterioles, while the short postganglionic neurons supply brown adipocytes (Schönbaum et al. 1970).

Composition of brown adipose tissue

Triglycerides account for 75–90% of the total lipid content of BAT. The remainder is chiefly phospholipid and cholesterol. The proportion of protein in BAT is over four times greater than in WAT (Steiner and Cahill 1964).

Function of brown adipose tissue

Johansson (1959) suggested that BAT is a possible source of heat. He found that 90% of free fatty acids (FFA) liberated in the brown adipocyte were metabolised within the cell, being either oxidised or re-esterified. Both these reactions are exothermic.

In the cold-exposed rat, BAT becomes the primary source of heat delivered to the brain stem and the heart (Smith 1962). About 40% of the maximal metabolic response to cold in the new-born lamb is derived from BAT (Alexander and Bell 1975). The temperature of the skin overlying BAT in the marmot exceeds its rectal temperature. In human infants up to 78 days old, weighing 1300–3720 g and exposed unclothed to 25.7°C, the nape skin was the warmest skin, and although the colon was warmer, the colonic temperature decreased more than did that of the nape skin, which strongly suggests that nuchal BAT is a source of heat in the newborn human (Silverman et al. 1964).

The paired lateral arteries supplying BAT lie in close apposition to the corresponding veins, thus forming a counter-current heat exchanger in which cool peripheral blood is heated before it reaches the BAT. Thus BAT gets progressively warmer, which further accelerates its metabolism. The central venous drainage warms the spinal cord and then passes to the thorax, utilising the body core as a heat sink (Smith and Roberts 1964).

In the triglyceride cycle, lipolysis produces fatty acids and glycerol with liberation of heat. The fatty acids are then re-esterified, liberating more heat. In cold-adapted rats exposed to cold, the rate of incorporation of labelled glucose increased, indicating an acceleration of the triglyceride cycle in the BAT, while in warm-adapted rats exposed to cold the rate increased only slightly. Cold adaptation did not alter the rate of operation of the triglyceride cycle in WAT or in liver lipids (Himms-Hagen 1965).

Addition of exogenous fatty acids to BAT inhibits fatty-acid synthesis from acetate (Steiner and Cahill 1966) and from pyruvate (Schönbaum et al. 1970). Fatty acid synthesis is extra-microsomal and extra-mitochondrial whereas fatty acid oxidation occurs within mitochondria (Hittleman and Lindberg 1970).

Effects of the sympathetic nervous system and catecholamines on adipose tissue

The responses of men and experimental animals to cold stress indicate that catecholamines play an important part (2.3 and 3.3). In men, acute cold exposure causes an increase in plasma FFA (Hanson and Johnson 1965) and also increased fat mobilisation and utilisation (Wilson, Laurell and Tibbling 1969). As nicotinic acid blockade of noradrenaline-induced fat mobilisation from WAT partly inhibited the thermogenic action of noradrenaline, Masoro (1966) considered the thermogenic action of noradrenaline to be due, at least in part, to this production of FFA and their subsequent oxidation. Noradrenaline and adrenaline are equally effective in altering WAT metabolism, but their concentrations must be high to achieve this

in vitro. The concentration of noradrenaline at sympathetic nerve endings is probably much higher than the circulating level, so it is likely to be the more important thermogenic hormone in vivo (Cahill et al. 1960).

Adipose tissue from cold-adapted rats is more sensitive to the lipolytic effects of noradrenaline and their tissues have an increased capacity to oxidise fatty acids (Hsieh et al. 1966). The high fatty-acid concentration then stimulates oxidation of FFA. Cold-induced hyperplasia of BAT may be caused by noradrenaline or some other sympathetic nervous effect (Cottle 1970).

Infants 6 to 12 days old and weighing 2600–3500 g exposed to an ambient temperature of 18–20°C showed increased plasma FFA and urinary noradrenaline excretion compared with infants in 32–34°C ambient temperature. Adrenaline excretion was in trace amounts in all instances. Infusion of noradrenaline while the infants were in warm surroundings more than doubled the plasma FFA concentration (Schiff et al. 1966).

Catecholamines promote thermogenesis by initiating a sequence of intra-cellular biochemical events within BAT, beginning with the activation of adenyl cyclase, which promotes the production of cyclic adenosine monophosphate (cyclic AMP) from adenosine triphosphate (ATP). Cyclic AMP activates lipase and lipolysis begins. Catecholamine-induced lipolysis in WAT is also mediated by cyclic AMP (Sutherland and Robinson 1966). The loosening of coupling between electron transport and phosphorylation leads to a reduction in the amount of energy going into the formation of high-energy phosphate bonds involved in ATP synthesis. More energy therefore appears as heat. This uncoupling of phosphorylation from oxidation is brought about by the increased concentration of FFA resulting from noradrenaline-stimulated lipolysis (Prusiner and Poe 1968). Brown fat mitochondria can also bring about coupling, leading to oxidative phosphorylation of fatty acids and thus reducing the heat output as required (Hittleman and Lindberg 1970). Noradrenaline and cyclic AMP serve only to mobilise fatty acids and do not affect their oxidation in the mitochondrion (Prusiner et al. 1970).

Cold-adapted rats living in the cold (4°C) excrete more cyclic AMP than do warm-adapted rats at 26°–28°C, but on re-exposure to cold after a few days in the warm, their excretion of cyclic AMP is similar to that of the warm-adapted rats exposed to cold for the first time. This suggests that acclimatisation to cold does not effect the adenyl cyclase system (Muirhead et al. 1974). Nevertheless, when BAT obtained from warm-adapted rats was incubated with noradrenaline, the cyclic AMP synthesis increased, correlating with triglyceride hydrolysis, whereas the increase in cyclic AMP in BAT from cold-adapted rats, treated similarly, was not associated with increased lipolysis possibly either ATP was depleted in the already active tissue or, the high concentration of FFA in BAT may inhibit further lipolysis (Dorigo et al. 1974).

The effects of cold-acclimatisation and noradrenaline on adipose tissues are summarised in Table 11.

Studies in the uptake of glucose-U-^{14}C by the lipids of rats showed that acclimatisation to cold was associated with an increase in glucose uptake by BAT, the uptakes being the same whether the animals were immunosympathectomised or not. No changes were observed in WAT or liver (Steiner et al. 1968). These findings suggest that cold-acclimatisation causes an intrinsic alteration in BAT, though alterations in blood flow in vivo are also important (Steiner et al. 1969).

Metabolic pathway		*CA*	*NA*
Glucose → glyceride-glycerol	WAT	unchanged	unchanged
	BAT	increased	unchanged
Glucose → CO_2	WAT	unchanged	increased
	BAT	increased	decreased
Glucose → fatty acids	WAT	unchanged	unchanged
	BAT	increased	decreased

Table 11. Metabolic activity of white (WAT) and brown (BAT) adipose tissue: effects of cold adaptation (CA) and noradrenaline (NA)

In explaining the action of the sympathetic nervous system and its relationship to adrenaline and noradrenaline, Ahlquist postulated in 1948 that they acted on two kinds of specific receptor sites in the effector organs. Stimulation of ∝-receptors resulted in activation of the organs while stimulation of β-receptors resulted in depression in most organs but activation in the heart. Adrenaline stimulates both ∝- and β-receptors, whereas noradrenaline stimulates β-receptors but not ∝-receptors, except in the heart (Gardiner 1969).

Stimulation of the long postganglionic fibres or administration of noradrenaline and adrenaline causes dilatation of the arteries and arterioles supplying BAT (Schönbaum et al. 1970), which is the opposite of the effect of noradrenaline on all other arteries and arterioles except the coronary arteries, which are unaffected by noradrenaline. Thus the receptors are not typically of β type. By using ∝ and β blocking agents during incubation of BAT with noradrenaline, the β-receptor was found to be associated with the activation of adenyl cyclase and the ∝-receptor inhibited adenyl cyclase and reduced cyclic AMP accumulation (Dorigo et al. 1974).

A possible mechanism of action of catecholamines at the receptor level appears to be the triggering of a response by an electrostatic interaction of the agonist and the receptor site. It seems that the primary triggering of a response involves the pairing of an ammonium ion with a negative charge on the receptor. The anionic species on the receptor appears to be a phosphate ion. Thus noradrenaline appears to trigger an excitatory response through a bimolecular interaction involving electrostatic field effects and van der Waal's forces (Belleau 1960).

Noradrenaline is present mainly in vesicles in sympathetic nervous tissue. There appears to be an active transport mechanism, with the transport site maintaining a higher concentration of noradrenaline in the cell than in extracellular fluid. BAT has a higher turnover of noradrenaline than have other tissues. Noradrenaline labelled with tritium was lost from heart and brown fat at a greater rate in rats kept at 5°C than in those kept at 25°C. The release of noradrenaline by sympathetic nerve endings in brown adipose tissue was greater in rats cold-adapted at 5°C than in rats kept at 25°C. The additional noradrenaline released in the cold was associated with a fall in the noradrenaline content of the tissue during the first 6-hour exposure at 5°C, but in rats adapted to 5°C, the tissue concentration of noradrenaline did not decrease during 6 hours at 5°C. The rate of noradrenaline synthesis does not, therefore, increase immediately on exposure to cold unless there has been previous cold adaptation (Kopin 1964).

6.1.2 Effects of noradrenaline on pulmonary function

In addition to promoting thermogenic reactions, noradrenaline was found to increase pulmonary function in rats. Noradrenaline caused an 85% increase in the minute volume in cold- and warm-adapted rats. In warm-adapted animals this increase was due to increased respiratory rate, but in the cold-adapted the increase was mostly due to increased tidal volume, with only a slight increase in rate. Apart from a changed pattern of response to noradrenaline, the cold-adapted rats had a greater efficiency of oxygen extraction before, during, and after noradrenaline infusion. Such enhanced pulmonary function facilitates combustion of subtrates mobilised by noradrenaline. This change in respiratory function may be a reflex response to increased carbon dioxide produced from noradrenaline-stimulated fatty acid metabolism. Nevertheless, the different kinds of pulmonary function responses observed in warm- and cold-adapted rats suggest that noradrenaline has a direct effect on pulmonary function, matching the increased oxygen demands brought about by the noradrenaline-stimulated increase in metabolism (Evonuk and Hannon 1963).

6.1.3 Neural integration of thermogenic mechanisms

The homeotherm's ability to prevent core hypothermia in the presence of a cooling load depends on a neural integration that activates thermogenic processes by nerve impulses from thermosensitive areas in the hypothalamus and the cervical spinal cord (Brück and Wünnenberg 1966).

The pre-optic anterior hypothalamic region is the location of warm-and cold-sensitive elements, but there are separate neuron pools, located in the pre-optic anterior hypothalamus and posterior hypothalamus that are concerned with the control of responses to heat and cold respectively. There is evidence of reciprocal inhibition between these two neuron pools. The location and function of the central thermosensitive areas in humans is not known. Clinical and necropsy studies suggest that they are in the hypothalamus (Bligh 1973a). There also appears to be fine and coarse thermal controls, the latter preventing extreme deviations from normal, the primary sensors being activated at 41°C and 36°C. This coarse control may depend on structures outside the hypothalamus (Bligh 1973b). Warming and cooling units have been located in the ventrobasal thalamus of the cat, receiving stimuli from the entire body surface (Martin and Manning 1971).

In the guinea pig, the onset of shivering depends on an inverse relationship between the temperature of the subcutaneous tissue and the shivering centre in the cervical spinal cord. If this shivering centre is kept at a sufficiently high temperature, shivering is suppressed at comparatively low surface temperatures (Brück 1970).

An inverse relationship has been found in the guinea pig between the temperatures of the body surface and the temperature-sensitive areas of the pre-optic and supra-optic area of the anterior hypothalamus. Cooling the anterior hypothalamus abolishes suppression of NST, permitting thermogenic activity in BAT at a surface temperature at which NST would not be elicited if the anterior hypothalamic centre had been warmer. This inverse relationship is similar to that between the tempera-

tures of the surface and of the spinal cord shivering-centre (Brück 1970). If the heat output of BAT is increased, the skin temperature can drop to a lower level before shivering starts because the spinal cord shivering-centre has been kept warm by efferent blood from BAT, and hence suppresses the hypothalamic shivering-centre. Further confirmation of this concept was obtained by a reverse experiment, in which warming the pre-optic area of the hypothalamus caused increased shivering in cold-exposed rats, presumably due to suppression of NST (Fuller et al. 1975).

The newborn miniature pig does not possess BAT and depends entirely on shivering for thermoregulation. Shivering can be suppressed by heating the anterior hypothalamus, in contrast to the effect in the guinea pig. The temperature of the cervical spinal cord does not appear to influence shivering.

It seems that in those species possessing BAT and the ability for NST, there are two functionally different sets of inner receptors, localised at different sites. In species not possessing BAT and hence lacking the ability for NST, there may also be two separate internal receptors, but they are used jointly to control shivering (Brück 1970).

Noradrenaline injections into the lateral ventricle of the ox did not result in increased heat production, which suggests that catecholamines are not involved in the cold-response of large animals (Findly and Thompson 1968).

The vascular anatomy of the interscapular BAT, as noted above for the rat (Smith 1962, Smith and Roberts 1964) and human infant (Aherne and Hull 1966), is such that in these species shivering is most probably suppressed by warm blood from BAT flowing near the spinal cord shivering-centre, activating warm sensors that suppress the hypothalamic shivering centre.

Observations on the cat indicate that temperature-sensitive structures are in the medulla oblongata which may be concerned with severe deviations in core temperature (Bligh 1973c).

The cervical spinal cord, and possibly also the medulla oblongata, may be important in the adult human response to acute cold stress. Rascher found that men immersed in a brine solution below 0°C had a longer survival time if the nape of the neck was kept clear of the brine (Mitscherlich and Mielke 1949). These experiments resulted in the death of the subjects, probably due to direct cold inhibition of vital centres in the medulla oblongata rather than interference with thermogenic mechanisms.

The longer delay in the onset of shivering associated with a lower skin temperature in the late Antarctic series than in the earlier ones therefore suggests NST, which suppressed shivering, had developed. As the human infant is well endowed with BAT and exhibits NST, it appears that chronic exposure of the adult to cold leads to proliferation of BAT and hence the redevelopment of the infantile capacity for NST. The establishment of this mode of thermogenesis appears to take about 8 months. Probably the rate of BAT hyperplasia is the limiting factor.

Shivering occurred in the Nepalese pilgrim when his rectal temperature fell below a critical level (Pugh 1963). Because of his mode of life, he almost certainly experienced more sustained and severe cold stresses than cold-climate indigenes hitherto investigated, and certainly his cold exposure was far greater than that of the subjects in this investigation. It may be supposed all his cold-adaptive mechanisms were optimally adjusted. In this situation rectal temperature may be a good index of the temperature of the spinal shivering-centre.

6.1.4 Responses to different kinds of cold exposure

Noradrenaline plays a very important part in thermogenesis and cold acclimatisation in those species endowed with BAT. Nevertheless, cold-adapted rats show greater variability in their oxygen consumption response to a cold stress than to endogenous noradrenaline liberated by intraperitoneal tyramine injection. The tyramine response can predict the maximal metabolic capacity, but not the metabolic endurance in a lethal cold exposure. Therefore factors other than noradrenaline-mediated NST are involved in sustained metabolic response to severe cold. The adrenals as well as peripheral vascular control in heat conservation (Heroux et al. 1975), appear to be essential.

Catecholamines, however, are important in the adaptation of the rat to continuous moderate cold exposure, and this is probably related to an enhanced response of beta receptors. Catecholamines do not appear to be important in the cold adaptation induced by intermittent severe cold exposures. The colonic temperature during prolonged cold exposure is sustained better in rats adapted by continuous moderate exposure than in those adapted by intermittent severe exposure (LeBlanc 1975).

Noradrenaline has a slight thermogenic effect in Korean Ama diving women, but it is regarded as too small to indicate the development of NST (Kang et al. 1970). These women are probably chiefly dependent on insulation derived from altered vascular dynamics. NST in adult humans may, therefore, represent only a small part of their total acclimatisation to cold. The system chiefly responsible for maintaining thermal balance may depend on the kind of cold stress. The Ama are subjected to intermittent immersions in cold water, whereas the Nepalese pilgrim, who was continuously exposed to cold air, appeared to maintain thermal balance mostly without shivering.

6.1.5 Catecholamines and heat conservation

Adrenaline, as noted above, is thought to provide not only an immediate response in the unacclimatised animal, but also a back-up facility when the noradrenaline-mediated system in the acclimatised animal is overloaded. Apart from any metabolic stimulation, adrenaline has well-known heat conservation effects, such as vasoconstriction of skin vessels, and it promotes ruffling of feathers in birds and pilo-erection in mammals (Cannon et al. 1927).

In humans, pilo-erection is shown by 'goose-flesh' on exposure to cold, but because of poor hair covering, this mechanism is ineffective in promoting heat conservation. On the other hand, skin vasoconstriction in response to cold is very conspicuous in humans. Reduction of blood flow through the skin reduces the heat loss from blood flowing near the body's surface.

Mice that were given β-receptor blocking agents just before a cold stress showed impaired heat production compared with untreated rats, but pilo-erection and shivering were still present (Estler and Ammon 1969).

The fractional blood flow to small bowel, caecum, liver, tail-skin, ears and feet was greater in cold-adapted than in warm-adapted rats. However, in cold-adapted rats, noradrenaline caused a greater reduction in fractional blood flow to tail-skin,

ears and feet, and a greater increase in fractional blood flow to heart, liver and brown adipose tissue than in warm-adapted rats (Evonuk and Hannon 1962).

The response to adrenaline and noradrenaline in rabbits acclimatised to 5°C showed less bradycardia. Ear blood flow was not so markedly reduced and ear temperature decreased less than in animals adapted to 27°C. Thus, although the classical effects on blood flow of adrenaline and noradrenaline were observed in both groups of animals, they were much less marked in the cold-acclimatised group (Honda et al. 1962).

Subsequent studies on the vascular response in the intact perfused ears of cold-adapted and warm-adapted rabbits showed that at an ambient temperature of 30°C, the vascular bed of the ear of warm-adapted rabbits was more sensitive to noradrenaline than was that of cold-adapted rabbits. This is in agreement with earlier work cited above. However, at 20°C there was no difference in the responses of the two groups, and at 10°C the cold-adapted rabbit was more sensitive to noradrenaline (Reite et al. 1966).

The role of noradrenaline in modifying haemodynamic responses during cold acclimatisation is uncertain, but the sensitivity of vascular musculature appears to be reduced as a consequence of cold acclimatisation.

The responses of blood pressure to the standard cold stress (3.5) did not change significantly, but there were linear increases over the year in the basal diastolic blood and basal pulse rates (4.1). These effects were probably related to the increase in adrenaline excretion in the latter half of the year, which was probably not a climatic effect (2.3).

Although animal experiments have given varying results, they do suggest — as do human experiments — that there is a reduction in vascular sensitivity to noradrenaline associated with cold adaptation. The lower rectal temperature and warmer skin cannot be explained solely as an effect of altered catecholamine responses. The changes observed in this work suggest there is a slight lowering of the body 'thermostat' and shunting of peripheral blood by mechanisms that do not appear to be related to catecholamines.

6.1.6 Adaptation of mammalian cells to cold

Apart from the cells of the highly specialised brown adipose tissue, which functions specifically to produce heat in response to a cold stress, there is evidence that other mammalian cells can undergo changes that make them more resistant to a cold environment. Using the tissue cultures of mouse fibroblasts, Michl and Svobodova (1968) showed that L-fibroblasts could be altered by repeated exposure to cold. The resulting cells were designated LC2 fibroblasts. They have a shorter lag after subcultivation and continue to multiply after 5 days at 37°C, when L cells enter the stationary phase. LC2 cells formed a greater pool of intermediates. There was increased stability of ribonucleic acid (RNA) in the LC2 cells. Ribonucleotides extracted from L and LC2 cells were added to the medium and the cold resistance at 4°C of unadapted L cells was determined and compared with L cells grown in the usual medium as a control. After 4 weeks at 4°C, about 25% of L cells survived, but about 60% survived in media enriched with ribonucleotides. This enhanced resistance to cold appears to be due to the size of the RNA-precursor pool, as the survival rate of the wild-strain L fibroblasts was virtually identical to that of the

LC2 cells, whether the medium was enriched with ribonucleotides from cold-adapted or unadapted fibroblasts. The difference in ^{3}H uridine pool between the two kinds of cells was greatest in the lipidic extract.

The nature of the usable lipidic extracts is not known, but there is some rather equivocal evidence that a high fat diet is beneficial for the life of the whole organism in the cold (Masoro 1966).

It seems, therefore, that cold-acclimatisation affects the functioning of all nuclei, which may enhance survival quite apart from the development of NST and changes in the vascular response to cold. Nevertheless, Michl and Svobodova (1968) pointed out that there was no evidence of whether cold-adapted animals showed RNA stabilisation. They unsuccessfully attempted to adapt human diploid cells, which contain highly unstable RNA, to cold.

6.2 SYNTHESIS AND CONCLUSIONS

6.2.1 Synthesis

The essential findings of this work have been summarised in the conclusions reached for each section. Here, these conclusions will be considered in relation to each other and interpreted in the light of the state of knowledge of thermoregulation as set out in the background to each section and the literature review in 6.1.

Thermoregulation is dependent on the combined effects of thermogenesis (shivering and non-shivering) and heat conservation (in a cold climate) or heat dissipation (in a hot climate).

The results strongly suggest that noradrenaline plays an important role in the NST of cold-acclimatised adult men. This is inferred from the similarity of the 24-hour excretion pattern to the pattern found in rats during prolonged cold exposure. Furthermore, the decrease in noradrenaline response to standard cold stresses during the year closely resembles the reduction in response observed in cold-adapted rats re-exposed to cold. These similarities to rats are important, because there is a substantial body of experimental evidence that noradrenaline is the mediator of NST in that species.

Animal experiments have also shown that an important source of the noradrenaline-stimulated heat is brown adipose tissue, though skeletal muscle may also be a source. Noradrenaline has been shown to mediate non-shivering thermogenesis in the newborn of many mammalian species, including humans, all of which are well endowed with brown adipose tissue. It is postulated from the findings of this work that after prolonged intermittent cold exposure, adult men partially re-establish this infantile thermogenic mechanism. This necessitates regeneration of brown adipose tissue, which was probably rather slow under the conditions experienced. From in vitro experiments, brown adipose tissue appears to develop metabolic alterations associated with cold acclimatisation.

Further evidence for development of brown adipose tissue was the increasing delay in the onset of shivering during the standard cold stresses. This was associated with a lower trunk skin temperature at the onset of shivering. Guinea pig experiments and anatomical studies of the vasculature of the human infant's brown

adipose tissue indicate that blood warmed by brown adipose tissue is directed over the spinal cord shivering-centre, suppressing shivering at a skin temperature at which shivering would occur in the unacclimatised subject. Although skeletal muscle may provide the greater proportion of NST, that derived from BAT is the most effective in suppressing shivering. The reduction during the year in the trunk-skin temperature at the onset of shivering refutes the widely accepted view that skin temperature alone stimulates shivering. It is the interaction of skin temperature and spinal cord shivering-centre temperature that determines when shivering will start. In addition, the onset of shivering was unrelated to rectal temperature: shivering often starting when the rectal temperature was rising.

The adrenaline excretion response to a cold stress was unaltered throughout the investigation, which suggests that, as with rats, adrenaline mediates non-shivering thermogenesis in acclimatised and non-acclimatised humans. The rising 24-hour adrenaline excretion over the latter half of the year suggests a response to non-climatic factors, probably ones associated with inner tensions related to living in a small, isolated group. The rising basal diastolic blood pressure and pulse rate noted during the year were probably a consequence of the raised adrenaline level. The low adrenaline excretion in the early months excludes psychological stress as a cause of the high noradrenaline excretion on arrival in Antarctica, giving further support to the view that the changes in noradrenaline excretion were of climatic origin.

A personality assessment of the subjects did not reveal any consistent catecholamine excretion patterns referrable to personality with the exception of one subject whose adrenaline excretion was somewhat higher than his usual level during a period of stress.

The plasma cortisol response to a cold stress diminished in Antarctica. Similarly, cold-adapted rats have a reduced corticosteriod requirement. This provided confirmatory evidence that the subjects had become cold-acclimatised, but the temporal relationship was quite different from that of catecholamines, fluctuations being more apparent than in catecholamines. It is not clear from this work how this reduced cortisol response affects the organism's ability to survive, nor how it is related to other acclimatisation mechanisms. It may be that the reduced cortisol response is simply due to the acclimatised body recognising the cold stress as less stressful than when unacclimatised.

Cold-acclimatisation would be expected to result in improved heat conservation. This could be enhanced by altered vascular dynamics and by deposition of subcutaneous fat to increase passive insulation. Although measurements of body temperature during standard cold stresses were intended to ascertain whether acclimatisation had taken place, they resulted in the unexpected discovery of early and late forms of acclimatisation.

The early form of acclimatisation is characterised by increased vasoconstriction, resulting in more pronounced peripheral cooling than in the unacclimatised subject, but rectal temperature was higher and better maintained during the cold stress. A late form developed in which the rectal temperature remained at a lower level during the cold stress than in the early acclimatised or unacclimatised states. In the warm phase before the cold stress, the late form exhibited a slightly, but not significantly, lower rectal temperature and a significantly higher toe-skin temperature than previously.

The smaller rise in rectal temperature in the late form of response to a cold stress and the trend, even though slight, for the rectal temperature to be set at a lower level have the advantage of reducing the temperature gradient between the core and the shell of the body. Rectal temperature maintenance was similar to that of the early form. The late form became fully developed at the end of the Antarctic year, with a pronounced reduction in peripheral skin cooling during the cold stress. Also, more rapid rewarming of the periphery occurred after the cold stress. Perhaps these changes are due to shunting of warm blood through the skin vessels, as in Eskimos, and improved counter-current heat exchange, as in Korean diving women, providing good peripheral circulation while conserving heat.

As was seen in the literature surveys, there is disagreement as to the cold-acclimatisation pattern in peripheral and rectal temperature responses to a cold stress. This may in part be due to the very small numbers of subjects used in previous work. Nevertheless, the elucidation of early and late forms of cold acclimatisation resolves some of the observed differences. The responses of the controls used in earlier work was similar to those in the early form, whereas the responses of cold climate indigenes closely resemble those observed in the late form of acclimatisation. The different responses previously observed in cold-climate indigenes may be due to different, and uncertain, levels of acclimatisation, as very few now live completely in the natural state.

There was a slight increase in the thickness of subcutaneous fat, but this appears to be due to an excessive food intake rather than to be a specific form of cold acclimatisation. The resultant insulation appears to be derived from the interplay of adjustment of vascular dynamics and the subcutaneous fat thickness. In one subject who drastically reduced the thickness of his subcutaneous fat, rectal temperature maintenance was impaired compared with his earlier responses, and was much inferior in this regard to subjects who had always been lean. It appears that the fat person relies on the insulative properties of fat, and in his case fat was lost faster than the rate of adaptation of vascular dynamics.

The early form of cold acclimatisation would increase the likelihood of loss of limbs through cold injury, but would give the organism a greater chance of survival than the unacclimatised organism. The late form, however, would improve the organism's ability to survive without loss of the peripheral tissue, and the lower core temperature would reduce energy demand.

Altered levels of physical fitness have been excluded as a possible cause of the changes in temperature response patterns to the cold stresses.

The development of NST and the late form of body temperature responses developed at about the same time, but there is no evidence from this investigation, or from the literature, to suggest they are inter-related. Nevertheless, vascular responses to cold, shivering and NST are under the control of the hypothalamus, so there may be some central nervous coordination of the various modalities of cold-acclimatisation.

There is evidence of racial differences in the ability to withstand cold, and cold exposure in early childhood may be even more influential than race. The subjects in the present study were of substantially the same race and had much the same early childhood thermal histories. Hence, it was not possible to clarify these points in this investigation.

The pattern of the 24-hour urine excretion suggests that a reduction in the volume of body water is part of the acclimatisation process. The diuretic response to a standard cold stress appears to have become more sensitive in Antarctica than in Melbourne. The advantage to the organism may be that it reduces the non-heat-producing mass, so less heat is required to maintain and restore the body's temperature.

The blood pressure increased and pulse rate decreased on exposure to the standard cold stresses, but no consistent changes in their response patterns were observed during the year in Antarctica. Likewise, no change was apparent in the increase in oxygen consumption due to the cold stress.

In Antarctica, the epidermis thickened and the number of granular cell layers increased in the skin from the dorsum of the hand, but no changes were observed in the skin from the lower abdomen. There was less elastica in the dermis from the dorsum of the hand. As the changes were found only in the skin from the intermittently exposed hand, it seems that they were due to direct effects of cold. The lack of changes in the covered skin of the abdomen shows that the cold climate did not induce systemic acclimatisational changes in the skin structure.

6.2.2 Conclusions

This study is unique, as never before has such advanced technology been used in studying human acclimatisation to cold in a remote, isolated area. This was the first occasion in which catecholamines have been determined in Antarctica. The number of subjects tested was far larger than before, much of the work involving all twenty-four members of the expedition. Furthermore, the subjects were tested at much more frequent intervals than in previous studies, thereby revealing changes in adaptational patterns throughout the year that would have been missed had the tests been sporadic.

This study of men from a temperate climate who spent 13 months in Antarctica shows that:

1. They exhibited changes in temperature responses to a cold stress; these are deemed to represent cold acclimatisation.
2. This cold acclimatisation develops early and late forms. The early form is characterised by increased peripheral cooling during a cold stress. The late form shows reduced peripheral cooling during, and enhanced rewarming after, a cold stress, compared with the early form and with the unacclimatised state. The rectal temperature is lower during a cold stress than in the early form of acclimatisation or in the unacclimatised subject. It is also better maintained in both forms of acclimatisation than in the unacclimatised state.
3. Non-shivering thermogenesis developed.
4. Noradrenaline mediates this non-shivering thermogenesis, the infantile mode of thermogenesis being partially re-established.
5. Adrenaline is equally involved in the response to cold of acclimatised and non-acclimatised subjects.
6. The conditions of life in a small, isolated group of men caused largely concealed psychological tensions, which resulted in increased adrenaline excretions, basal diastolic blood pressure and basal pulse rate.

7. There was reduced corticosteroid response to cold stress.
8. The epidermis thickened and the elastica in the dermis was reduced as a direct response to cold. No systemic effects of cold on skin structure were observed.

It is considered that these results make a significant contribution to understanding the acclimatisation of humans to cold in Antarctica.

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Appendix I. Statistical Methods

The paired t test was used to assess the significant levels of differences occurring in the variables measured before and at the end of the standard cold stress in each series. This method of analysis was also used to compare the pre-Antarctic values with those of the Antarctic, follow-up and control group series. The two-tailed probability level was used, as no particular direction of change could be presumed.

Regression analyses were used to evaluate trends in the Antarctic data. The value of a particular variable for subject i at t, the time spent in Antarctica, may be denoted by $y_i(t)$. For each subject assuming a quadratic regression Q1 --- $y_i(t) = a_i + b_i\, t + C_i\, t^2$.

The coefficients a_i, b_i and C_i are estimated by least-squares from the measurements taken at times t = March, June, September and November. If, apart from absolute individual differences, each subject acclimatises in the same way, this can be represented by an equation Q2 where the coefficients b and c are constant. Q2 --- $y_i(t) = a_i + b\, t + c\, t^2$. The adequacy of this simpler equation can be tested by comparison with Q1 by analysis of variance. If Q2 is not significantly different from Q1, a common form of acclimatisation is indicated for each subject. If a common a_i co-efficient produces an adequate fit, the subjects may be regarded as having come from a homogeneous population with model
Q3 --- $y_i(t) = a + b\, t + c\, t^2$.

Similarly, it can be determined whether the quadratic term is necessary or whether a straight line would provide an adequate model

L_1 --- $y_i(t) = a_i + b_i\, t$ (which allows each subject to have a different straight line response)

L_2 --- $y_i(t) = a_i + b\, t$ (which allows each subject to have different absolute values but a common slope) and

L_3 --- $y_i(t) = a + b\, t$ (which corresponds to the subjects behaving in a homogeneous way).

Constant models correspond to no change in the variable with the time in Antarctica, that is, there is no acclimatisation.

C_1 --- $y_i(t) = a_i$ (different constants for each subject) and

C_2 --- $y_i(t) = a$ (the same for each subject).

The regression analyses were carried out using GENSTAT on the CSIRO Cybar 76 computer.

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