Energy exchanges and injury

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The practical application of calorimetry to the study of energy expenditure after injury did not begin until 1930, with the work of Cuthbertson in Glasgow, who used relatively crude indirect calorimetry techniques to study patients with bone fractures. During the late recovery phase, he found increases in oxygen consumption of 15–25% above resting values, associated with a rise in rectal temperature (Cuthbertson, 1932). These increases in metabolic expenditure were linked with evidence of increased protein breakdown (Cuthbertson, 1930, 1931), thought to originate in muscle (Cuthbertson, 1936). The injured rat displayed similar changes (Cuthbertson, 1939). Over the next two decades interest in the energetic consequences of injury waned, largely as a result of the poor accuracy and cumbersome nature of the calorimeters then available. Using radioisotope tracers and other advances, research into the metabolic response to injury centered upon biochemical, hormonal and cellular changes (Richards, 1977). Further indirect calorimetry studies were made (Cope, Nardi, Quijano, Rovit, Stanbury & Wright, 1953) in the early 1950s but they could not identify the cause of the hyper-metabolism following injury.

Advances in the study of energy expenditure after injury did not take place until the development of gradient-layer calorimetry (Benzinger & Kitzinger, 1949). The adiabatic principles which had been responsible for the slow response time and relatively poor performance of earlier calorimeters were replaced by direct measurement of the instantaneous flow of heat through the walls of the chamber. The sensible and insensible heat loss of the subject could be measured continuously and automatically. A calorimeter of the Benzinger type was constructed at the Rowett Research Institute (Pullar, 1956, 1957, 1969), and later was used by Cairnie, Campbell, Cuthbertson & Pullar (1957) to measure energy expenditure after bone fracture in groups of rats. They found increases in metabolic rate of up to 7% above normal resting values in rats kept at an ambient temperature of 19° after unilateral femur fracture. By direct and indirect measurement, the total metabolism of rats fed on 18·1% protein diet was 608 kJ/$kg^{0.75}$ per d before injury. This increased to a maximum of 637 kJ/$kg^{0.75}$ per d on the third post fracture day and was closely paralleled by an increase in urinary nitrogen losses from 360 mg/d before, to a maximum of 478 mg/d on the third day after injury. The calculated

energy provided by oxidation of the quantity of protein was 126 kJ/kg$^{0.75}$ per d before and 155 kJ/kg$^{0.75}$ per d after injury. This difference was exactly sufficient to explain the 29 kJ increase in total metabolic expenditure measured by calorimetry. There was no evidence of an increase in fat or carbohydrate metabolism, and it was concluded that the post-injury increase in resting metabolism was 'heat resulting from the process of deamination of protein amino acids and from the use of the non-nitrogenous residue as a source of energy' causing a form of endogenous specific dynamic action. Catabolism of muscle protein stores in these circumstances was seen as an obligatory part of our primitive metabolic response to severe injury providing energy and water to sustain life and to repair tissues, where the ability to provide adequate nutrition might be restricted.

Cairnie's views on the importance of post-injury protein catabolism were challenged by Miksche & Caldwell (1967) who studied energy expenditure after severe bone injury in the rat using a specially constructed, gradient-layer calorimeter (Caldwell, Hammel & Dolan, 1966). After bone fracture, fasted male albino rats had a significant increase of 14.20% in heat production with increased sensible heat losses and an increase in average body temperature (0.6–0.76°). Between 40 and 70% of the increase in heat production after injury could be accounted for by the van't Hoff Q_{10} effect, secondary to elevation of the average body temperature. The energy equivalent of the increased urinary N excreted (allowing 110 kJ/g N, West, 1961) accounted for only 24% of the increase in heat production over the first 4 d after injury. Respiratory quotient (RQ) measurements indicated that control and injured rats alike utilized fat as their main energy source (RQ 0.7–0.73).

Direct comparison of Cairnie's and Miksche's observations is misleading because of significant differences in experimental design. These are presented in detail (Table 1) to illustrate the problems of interpretation which exist in much of the literature regarding changes in energy after injury.

The significance of changes in protein degradation after bone fracture in the rat and their relationship to energy expenditure remain unresolved. However, considerable indirect evidence has been obtained in nutritional studies on rats with burn wounds (Caldwell, 1961, 1962; Caldwell, Osterholm, Sower & Mayer, 1959) and in calorimeter studies of burned rats (Caldwell et al. 1966) that increased proteolysis alone does not account for the measured post-injury increase in energy expenditure. Evaporative heat losses increased up to five times control values after thermal injury but no increment in sensible heat loss occurred. Total heat losses increased 47.6% above control values and were sustained, continuing beyond the 43rd post-burn day. The increased protein excreted contributed only 20% of the total energy expenditure before and after burning (Caldwell, 1970). Cuthbertson's early hypothesis that protein was utilized exclusively as a fuel in order to meet increased energy needs after injury was therefore not supported by subsequent experimental evidence.

In man, studies were carried out with a practical indirect calorimetry system constructed for use in a clinical situation (Kinney, Morgan, Domingues & Gildner,

Table 1.   *Comparison of the experimental conditions of rat calorimetry used by two sets of investigators (Cairnie et al. 1957; Miksche & Caldwell, 1967)*

	Cairnie *et al.* (1957)	Miksche & Caldwell (1967)
Calorimeter	23 kg in adapted gradient layer	Single rat size, purpose-built gradient layer
Measurement period	22 out of every 24 h continuous measurement	3 periods of 20 min per 24 h
Rats, number in calorimeter	9 rats packed into boxes within calorimeter chamber	Single
Rats, number in study	9 in calorimetry group, 6 in separate metabolic cages. Urine nitrogen estimated on the latter	12 in calorimetry group. 12 in a separate metabolic group. Urine nitrogen estimated on latter
Injury	Single femur fracture, open method	Bilateral femur fracture, closed method
Feeding regime	Rats restricted after fracture to mean pre-injury *ad lib.* intake	*Ad lib.* throughout
Duration of urine collections	24 h period	(14·01–23·59 hours) 10 h post-prandial period
Animal house temperature	19±2°	25±1°
Calorimeter operating temperature	19±0·3°. Below rat thermoneutral zone	28·4±0·23°. Within rat thermoneutral zone
Dietary status of rats during calorimetry	Not fasted, fed during calorimeter runs	18 h post-absorptive during calorimeter run

1964) on energy expenditure in relation to metabolic changes in a wide variety of surgical conditions (Kinney, Long, Gump & Duke, 1968; Kinney, Long & Duke, 1970*a*; Kinney, Long, Duke & Gump, 1970*b*). Very small increases in energy expenditure, less than 10%, occurred after elective surgery. Multiple bone fractures were followed by increases of 15–30% for up to 2–3 weeks. Major sepsis, such as peritonitis, caused increases of 20–50% and extensive full skin thickness burns caused the greatest and most sustained increases of up to 125% above predicted normal values.

Surgical patients (Kinney & Roe, 1962; Roe & Kinney, 1965) showed variations between their rise in body temperature and increase in resting metabolic rate similar to those observed in certain clinical infections (Du Bois, 1924) so making the application of the van't Hoff factor uncertain in the individual case.

In extensive burns, and other severely catabolic injuries, Kinney and his colleagues found that the increase in metabolic expenditure determined by daily indirect calorimetry was insufficient to account for the sustained weight loss of 0·3–0·6 kg/d which took place. As with rats, protein provided only a small (12–22%) proportion of the increased energy expenditure after injury, the majority of the energy requirements being met by oxidation of fat. Taking full allowance of

all factors, they were able to account for only 60% of the weight lost after severe injury. They suggested that the remainder of the weight loss was due to the excretion of excess body water, but could not corroborate this.

Kinney presented evidence (Kinney *et al.* 1970*a,b*) that increased protein degradation occurred after injury in order to meet the body's increased demand for carbohydrate intermediates in the Krebs cycle via hepatic gluconeogenesis, rather than to meet increased whole-body energy requirements. Additional indirect calorimetry studies were devised to measure whole-body and splanchnic blood flow and oxygen consumption in patients with burns, and in patients with peritonitis to determine the aetiology of the hypermetabolism of injury (Gump, Price & Kinney, 1970*a,b*). If due solely to the deamination of labile body protein it was argued that this would occur mainly in the liver. As the increased blood flow and oxygen consumption across the splanchnic viscera after injury and in infection accounted for only 40–50% of the total increase, it was thought that the hypermetabolism of this form of surgical fever involved tissues other than liver, and chemical reactions other than deamination and urea synthesis. Fat stores could be oxidized to meet energy needs in the state of semi-starvation often associated with injury, but protein represented the only sizeable reserve of carbohydrate intermediates and glucose precursors also essential for survival. Carbohydrate stores within the body were insignificant, and after even short periods of starvation body glucose needs are met by an increased liver production of glucose from protein precursors. Using an isotopic tracer technique (Spencer, Long & Kinney, 1971), it was established that in the late recovery phase following severe injury, and in sepsis, both glucose oxidation and gluconeogenesis were increased (Long, Spencer, Kinney & Geiger, 1971; Gump, Long, Killian & Kinney, 1974) and that gluconeogenesis could not be suppressed by intravenous infusion of glucose (Long, Kinney & Geiger, 1976). This suggested that after injury the normal neurohumoral regulation of gluconeogenesis with muscle and liver had ceased to be effective. The explanation may lie in the upset hormonal balance known to follow severe injury, in which increases in the 'catabolic' hormones, adrenaline, noradrenaline, corticosteroids, glucagon, and growth hormone predominate (Allison, 1974; Moore & Brennan, 1975; Wilmore, Long, Mason & Pruitt, 1976*a*; Alberti, Batstone & Johnston, 1977).

It has been suggested that the increased proteolysis and gluconeogenesis of injury is caused by catecholamine-induced 'insulin resistance' in muscle tissue, which leads to impaired glucose and fat utilization (Clowes, O'Donnell, Ryan & Blackburn, 1974; Clowes, O'Donnell, Blackburn & Maki, 1976). Energy needs in muscle are then met by oxidation of the branch-chain amino acids producing excess alanine and other gluconeogenic precursors in the blood stream. Hepatic gluconeogenesis and ureagenesis is thereby stimulated (Odessey, Khairallah & Goldberg, 1974). Therefore 'insulin resistance' and its effect on metabolic pathways in muscle makes available large quantities of glucose derived from muscle amino acid pools, providing in the short term, brain and other essential organs with a readily assimilated energy supply. This response becomes disadvantageous in the long term if muscle protein pools become depleted without

replenishment, a situation which occurs in serious injury if a 'catabolic' endocrine climate with high circulating catecholamine levels is prolonged.

In the midst of these recent, complex and exciting biochemical developments does the calorimeter still have a role? The work already described represents what might be termed long term nutritional–energetic studies after injury. Used in this way, in rats, direct gradient layer, with combined indirect calorimetry has provided an essential first step in our detailed knowledge of the energetic consequences of injury. It is difficult to assess the accuracy of indirect calorimetry studies in patients, largely because of the problems of interpreting measurements where so many factors can vary.

The large insensible water losses from burned patients, resulting from heat damage to dermal vapour barriers (Moyer & Butcher, 1967) has been assumed, since its discovery, to be the major cause of post-burn hypermetabolism. Gump & Kinney (1970) challenged this view, quoting the work of Zawacki, Spitzer, Mason & Jones (1970) who appeared to demonstrate no link between increases in evaporative water loss and increased metabolic rate after burn injury, though the indirect methods of measurement used make it difficult to draw firm conclusions from any of these studies. More recently Wilmore and his co-workers (Wilmore, Orcutt, Mason & Pruitt, 1975; Wilmore *et al.* 1976a; Wilmore, Taylor, Hander, Mason & Pruitt, 1976b) revived this notion. They built a special insulated chamber for heat exchange measurement in burned patients and, using indirect calorimetry and skin/core temperature measurements, concluded that these patients behaved as if their hypothalamic centre had been reset at a higher level than that required to counteract the evaporative heat losses of the burn. However, in the absence of accurate partitioned heat-loss studies using simultaneous direct and indirect calorimetry in burned patients, this indirect evidence cannot be regarded as conclusive.

Arturson, Danielson & Wennberg (1977) continue to view the cooling load of accelerated water and heat losses as the most important single factor leading to a compensatory catecholamine-mediated increase in heat production inducing post-burn hypermetabolism. The anticipated protein and weight losses did not occur after severe burns when patients (Davies, Liljedahl & Birke, 1969; Davies & Liljedahl, 1970; Davies, 1977) and experimental animals (Caldwell, 1970) were nursed at thermoneutral ambient temperatures (30–32°) (Tilstone, 1974). But Campbell & Cuthbertson (1967) noted that this also occurred in rats after closed femur fracture where the cooling load was absent, and in patients with fractures (Cuthbertson & Tilstone, 1968). The mechanism of this effect is not fully understood (Wilmore *et al.* 1976a,b). Although some of the reduction in weight loss found at the thermoneutral environmental temperatures could be related to reduced sensible heat losses (Caldwell, 1970), it is interesting that Liljedahl (1972) observed a marked reduction in the excretion of catecholamines when burned patients were treated at the higher ambient temperature. As Clowes *et al.* (1976) have suggested, this may decrease muscle insulin resistance, thereby sparing

muscle protein. If valid the prospect of environmental control of body hormonal climate is appealing.

Kinney (1976) has summarized the current situation. The hypermetabolism of catabolic injury states might be due to alterations in any of the following categories: (A) Intermediate metabolism involving: protein turnover; specific dynamic action; gluconeogenesis; triglyceride metabolism. (B) Utilization of high energy bonds involving: increased demand for work; reduced work efficiency. (C) Heat metabolism involving: fever; increased heat losses. (D) Abnormal 'setting' of hypothalamic centre.

For practical reasons the application of calorimetry, even in categories C and D, remains limited in injured patients to indirect techniques. At the present, accurate partitioned heat-loss studies can only be effectively performed in experimental animals. To be most informative, calorimetry must be carried out together with biochemical and other analyses in a multi-disciplinary approach to the problem of the response to injury.

Experimental conditions

In view of the widely differing environmental conditions and techniques previously employed in measuring the rat's response to thermal injury, a carefully controlled, reproducible rat model of untreated injury was sought which might be a useful parallel to the human response. This required at least a laboratory and animal unit with a closely controlled environment, a calorimeter to measure energy loss, accurate dietary intake information and continuous metabolic collections for biochemical estimations.

A purpose-built laboratory and animal house were designed to minimize stress from noise and the effects of handling. The animal house was air-conditioned to give controlled temperature and humidity, and continuous separate collection of urine and faeces was possible in ten of the cages. The animals were trained to accept the handling procedures, the feeding regime and the calorimetry conditions before the experiment began (Drury, Richards & Brown, 1977). A common steady weight growth was taken as the criterion of successful training.

Because a thermal injury involves a large amount of evaporative water loss, an indirect and direct calorimetry system able to measure heat production and partition energy loss was designed (Carter, Shaw, Richards, Boyd & Harland, 1975; Carter, Drury & Richards, 1976). The calorimeter shown schematically in Fig. 1 has at its heart a gradient-layer box large enough to take a single rat. Three thousand thermocouples in the walls of the box give it a high enough sensitivity (1 mV/0.361 W) to make it suitable for rat energy determinations. The box is ventilated by air flowing at a rate of 6–8 l/min which has been conditioned for humidity via a humidifier and dew-point bath and for temperature using a water bath and heat exchangers. The energy loss to the air is calculated from the temperature difference across the box and added to the larger energy flow through the box walls gives, apart from small correction, the sensible heat loss of the animal. A differential hygrometer measures the water evaporating from the rat,
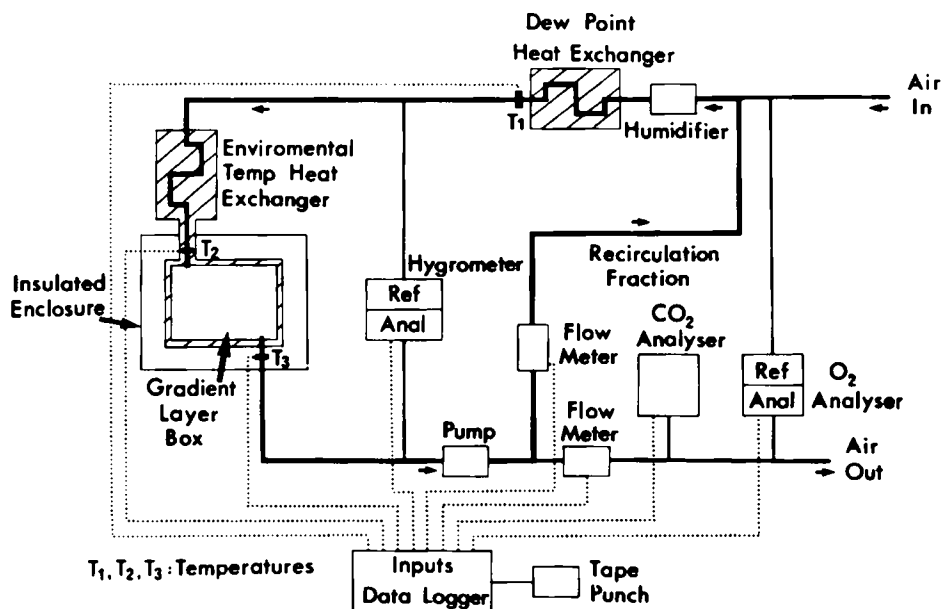
Fig. 1. A schematic diagram of the direct and indirect calorimetry system.

allowing calculation of its insensible heat loss. Oxygen consumption and carbon dioxide production are monitored to give the rate of heat production. All the instrumental outputs are scanned periodically using a data logger, the values being punched onto paper tape for later computer processing.

Calorimetry runs were made at the same time each day, lasting a minimum of 3 h with the rat at least 12 h post-absorptive. Comparison of direct and indirect calorimetry has shown that if the values obtained during the first 40 min while the rat is settling and the apparatus equilibrating are ignored, agreement to better than 1% can be obtained. This suggested that the heat content of the animal was stable, at least after the first 40 min. In their post-absorptive state the respiratory quotients of both burn and control groups fell in the range 0·69–0·73.

The experimental animals used were male Wistar rats from a semi-inbred closed colony. A standard thermal injury of a 25% body surface area full thickness burn was used in all the experiments, the surface area being calculated using the formula:

$$\text{surface area} = 10 \cdot 1 \times (\text{weight})^{2/3}$$

The thermal injury was controlled in temperature, pressure and time using a Bunyan contact burn apparatus. The anaesthetized animals were branded only on shaved areas of anagen hair growth (Zawacki & Jones, 1970) and the controls were also shaved.
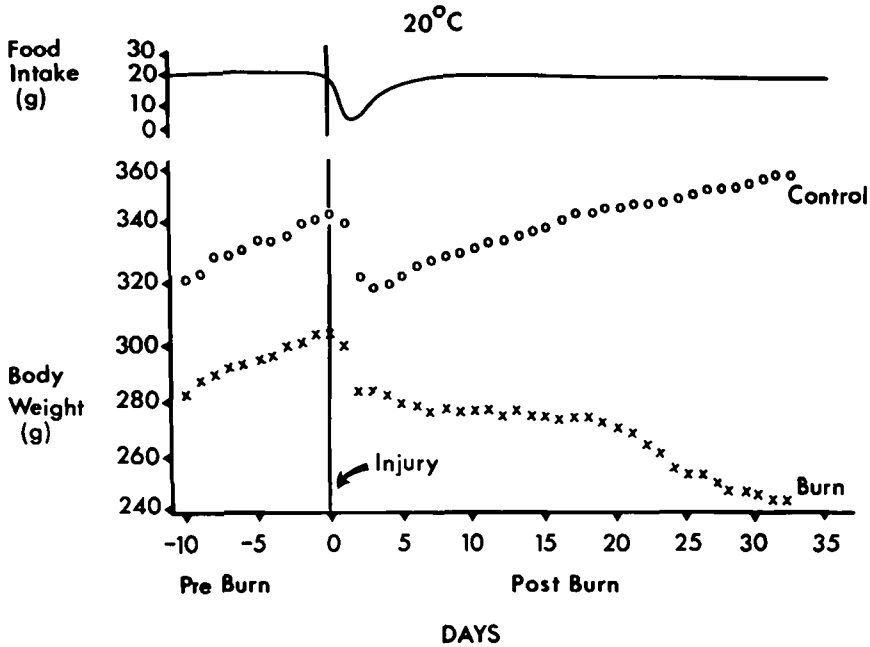
Fig. 2.   The food intake and weight graphs of burn and pair-fed control rats kept at 20°.

Experiments and results

In the first experiment, five rats with the standard injury and five pair-fed control rats were kept at 20°. Their food intake was restricted to 94% of their preburn average intake. There was an initial weight loss phase for both sets of rats as shown in Fig. 2. This was mainly caused by the decreased food intake of the injured animals and the pair-feeding. There followed a phase of slow weight loss by the burned animals while the controls resumed their preburn growth rate of approximately 2 g/d. After about 20 d the burned rats entered a third phase of faster weight loss. There could be three main possibilities for this increased rate of loss. (1) The energy losses of the animals increased requiring greater usage of energy substrates. (2) A larger proportion of the energy demands were being met from a lower energy/g source e.g. less fat and more protein being catabolized. (3) More water being excreted.

Fig. 3 shows the partitioned energy losses of the rats (the bars represent insensible heat loss and the height of the base of the bars sensible heat loss) with the dietary non-protein energy intake superimposed as an index of dietary energy available to the animal. The insensible heat losses rose to a steady level by about 10 d and thereafter showed no dependence on the state of the eschar which lost large amounts of water even though it was dry to the touch (Moyer & Butcher, 1967). There is no increase in the apparent energy deficit over the 20–30 d period to account for the increased rate of weight loss. Figs 4 and 5 show the same experiment but following the progress of the rats over a longer period. The burned
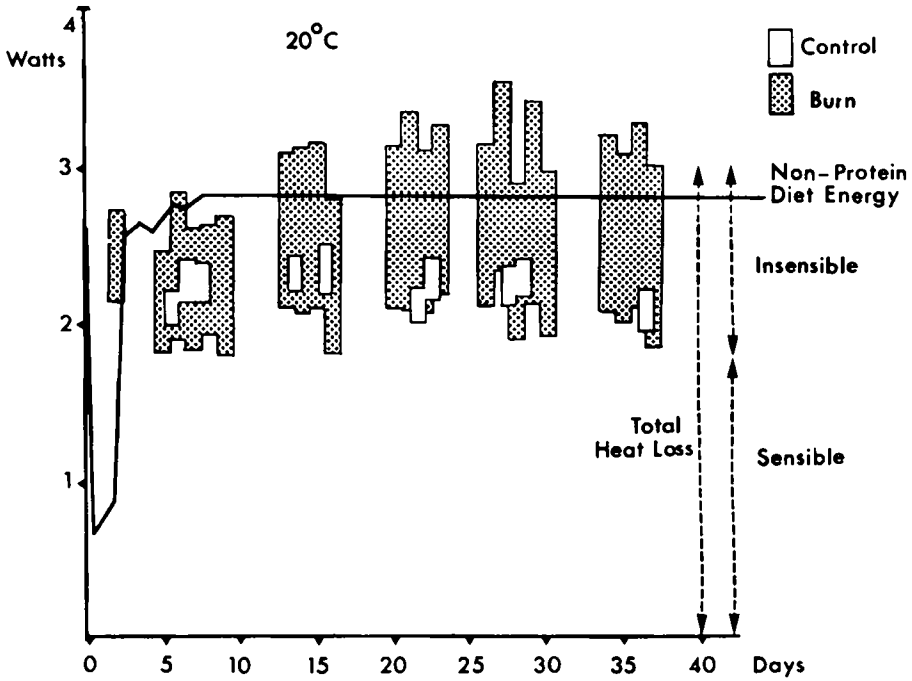
Fig. 3. The partitioned energy losses obtained from direct calorimetry on burn and control rats kept at 20°. The daily non-protein dietary energy intake is also shown.

animals, by losing a large portion of their mass, decreased their sensible heat loss until the total calorimetered loss approximately equalled their dietary intake. At this point of apparent energy balance their weight loss ceased, tending to support the representative nature of the calorimetry results.

Metabolic collections allowed continuous estimation of nitrogen balance in the rats. Unlike man, the rat is a continuously growing animal with no definite adult size, so that the animal is normally in a state of nitrogen retention. This was seen in both the burned and control animals, though the burned animals retained 20% (2·2 g nitrogen) less than the controls over 35 d.

At an environmental temperature of 30° the response to the injury is different, as shown in Fig. 6. The animals were transferred from a temperature of 20° to 30° at the time of burning and were offered 94% of their average preburn *ad lib.* diet though their intake fell to approximately 70% of their 20° consumption. The burned rats still did not grow as well as the controls but only a phase of slow weight loss was seen. There was no late phase of rapid weight loss. The energy values are shown in Fig. 7 with the dietary non-protein intake superimposed. The heat losses of all the animals at 30° were consistently lower than the 20° experiment.

The control animals had a period of raised heat loss over the first 10–15 d mainly due to an increment in sensible losses. This suggests that the rats require a period of acclimation on transfer from 20° to 30°, though the shaving to which all
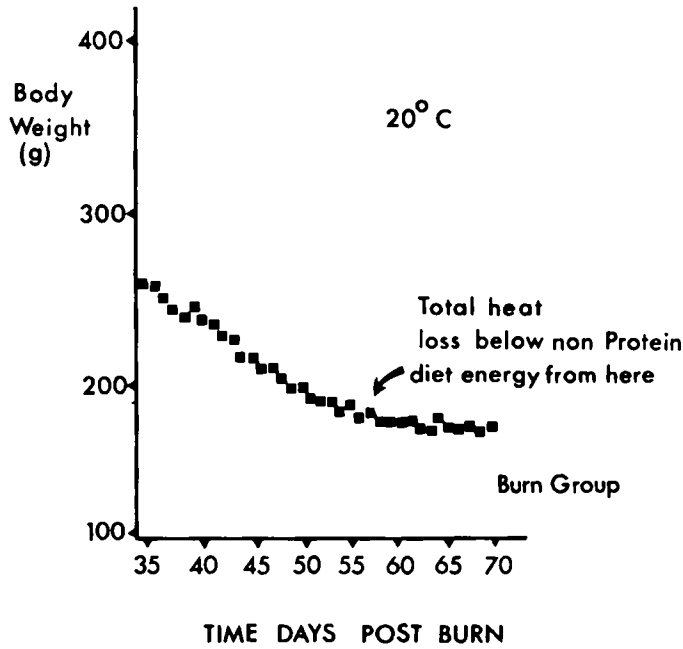
Fig. 4. The weight graph of the burned rats kept at 20° from 35–70 d post-burn.
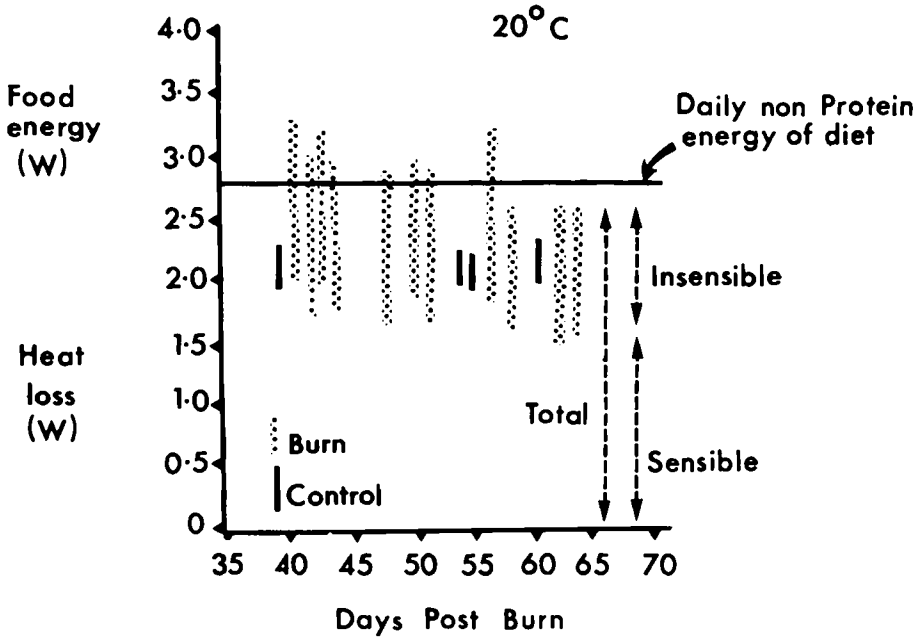

Fig. 5. The partitioned energy losses obtained from direct calorimetry on burn and control rats kept at 20° over the period 35–70 d post-burn. The daily non-protein dietary energy intake is also shown.
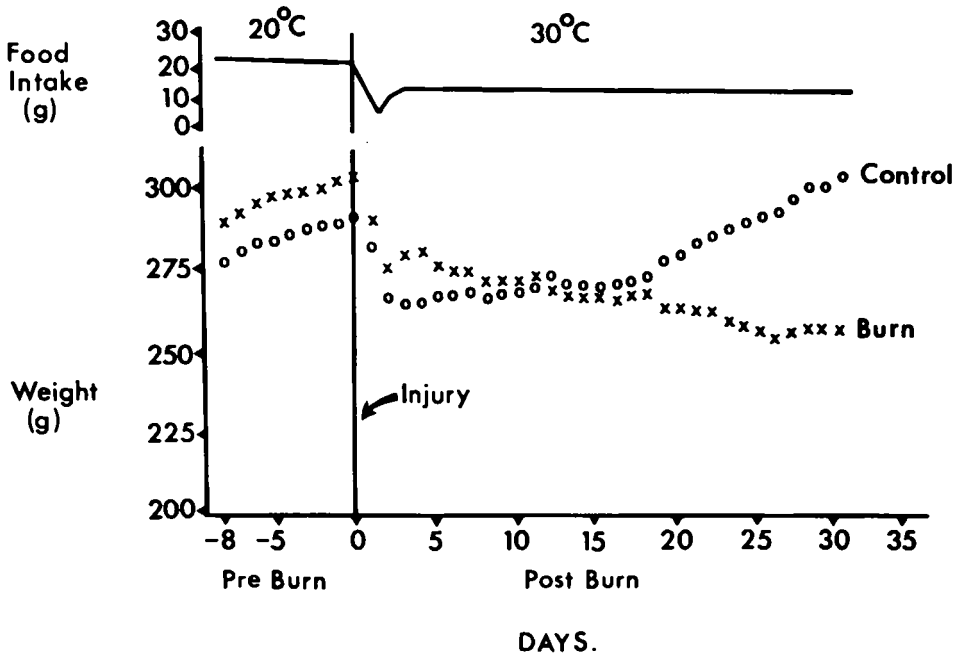
Fig. 6. The food intake and weight graphs of burn and control rats kept at 20° preburn and 30° post-burn.

animals are subjected at the point of injury will also increase the controls' sensible losses. The weight and energy balance relationships can be interpreted in a similar way to the 20° experiment. When the calorimetered losses are approximately equal to the non-protein dietary intake, as with the control animals over the first 10–15 d, the growth rate is small. They only begin to grow at their preburn rate when they go into significant positive energy balance at around 15–20 d. The slow rate of weight loss seen in the burned animals appears to be consistent with their continuing small energy deficit. Unlike the 20° experiment, at 30° both burn and control animals were in equal positive N balance.

The effects of energy changes on body composition were investigated in an experiment similar to the previous 20° run. Six burned and five pair-fed controls gave a similar weight curve to Fig. 2, but the animals were sacrificed after 25 d. Body composition analysis (Entenman, 1957; Folch, Lees & Sloane-Stanley, 1957; Varley, 1967; Fleck, 1967) gave the amounts of water, fat and protein present in the carcases shown in Table 2. Compared with the controls, the burned animals had approximately 90% less fat, 30% less protein and 20% less water. Fat appears to be the major energy substrate utilized, protein only providing a small percentage of the tissue energy difference. No excess water has been lost, the ratio of total body water to protein being 3·06:1 in the control animals and 3·42:1 in the burned.
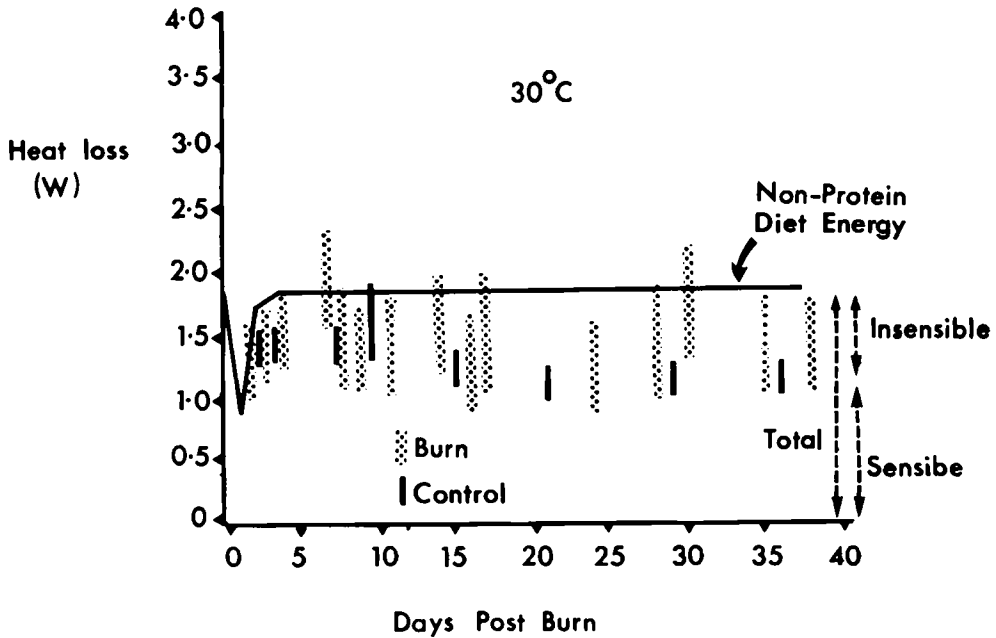
Fig. 7. The partitioned energy losses obtained from direct calorimetry on burn and control rats kept at 20° preburn and 30° post-burn. The non-protein dietary energy intake is also shown.

Table 2. *Body composition of control and burned rats kept at 20°C under similar conditions, except that 6 of the rats were burned 25 days before sacrifice*

	Water g	Water %	Protein g	Protein %	Fat g	Fat %	Residue g	Residue %
Control mean	157·5	61·9	51·4	20·2	30·3	11·9	15·2	6·1
N=5 s.e.m.	9·97	0·58	3·55	0·27	2·57	0·59	1·54	0·70
Burn mean	124·3	69·2	36·4	20·4	3·8	2·1	15·0	8·2
N=6 s.e.m.	6·05	0·27	1·58	0·82	0·90	0·38	1·70	0·63
Control mean—								
Burn mean	33·2	−7·3	15·0	−0·2	26·5	9·8	0·2	−2·1
Significance (P) of difference between means								
(Wilcoxon Test)	<0·01	<0·01	<0·01	N.S.	<0·01	<0·01	N.S.	N.S.

The measurement of catecholamines (Wood & Mainwaring-Burton, 1975) at 20° showed elevated values over an extended period for the burned animals compared with the controls (Fig. 8), in a similar way to man. Also shown on the figure are the insensible heat losses of the animal which decline at about the same point as the catecholamines. At this time the wound began to heal and the animal also started to put on weight and generally recover.
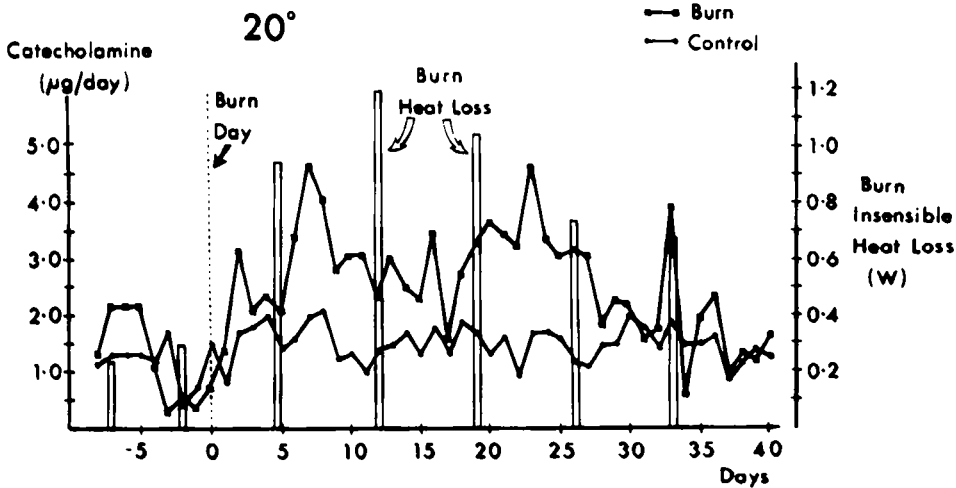
Fig. 8. The catecholamine excretion of two pair-fed rats, one burn and one control both kept at 20°. Also shown are the insensible heat losses of the burned animal.

Discussion

The experiments at both 20° and 30° show a close relationship between energy balance and growth rate in the rat. The ability to partition heat loss allows the identification of the unremitting evaporative heat loss from the burn and the response of the sensible heat loss to acclimation and partial shaving of hair. The third phase of increased weight loss in the 20° experiment points to a change in energy substrate usage, as excess water excretion and increased energy deficit do not appear to be involved. Further work remains to be done to decide if the increased catecholamine excretion is linked directly to the evaporative water loss, as suggested by Arturson *et al.* (1977), to the whole body energy deficit or to some other stimulus.

Because calorimetry disturbs the feeding pattern and the rat's energy balance is only being measured at one point in its circadian rhythm, the values obtained may not be representative of the animal's average heat state. Differences in energy loss between animals and, in a longitudinal study, the change in energy losses in a particular animal, are not so markedly affected by these considerations.

The rat has major physiological differences from man including those of continuous growth and N retention already mentioned. This requires that control animals must be used to give an idea of the rat's normal response to feeding, calorimetry, etc. In man, injury response is normally judged against a control, though in this case it is a control with N balance and little weight change. However, the reproducibility of the rat's response along with the ability to study untreated injury with accurate body composition analysis should make it a useful analogue. Further investigation into the effects of wound covering, raised environmental temperature, direct biochemical intervention (e.g. administration of insulin) and dietary input are planned. The effects of these on energy substrate

usage, protein synthesis and catabolism and general recovery will hopefully be of eventual use in patient management.

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