Changes in body composition after thermal injury in the rat

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1. The effects on body composition, measured by direct techniques, of a controlled 25 % body-surface-area thermal injury have been studied in two groups of forty male Wistar rats.

2. The extent of weight loss in the animals was directly related to their energy deficit resulting from a combination of injury, food intake and rate of wound healing.

3. Body fat proved the most labile source of tissue energy, decreasing to a minimum of approximately 30 g/kg body-weight.

4. Relationships between water and fat, and water and protein seen in control animals were not significantly different in the traumatized group.

Major injury in man is commonly followed by extensive weight loss associated with a negative nitrogen balance and an increase in the basal metabolic rate (Richards, 1977). In man only indirect methods can be used to determine changes in body composition during the period of weight loss after injury (Moore & Brennan, 1975). Recent long-term studies using these techniques were unable to fully explain the nature of the tissue fuels utilized after severe injury (Kinney *et al.* 1970).

Direct chemical analysis of body composition has been carried out extensively on animals, primarily in the field of animal husbandry (Brozek, 1968).

Similar direct methods of carcass analysis may be employed in the studies of changes in body composition after severe injury in experimental animals.

The effects of serious burn injury were studied in rats using serial whole-body analyses. The response of the animals in closely controlled environmental conditions was studied at two levels of dietary intake.

EXPERIMENTAL PROCEDURE

Experimental animals

Forty male rats (aged between 10 and 12 weeks and approximately 200 g in body-weight at the start of the experiment) were used in each of the two experiments (A and B). These rats were from the semi-inbred closed colony of the Institute of Physiology, Glasgow University.

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Table I. *Composition (g/kg) of diet* for rats*

Dextrin†	546·2
Glucose	100
Arachis oil	100
Lactalbumin‡	200
Minerals§	51·1
Vitamins‖	2·7

* Diets were made in 5 or 10 kg lots and mixed using an industrial food blender (Hobart Model SF 50, the Hobart Mfg Co. Ltd).

† Dextrin Type II, Sigma Chemical Co. Ltd, London.

‡ Lactalbumin, Practical Grade, Sigma Chemical Co. Ltd, London.

§ Contained (g/kg diet): $CaCO_3$ 14·6, KH_2PO_4 17·2, NaCl 12·5, $MgSO_4.7H_2O$ 4·99; also (mg/kg diet): $CaHPO_4.2H_2O$ 220, Fe $(C_6H_5O_7).6H_2O$ 310, $CuSO_4$ 80, $MnSO_4.H_2O$ 60, $ZnCl_2$ 10, KI 0·25, $(NH_4)_6Mo_7)_{24}.4H_2O$ 1·25, $Na_2SeO_3.5H_2O$ 0·75. These constituents were supplied as Rogers and Harper salt mixture (ICN Pharmaceuticals) (50 g/kg diet) and were supplemented with the following (mg/kg diet): $CrK(SO_4)_2.12H_2O$ 48, $MnSO_4.4H_2O$ 124, $ZnSO_4.7H_2O$ 155, $NiCl_2.6H_2O$ 4·1, $SnCl_2.2H_2O$ 3·8, $CoCl_2.6H_2O$ 0·81, NH_4VO_3 0·46, NaF 5·5 KIO_4 1·5, $Na_2SiO_3.5H_2O$ 755.

‖ Contained (mg/kg diet): p-aminobenzoic acid 10, D-biotin 5, folic acid 5, myo-inositol 400, nicotinic acid 30, Ca D-pantothenate 20, pyridoxine 10, riboflavin 10, thiamine 10, cyanocobalamin 0·03, choline chloride 200, retinyl acetate 8, cholecalciferol 0·25, α-tocopherol 200, menadione 5.

They were individually housed in a specially constructed soundproof animal unit which permitted close control of environmental conditions (Drury, 1976). The ambient temperature was maintained at 20 ± 0.5 °, with a relative humidity of 55% throughout both experiments.

A 12 h light–dark cycle was used and the animals were weighed daily at a set time using the technique described by Richards, Drury & Brown (1976).

The diet constituents are shown in Table 1. The complete diet was randomly sampled to check homogeneity of its major constituents. A measured quantity of diet (18 g in Expt A and 15 g in Expt B) was offered to the animals daily in paste form. The rats had free access to the food over the 24 h period. Any dietary residue was collected, dried and weighed to give daily food intake of each animal.

Experimental design

The rats were allowed to acclimatize and establish steady weight gain for not less than 14 d before injury. The hair was shaved from the dorsum of all rats in order to determine the areas of anogen and telogen skin (Zawacki & Jones, 1967). Animals showing large areas of dorsal anogen skin growth were selected for injury, and the remainder used as controls. A small group of rats were killed immediately before the time of injury in order to provide an estimate of the starting body composition of the animals. A full skin thickness burn of 25% of body surface area was produced under deep pentobarbitone anaesthesia using the Bunyan contact burn apparatus (Wilkinson Sword Research).

The uninjured animals were subjected to anaesthesia, and group pair-fed 24 h after the burned animals. Animals from both groups were killed at intervals for carcass analysis.

Body composition method

The chemical body composition was determined as follows.

The rat was weighed and then killed by intraperitoneal injection of 60 mg pentobarbitone sodium. The animal was then dissected, the intestine excised and the gut contents removed. The empty intestines were replaced and the carcass weighed. This constituted the empty body-weight (EBW). The carcass was dried to a constant weight at 70 ° for 48–60 h in a hot-air oven. The decrease in weight was taken as the total body water of the sample.
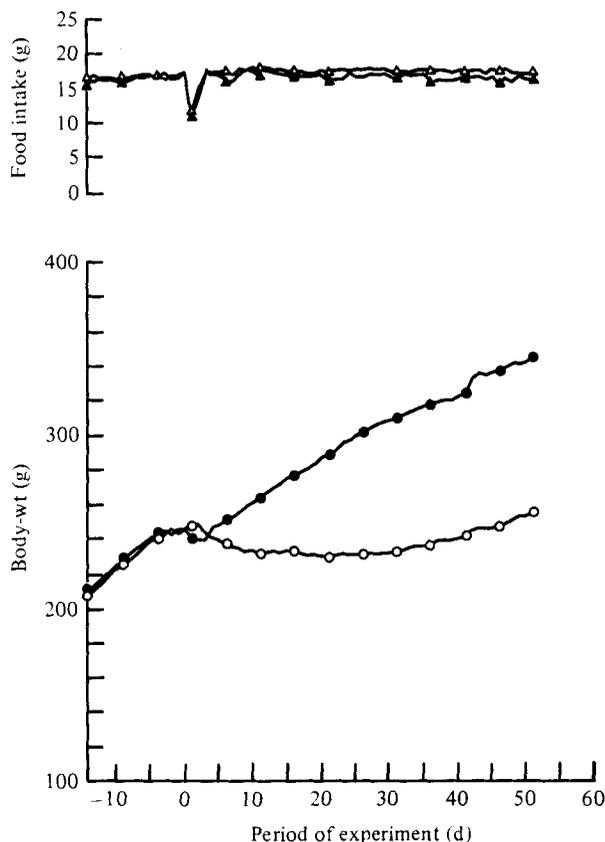
Fig. 1. Expt A. Food intake (g/24 h) (▲, △) and body-weight (g) (●, ○) for injured (○, △) and control (●, ▲) animals. For clarity only values for each 5th day are shown.

Digestion of the whole rat was carried out under reflux with 2–4 l of ethanolic sodium hydroxide (300 g/l) as described by Entenman (1957). The alkaline digest was brought to a known volume.

The lipid content was estimated by acidifying a 20 ml portion of the alkaline digest with 5 M-sulphuric acid. This was then extracted three times with equal volumes of light petroleum (b.p. 30–60 °) to isolate the lipid component. The extracts were then purified by shaking with distilled water as described by Folch *et al.* (1957). The fatty acids in the extracts were then titrated against 1 M-sodium hydroxide following the method of Varley (1967). The acidification and purification stages were performed on duplicate samples.

A separate sample of the alkaline digest was used for protein estimation by the micro-Kjeldahl method, as modified by Fleck (1967).

RESULTS

Expt A

Both the burned and the uninjured animals had similar total food intakes over the duration of the experiment (Fig. 1). The growth rate of the control rats was similar before (2·3 g/d), and after (2·1 g/d) the period of anaesthesia. The injured animals showed a response characterized by weight loss from day 0 to day 20 (1·0 g/d). Thereafter a slow weight gain

Table 2. *Expt* A. *The body compositions of injured and control groups of animals given* 18 g *diet* */d at various intervals after injury*

(Values are means with their standard errors)

Period after anaesthesia (d)	Treatment	No. of rats	EBW (g) Mean	SE	Water (g/kg EBW) Mean	SE	Fat (g/kg EBW) Mean	SE	Protein (g/kg EBW) Mean	SE
0	C	4	228·1	4·7	661	6	89	7	234	3
7	C	3	245·7	14·8	656	13	89	10	225	7
	B	3	230·6	8·3	653	13	90	21	226	1
22	C	3	275·0	19·6	652	16	83	8	217	12
	B	3	218·3	24·5	681	11	60	11	226	4
41	C	3	302·2	7·9	593	5	160	4	226	2
	B	3	228·4	18·0	660	23	76	26	223	1
51	C	7	336·8	11·7	615	15	131	19	221	4
	B	11	247·6	5·8	671	6	79	7	220	4

B, burn animal; C, control animal; EBW, empty body-weight (or ingesta-free body-weight).
* For details, see p. 368 and Table 1.
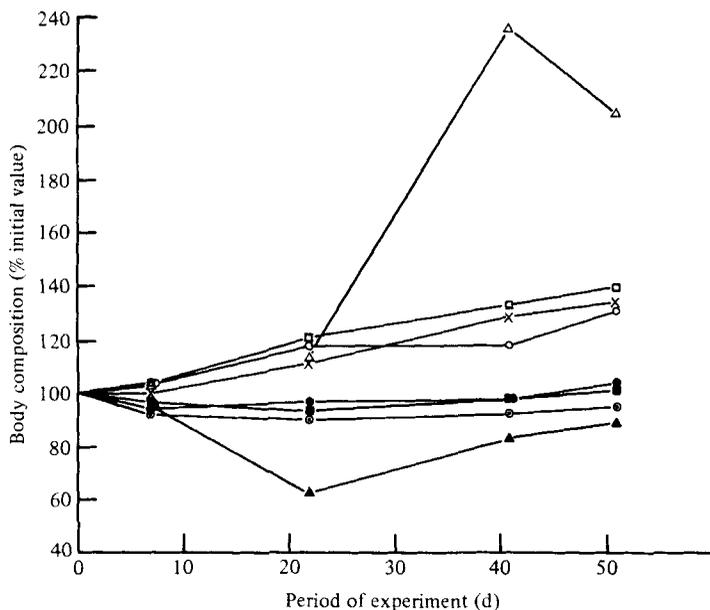
Fig. 2. Expt A. Changes in body composition (% initial value) of (*a*) control rats and (*b*) injured rats. (○, ●), total body water (□, ■), empty body-weight (EBW); (△, ▲), body fat; (×, ○), total body protein of control and injured animals respectively.

occurred (0·5 g/d) associated with early evidence of marginal wound healing and a decrease in burn area.

Body composition analyses for groups killed at intervals after injury are shown in Table 2. At each interval after injury the average percentage EBW for each tissue of those animals killed was taken as the best estimate of the body composition of those animals that lived beyond the sample point. The EBW was derived from the live weight by subtracting 5·8 g, which was the average difference over the experiment for the animals killed.

Fig. 2 shows the body composition of the animals at the day of killing, as a percentage
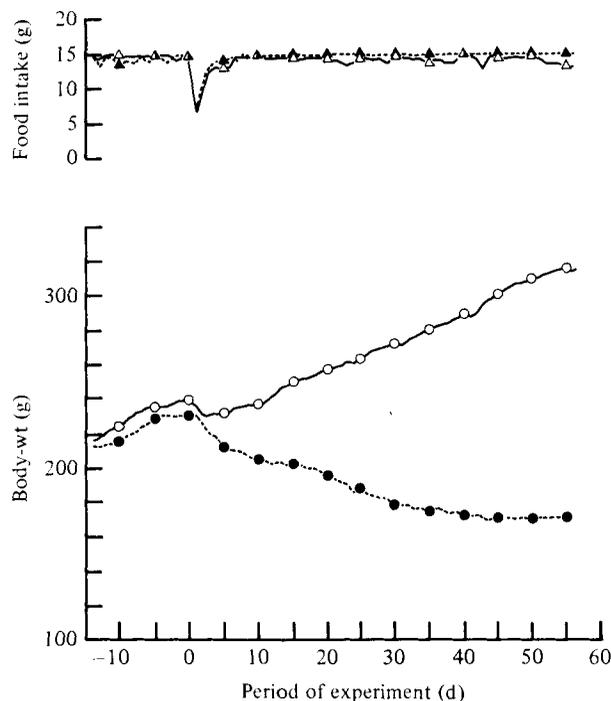
Fig. 3. Expt B. Food intake (g/24 h) (▲, △) and body-weight (g) (●, ○) for injured and control animals respectively. For clarity only values for each 5th day are shown.

of their initial values. The control animals put on a large proportion of fat over the experiment. Protein and water were added at a much lower rate, but in an almost constant ratio. In the burned animals, the fat was again the tissue most subject to change. Over the period of weight loss the fat content decreased to 64 % of the original value whilst protein only decreased to 89 %. At 30 d after injury the burned rats showed evidence of recovery, demonstrated by return of weight gain. By the end of the experiment, 51 d after injury, the percentage fat content approached preburn levels and in general the burned animals had regained initial body composition proportions. There were no significant differences (Wilcoxon test) in the relative content of water, fat or protein between the day 0 animals and the burned animals killed at the end of the experiment. The same measurements compared between the day 51 controls and burned animals showed significant differences in the water and fat contents ($P < 0.002$ and $P < 0.01$ respectively), but not between the protein contents. The relative protein content was expected to demonstrate the least change. The differences between the absolute amount of the three body components were all highly significant ($P < 0.002$), there being a difference of 89·2 g in EBW between burned and controls at day 51.

Expt B

Fig. 3 shows the food consumption and weight changes for the animals with higher temperature branding and lower dietary intake of the two groups. The growth rate of the control animals was again similar before and after anaesthesia (1·8 g/d up to day 0 and 1·6 g/d from day 4 to day 56). The burned animals demonstrated a persistent weight loss throughout the experiment, with no evidence of significant reduction in wound size. By day 56 they had lost 30 % of their initial body weight from the day of burning.

Table 3. *Expt B. The body composition of injured and control groups of animals given 15 g diet d/* at various intervals after injury*

(Mean values with their standard errors)

Period after anaesthesia (d)	Treatment	No. of rats	EBW (g) Mean	SE	Water (g/kg EBW) Mean	SE	Fat (g/kg EBW) Mean	SE	Protein (g/kg EBW) Mean	SE
0	C	5	228·6	6·5	649	10	97	10	223	7
13	C	2	235·6	18·6	670	1	66	3	224	12
	B	3	193·8	6·9	656	12	83	10	201	6
20	C	2	252·2	8·3	617	1	125	7	210	3
	B	3	185·6	7·9	694	10	38	11	230	3
31	C	2	277·0	13·5	629	12	115	15	207	16
	B	3	166·2	3·7	697	8	28	5	230	8
43	C	2	274·5	4·4	608	30	140	39	224	5
	B	3	165·6	3·5	688	2	44	2	217	8
57	C	6	308·3	12·2	606	12	139	15	231	6
	B	8	171·0	7·7	695	4	35	5	221	4

B, burn animal; C, control animal; EBW, empty body-weight (or ingesta-free body-weight).
* For details, see p. 368 and Table 1.
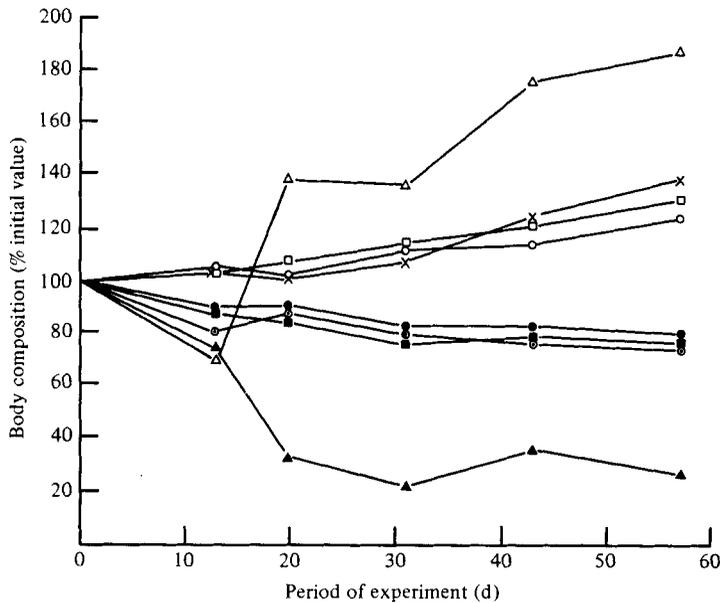
Fig. 4. Expt B. Changes in body composition (% initial value) of (a) control rats and (b) injured rats. (○, ●), total body water (□, ■), empty body-weight (EBW); (△, ▲), body fat; (×, ○), total body protein of control and injured animals respectively.

The tissue changes were calculated as in Expt A and are shown in Table 3. The difference between live body-weight and EBW was 4·5 g in this experiment. The percentage increases in the various body components of the control rats showed a similar pattern of change to those found in the control animals in Expt A, but of smaller magnitude.

In the burned animals (Fig. 4) a rapid loss of fat occurred over the first 30 d after injury. The fat content fell to 20–30% of its starting value. This represents a reduction from
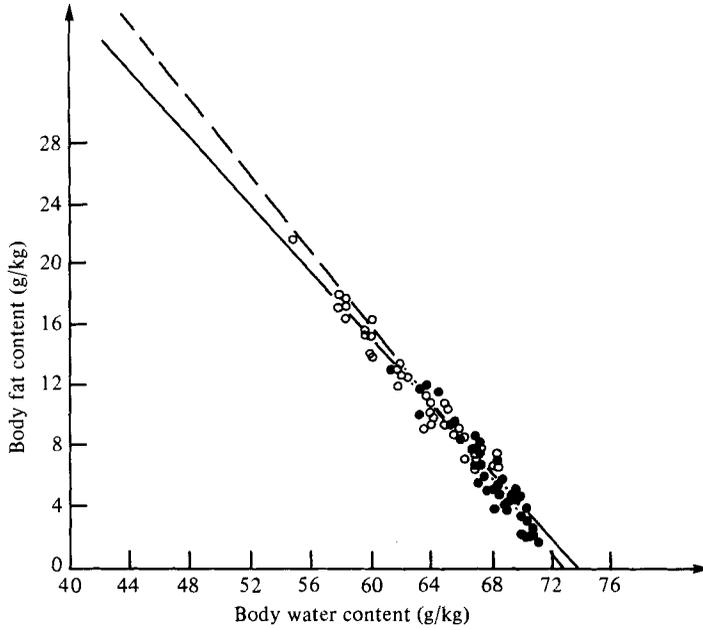
Fig. 5. Expts A and B. Relationship between body fat content (g/kg) and total body water (g/kg) for (*a*) animals receiving 25 % surface area full-thickness burn (●) (*n* 40) and (*b*) control animals (○) (*n* 36).
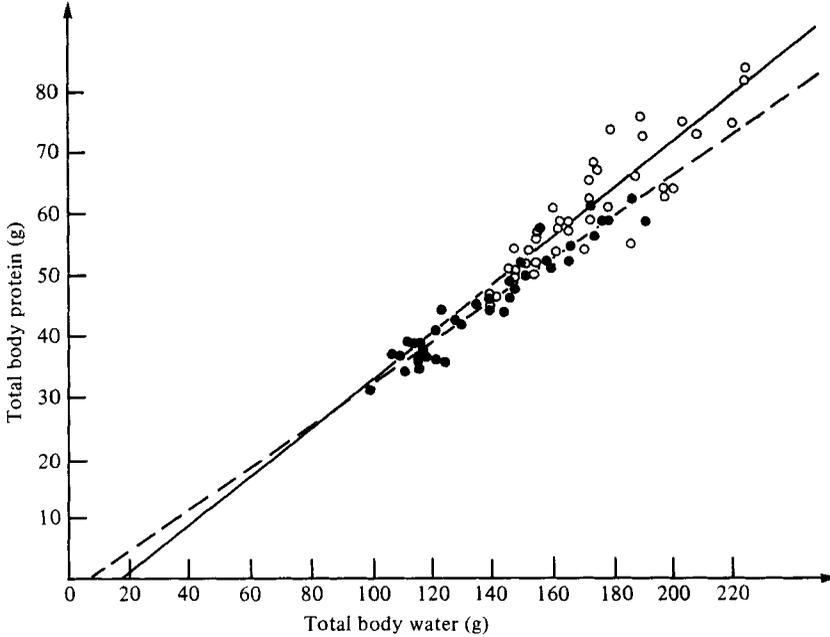
Fig. 6. Expts A and B. Relationship between total body protein (g) and total body water (g) for (*a*) animals receiving 25 % surface area full-thickness burn (●) (*n* 40) and (*b*) control animals (○) (*n* 36).

approximately 9 to 30 g/kg body-weight. The protein loss was also more rapid than in Expt A over the first 30 d, falling to 75% of initial value. The ratio, protein:water of the lost tissue appeared to be the same as in Expt A. The relative water and fat contents in the injured rats at the end of the experiment were significantly different from those found in control animals at the beginning and end of the experiment ($P < 0.01$), contrary to the findings in Expt A. The relative protein content was not significantly different from that in either group of control animals. The burned rats reached a minimum fat content of approximately 30 g/kg body-weight by the 20th day after injury, and maintained this level for the remainder of the experiment.

Fig. 5 demonstrates a linear relationship between relative body fat and relative total body water for injured and control animals. The coefficients of linear regression were -0.956 and -0.978 for burns and controls respectively, there being no significant difference between these two. A linear relationship between the amounts of water and protein was also demonstrated (Fig. 6). Again there was no significant difference in the regression statistics for injured and control rats (R 0.901, R 0.899 respectively).

DISCUSSION

The body composition method of analysis used in this study was selected for several reasons. The alkaline digest step gives a homogeneous solution of the entire rat with no preliminary treatment other than drying. This eliminates sampling errors, particularly when extraction of lipids by organic solvents is attempted. Oven-drying at 70° and digestion do however result in a measured loss of protein (11%) when compared with a freeze-drying method (Al Shamma, unpublished results). Similar observations have been reported by Lofti *et al.* (1976). This correction of 11% has been applied to our results. In addition this method is not suitable for direct determination of ash and other residues.

The experimental design was chosen to show, by sampling, the time-course of major body composition changes after injury.

In Expt A the change from slow weight loss over the first 20 d to that of growth after 30 d may be assumed to be a direct result of marginal wound healing and hence decreased energy requirements. Tissue losses after thermal injury were the product of an energy deficit brought about by hypermetabolism caused by the burn (Richards, Drury & Bessent, 1976), and the decreased food intake immediately after injury. The injured animals in this experiment, though they suffered a large growth retardation (26% compared with the controls on day 51) were able to return their body composition to that of an uninjured animal of the same weight. The fat which they were able to utilize quickly to meet energy demands was replaced as soon as a positive energy balance allowed, i.e. shrinkage of wound area leading to reduced energy losses.

In Expt B the lack of wound healing, and hence sustained energy losses (Richards *et al.* 1978), plus a lower dietary intake, led to a weight loss of 22% (50 g) in the injured animals over the 50 d experimental period. In comparison to their pair-fed controls, injured animals suffered a growth retardation of 44% (134 g). The reduction in rate of weight loss by the injured animals seen towards the end of the experiment was due to the reduction in metabolic body size of the animal, such that an energy balance situation was imminent (Richards, Drury & Bessent, 1976). The minimum fat reached in the injured animals appears to be an indispensable or structural fat tissue (Keyes & Brozek, 1953). From the 20th day after injury all energy derived from tissue had to be provided from protein which has a relatively low intrinsic energy value per unit mass. This coincided with an increase in the rate of weight loss as shown in Fig. 2.

Even though the rats used were of a similar age and weight and were from an inbred colony, a considerable variation was seen in their relative fat contents. For the animals killed

at day o in Expts A and B the coefficients of variation for relative fat content were 7·87 and 10·31 respectively, while those for water were 0·91 and 1·54, and for protein 1·28 and 3·14 respectively. The marked dependence of fat content on factors such as dietary input, feeding frequency and other diverse influences has been reported by others (Cohen, 1963; Reid *et al.* 1968).

Kinney *et al.* (1970) have postulated loss of water unassociated with protein loss in injured man. Indirect techniques of body composition analyses were used in his study. From our studies there is no evidence for loss of this 'excess body water' as shown in Fig. 6.

Our results with injured animals show how rapidly fat reserves can be exhausted or replaced, depending on the extent of thermal injury and the nutritional status of the animal. Selective utilization of fat as an energy source failed to prevent depletion of lean body mass in these animals. The relationships between fat and water and protein and water were not significantly altered by trauma severe enough to inhibit growth and cause marked weight loss.

The authors are not aware of any direct body composition measurements after a standardized injury. Such measurements of absolute body tissue changes are an essential first step towards the understanding of the alterations of the kinetics of body protein and fat stores. These findings, if applicable to man, may prove important in the development of rational nutritional therapy after injury.

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