TISSUE PROTEIN BREAKDOWN IN BURNS

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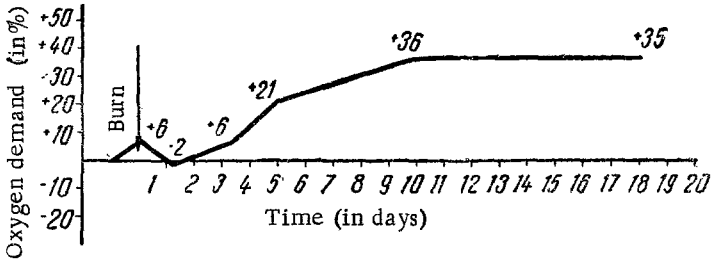
It is generally accepted that following burns there is an increase in the intensity of catabolic reactions and in protein breakdown, leading to the development of a negative nitrogen balance in the body. It has been stated [1, 2, 3, 6, 8, 11, 24] that a generalized breakdown of the proteins of various organs and tissues is observed in burned patients and experimental animals, and that this is a decisive factor in the development of toxemia, of the emaciation found in burns, and of the unfavorable prognosis associated with this condition. Nevertheless, a careful scrutiny of the literature reveals that the only conclusion regarding the increased tissue protein destruction is based mainly on results which do not allow the catabolic disturbances in the different tissues and organs to be judged separately.

Various workers have assumed an intensification of tissue breakdown on the basis of an investigation of the excretion of products of nitrogen metabolism [7, 18, 19], of aminophorases [12, 13], of the autolytic activity of the tissues [6], and determination of the proteolytic enzymes formed at the site of a burn [14, 22, 23, 27, 32]. Some workers have studied tissue disintegration by the method of radioactive indicators both experimentally [8] and clinically [15, 16, 17]. The results obtained have not revealed changes in the enzymic activity of individual tissues and do not permit conclusions to be drawn regarding the order in which the various organs react to burns.

The object of this investigation was to study the activity of the enzyme systems catalyzing protein breakdown in burns in different organs and at different time intervals after the burn injury, and to compare these findings with the level of the oxygen demand.

EXPERIMENTAL METHOD

Experiments were carried out on male rabbits weighing from 2 to 3 kg. Burns were produced by immersing the rabbit's hind limbs in boiling water (100°) for 20 sec or by directing a stream of hot air on to the animal's back and by irradiation using the technique of N. I. Kochetygova and co-workers [5], giving equivalent results in both cases. The area of the resulting third degree burn was approximately 20% of the body surface. The intensity of tissue breakdown and the oxygen demand were investigated 1, 4, and 20 h after infliction of the burn. The intensity of breakdown of the proteins of the liver, heart, skeletal muscle, and brain and of the plasma proteins was estimated by



Oxygen demand after experimental burn trauma.

TABLE 1. Intensity of Autolysis in Organs of a Rabbit after Experimental Burn Trauma (in μg tyrosine/100 mg fresh tissue *)

Organ	pH	Duration of incubation (minutes)	Control	Time after infliction of burn (days)		
				1	4	20
Liver	4,0	30	44	44	86	91
	8,6	30	0	0	0	0
		30	3,2	3	10,8	8,7
Skeletal muscle	4,0	120	8,8	—	16	17
		1 440	12	—	34	48
	8,6	30	0	0	0	0
Heart muscle	4,0	30	39	36	46	49
	8,6	30	Traces	Traces	Traces	Traces
Brain	4,0	30	13	—	41	27
Plasma	4,0	30	0	0	0	0
	8,6	30	0	0	0	0
	7,2	30	0	0	0	0

*Each figure is the mean result of 8-15 experiments. Differences statistically significant: $P < 0.05$.

TABLE 2. Changes in Transaminase Activity of Blood Serum in Burns*

Test object	Control	Time after infliction of burn (in days)				
		1	4	15	20	27
Glutamico-oxaloacetic transaminase	18	40	23	16	25	14
Glutamico-pyruvic transaminase	10	20	15	6	5	6

*The differences found 24 h after burning are statistically significant: $P < 0.05$.

determination of the autolytic activity of these tissues. Consideration was also paid to the results of various investigations in which an increase in transaminase activity was associated with increased tissue breakdown [4, 9, 12, 13, 21], and the activity of glutamico-oxalacetic and glutamico-pyruvic transaminases in the blood serum was also determined. The autolytic activity was determined with Folin's reagent from the increase in the concentration of protein breakdown products in a homogenate of the tissue after incubation (a 20% homogenate of the tissue was prepared in 2% KCl solution in the cold). The homogenate was added in a volume of 1 ml to each of two parallel tubes containing buffers of pH = 4.0 and pH = 8.6 respectively. The enzymic processes in one tube were halted at once by the addition of 10 ml of 0.3 M trichloroacetic acid. The tubes were incubated for 30 min at 37°. Trichloroacetic acid was then added to the second tube also, and the increase in the concentration of protein breakdown products in the protein-free filtrates was investigated. The aminophorase activity of the serum was determined by the method of Tonharzy and co-workers [30] and by a modification of the method of Friend and co-workers [21]. The oxygen demand was investigated in a closed chamber by a method enabling determinations to be made in short intervals of time [10]. The temperature, respiration rate, and change in body weight of the rabbits were recorded throughout the experiment.

EXPERIMENTAL RESULTS

The burn trauma inflicted on the animals caused death in a high proportion of the animals during the 3-week period of the experiments. As a rule severe shock was not observed in the rabbits. The body temperature rose by 0.5-1.0° and the respiration rate rose to 100-160/min. The oxygen demand (see figure) rose slightly immediately after the burn to an average level of + 6%. On the second day the oxygen demand both rose and fell, so that its

average value was unchanged. On the 3rd-4th days the metabolic rate increased in most rabbits (on the average + 9 %), and subsequently it rose to + 36%. After the burn the rabbits as a rule lost weight although they ate the usual amount of food. At the end of the third week the loss of weight in severe cases reached 30%.

To increase the reliability of the results, the investigations of the autolytic activity of the muscles, which is normally very small, were carried out at three stages of incubation: after 30, 120, and 1440 min.

It is clear from Table 1 that the body tissues contain enzymes mainly active in an acid medium (of the cathepsin type). The development of burns in experimental animals is characterized by an increase in the activity of enzymes catalyzing breakdown of the proteins of such tissues as muscle (the striped muscles and, to a lesser degree, heart muscle), nerve (brain), and parenchymatous organs (liver).

It is interesting to compare the results described above with those obtained during determination of the transaminase activity (Table 2).

Attention is drawn to the discrepancy between the times of the increases in the serum transaminase activity and the autolytic activity of the tissues. Whereas the activity of the glutamico-oxaloacetic and glutamico-pyruvic transaminases rose sharply 24 h after infliction of the burn, the autolytic activity of the tissues remained at its normal level at this time. Four days or more after the burn the transaminase activity was practically restored to normal, whereas at this time the autolytic activity of the tissues was increasing in intensity.

Some workers regard the increased activity of the serum glutamico-oxaloacetic and glutamico-pyruvic transaminases as indicating necrosis of the heart muscle in myocardial infarction [4] or necrosis of liver tissue in various diseases [9, 12, 13, 21, 28]. In the present investigation no evidence of necrosis of the liver tissue was obtained by histological examination of the liver of animals sacrificed 24 h after being burned*. The only source of the aminophorases at this period could be the cells destroyed in the burner zone. However, destruction of the burned skin and muscles continued throughout the investigation, and it was supplemented by breakdown of the proteins of organs not directly injured by the burn. Yet, at this period, the transaminase activity returned to normal. We suggest that in our experiments breakdown of tissue proteins was not yet taking place outside the zone of the burn 24 h after its infliction, but that the increase in transaminase activity was nonspecific and entirely due to the destruction of tissue structures, where it was apparently associated with the direct disturbance of amino-acid metabolism.

Marked changes take place in the function of the adrenals in burns, and the effect of their hormones on the transaminase activity of the serum cannot be ruled out in these conditions. There are reports in the literature [25, 29] that transaminase activity is dependent on the functional state of the adrenals. Our results show clearly that on the 5th day after infliction of a burn or later the activity of the proteolytic enzymes of the liver and muscles is increased. If this conclusion is compared with the results of investigations using the method of radioactive indicators, reflecting the process taking place in vivo [7, 8, 15, 16, 17], and with the results of other researches described above, it may be assumed that the processes of protein breakdown are intensified in burned animals.

It would be very illuminating to discover the source of this increase in proteolytic enzymes. They may be either activated or newly formed enzymes from unburned tissues, or enzymes induced into the tissue, having been formed in the burned area and then carried by the blood stream to other organs. Following extensive injury to the tissues of the burned zone, the integrity of a very large number of cells is disturbed and it is highly probable that cytoplasmic and mitochondrial enzymes are liberated into the blood stream. The fact that we found no autolytic activity in the blood plasma, which seems to oppose the latter suggestion, does not, in fact, refute it categorically because the activity of the enzymes in the plasma may have been abolished by an inhibitor. Nor can the suggestion be ruled out that the tissue cathepsins are activated or induced enzymes formed in unburned tissues.

The increased protein breakdown is often related to the inadequate supply of oxygen to the tissue. In the present experiments, however, the oxygen consumption of the intact organism was not diminished but, as pointed out above, it rose in proportion to the increased intensity of proteolysis. Hence, this leaves for consideration only the hypoxia resulting from failure to keep pace with the increasing oxygen demand of the tissues.

SUMMARY

A study was made of the autolytic activity of tissues and transaminase activity of rabbit serum with experimentally induced burns. As established, on the 2nd day after the burn there was a considerable rise in the activity

*The histological investigations were conducted by R. I. Kai of the Pathomorphological Laboratory.

of glutamico-oxaloacetic and glutamico-puropyruvic transaminases with a subsequent normalization of these indices. The autolytic activity of the tissues studied remained within the normal range on the 2nd day. Four days after the burn and during the subsequent periods the autolytic activity is considerably intensified in the skeletal muscle and in the liver at the expense of the enzymes acting in the acid medium. Oxygen absorption by the body rose directly after the experiment, normalized on the 2nd day and increased (+ 35%) during the 2nd and 3rd weeks of the disease. A rise of transaminase activity is considered to be the sequence of direct disturbance of amino acid metabolism, whereas autolysis intensification – the reflection of increased disintegration of intact tissues.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
