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Serum elastase and its inhibitors in the blood of heavily burnt patients*

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SUMMARY Serum elastase and its inhibitors were determined in the sera of heavily burnt patients. Serum elastase levels were elevated at two to eight days after a severe burn-accident and returned towards normal values from the 10th day on. Both α_1-antitrypsin and α_2-macroglobulin levels were also elevated in the sera of heavily burnt patients. α_1-Antitrypsin showed a parallel evolution to the elastase level but α_2-macroglobulin followed a somewhat different time curve. Plasminogen and antithrombin were not elevated significantly. It is suggested that serum elastase may play a role in tissue degradation in burnt patients.

The study of proteins and enzymes in the blood serum of heavily burnt patients yielded interesting information on the nature of modifications produced by this kind of trauma. Protease activity was reported to be elevated (Zamecnik *et al.*, 1945; Tokaji, 1971) as well as some of the protease inhibitors, such as α_1-antitrypsin (Nauroy *et al.*, 1972), α_1-antichymotrypsin (Daniels *et al.*, 1974), and α_2-macroglobulin (Hainaut *et al.*, 1971; Farrow and Baar, 1973). The variations recorded by different authors were sometimes contradictory, as, for instance, those concerning the modifications of α_2-macroglobulin (no modification found by Daniels *et al.* (1974), strong increase by Farrow and Baar (1973), and variable results according to the clinical picture by Hainaut *et al.* (1971)). No studies were performed, to our knowledge, on serum elastase activity after burn injury.

Elastase was demonstrated in the blood serum, originally by Hall (1963, 1966). It may originate from any of the elastase-producing organs such as the pancreas (Baló and Banga, 1949; Banga, 1952), leucocytes (Janoff, 1972), macrophages (Werb and Gordon, 1975; De Cremoux *et al.*, 1978), thrombocytes (Robert *et al.*, 1969; Legrand *et al.*, 1975), or smooth muscle cells of the arterial wall (Robert *et al.*, 1974; Hornebeck *et al.*, 1975). Recently, Geokas *et al.* (1977) demonstrated pancreatic type II elastase in human serum. Besides its lytic action on elastin, elastase may act on other proteins. It possesses a relative specificity for aliphatic, hydrophobic amino-acids (Thomas and Partridge, 1960; Naughton and Sanger, 1961). Although several of the serum protease inhibitors do act on elastase, α_1-antitrypsin and α_2-macroglobulin were shown to be the most potent inhibitors (Heimburger and Haupt, 1966; Baumstark, 1967, 1970; Bieth *et al.*, 1970; Lieberman and Kaneshiro, 1972; Katayama and Fujita, 1974; Turino *et al.*, 1974). Proteases probably play an important role in tissue degradation after burn injury (Lewis *et al.*, 1970; Davies and Fell, 1974). They may be involved in the catabolism of proteins in oedema fluid (Piller, 1976) and in the *in vivo* breakdown of immunoglobulins (Goldberg and Whitehouse, 1970). These problems were recently reviewed by Bieth (1978).

Of particular interest in this respect are the proteolytic enzymes capable of attacking the fibrous proteins of intercellular matrix such as collagenases and elastases. Such proteases may play a predominant role in tissue degradation as well as in the liberation of toxic peptides (Moati *et al.*, 1977). It appeared therefore of particular interest to study such enzymes in the sera of heavily burnt patients and to determine also the level of some of the protease inhibitors that may play a role in the regulation of their activity. We present here the results obtained

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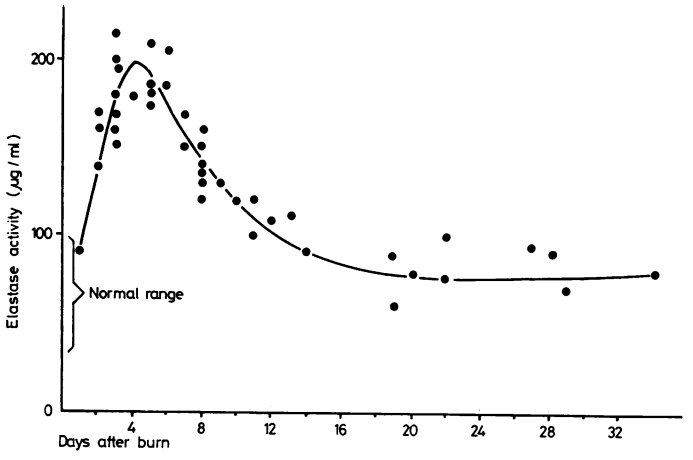

Fig. 1 *Kinetics of increase of serum elastase in serum of heavily burnt patients. Ordinates: serum elastase activity expressed as equivalent of pancreatic elastase, µg/ml serum. No distinction is made on this graph between the different patients studied at different times after the injury.*

on serum elastase and some of its inhibitors such as α_1-antitrypsin and α_2-macroglobulin determined in the sera of heavily burnt patients. Our studies on serum collagenase activity will be described separately (Moati *et al.*, 1978).

Material and methods

Sera from heavily burnt patients (more than 30% of the body surface covered with second- and third-degree lesions) were obtained from the Percy Military Hospital, cooled in ice.

Elastase was determined by the κ-elastin-agarose gel method[1] as previously described (Robert *et al.*, 1974; Bellon *et al.*, 1978). Ten microlitres of serum was deposited in the trough and the gels were incubated for five hours, fixed in trichloracetic acid for 10 minutes, then in acetic acid for 60 minutes, and rinsed in water, and the lysis areas were estimated on photographic enlargements. Enzyme activity was expressed as equivalents of pancreatic elastase (Sigma Chemical Co, St-Louis, Mo, USA or EURORGA) in micrograms per millilitre serum. Standard curves were prepared with the above crystalline pancreatic elastase under the same conditions.

Plasminogen, antithrombin III, α_1-antitrypsin, and α_2-macroglobulin were estimated by radial immunodiffusion according to Mancini *et al.* (1965) using the immunodiffusion plates of Behringwerke (Partigen).

Results

Figure 1 shows the results of about 50 individual elastase determinations carried out at different times after the burn-accident. The normal range of serum elastase activity, as obtained with the present method, is also indicated. It can be seen that elastase activity increases steeply up to the fourth to sixth day post-burn to reach values about double the normal activity. The maximum is followed by an approximately first-order decrease, and normal levels are attained about 20 days after the accident. The apparent half-life of the elevated enzyme level, estimated from the slope of the semi-log plot between days 4 and 20, is approximately four days.

When the evolution with time of the serum elastase and its principal inhibitors, α_1-antitrypsin and α_2-macroglobulin, is followed in individual patients, the α_1-antitrypsin levels follow rather closely the serum elastase levels. This is not the case for α_2-macroglobulin, which behaves differently; its level does not follow those of elastase and α_1-antitrypsin.

Figure 2 shows that the evolution of the serum α_1-antitrypsin level follows a similar kinetics to the one found for serum elastase activity (Fig. 1) with peak values about the fourth day post-burn followed by a decrease to still elevated levels. The above results suggested a correlation between serum elastase and α_1-antitrypsin levels.

Figure 3 shows this correlation between the elastase activity and the α_1-antitrypsin content of control and burnt patients' sera. A significant linear correlation between all values was found (correlation coefficient $r = 0{\cdot}773$, $P < 0{\cdot}001$). The straight line fitted according to the least squares method fits well the data of the normal controls as well as those of the burnt patients.

This was not the case for the relationship between α_2-macroglobulin and serum elastase activity (Fig. 4.)

[1]Commercially available from EURORGA, Villeras-Saclay, France.

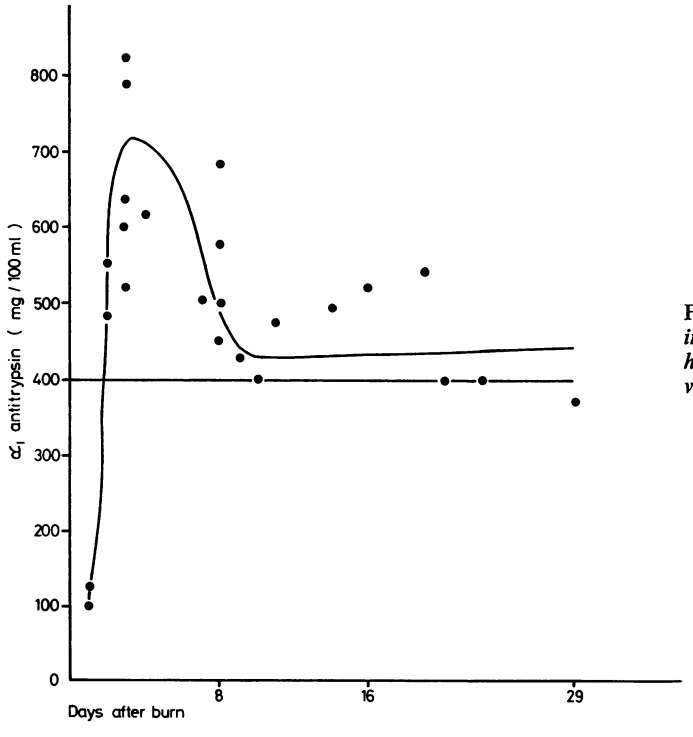

Fig. 2 *Time course of α_1-antitrypsin levels in the sera of severely burnt patients. The horizontal line represents the average normal value.*

The data could best be fitted by three separate lines, one going through the normal controls ($r = 0{\cdot}432$, $P < 0{\cdot}10$), the second of a low slope through part of the data of the burnt patients ($r = 0{\cdot}792$, $P < 0{\cdot}05$), and the third, with a high slope, through the other half of the data of burnt patients ($r = 0{\cdot}65$, $P < 0{\cdot}05$). No evident explanation was found in the clinical data of these patients for this peculiarity of the elastase-α_2-M-correlation.

When the average value of all the determinations is compared for elastase and its two major inhibitors (Table) independently of the time elapsed after the accident, it appears that elastase levels are on the average three times higher in burnt patients' sera than in normal sera. Another proteolytic factor of serum, plasminogen, showed a significant elevation in only five sera out of 15; the others showed normal values. α_1-Antitrypsin was found to be elevated in all sera but α_2-macroglobulin was increased in only six out of 15 sera. No significant rise was observed for antithrombin III determined in the same sera.

Discussion

The presence of an elastase-like enzyme in blood serum was postulated by Hall (1963, 1966), Geokas

Table *Elastase, plasminogen, and protease inhibitors in the sera of heavily burnt patients and in normal controls* Elastase determined by the κ-elastin-gel-lysis method, the other proteins by radial immunodiffusion. Average values ± standard error of the mean for the 15 different patients. Elastase expressed as equivalents of pancreatic elastase, μg/100 ml serum. The other proteins as mg prot. per 100 ml serum.

Source of serum	*Elastase*	*Plasminogen*	*α_1-Antitrypsin*	*α_2-Macroglobulin*	*Antithrombin III*
Normal control	65 ± 35	28 ± 18	256 ± 105	325 ± 155	27 ± 17
Burnt patients	185 ± 102	180 ± 95 (n = 5)	650 ± 142	315 ± 105 (n = 9)	25 ± 10
		25 ± 10 (n = 10)		605 ± 102 (n = 6)	

Different sets of values found in pathological sera; n indicates number of patients with values clustering around the indicated average.

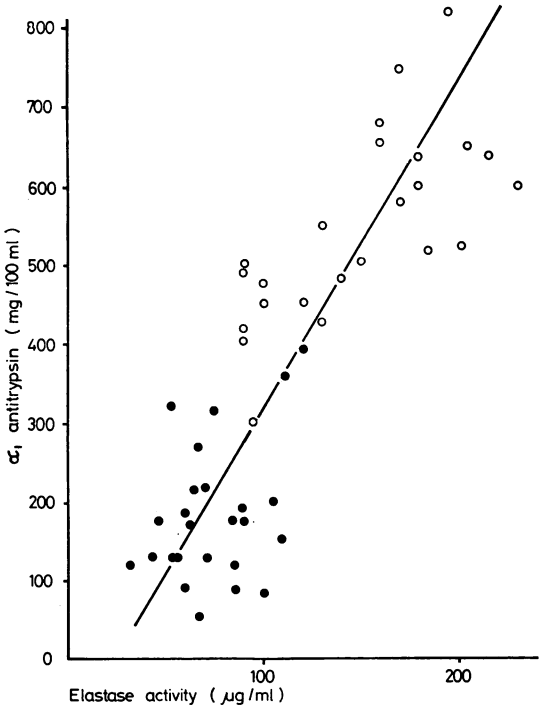

Fig. 3 *Correlation between serum elastase activity and serum α_1-antitrypsin levels in control (●) and burnt (○) sera.*

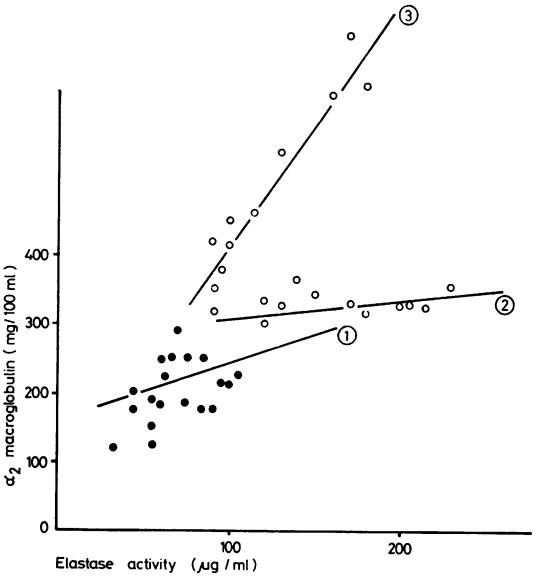

Fig. 4 *Correlation between serum elastase activity and α_2-macroglobulin levels in control (●) and burnt (○) sera. The results cluster according to three different correlations: (1) normal controls; (2) 12 burnt patients; (3) 10 other burnt patients.*

et al. (1977), and others (for a review, see Bieth (1978)).

Since the demonstration of elastolytic enzymes (elastases) in leucocytes (Janoff, 1972), blood platelets (Robert *et al.*, 1969; Legrand *et al.*, 1975), alveolar macrophages (Werb and Gordon, 1975; De Cremoux *et al.*, 1978), and vessel wall (aortas) (Robert *et al.*, 1974; Hornebeck *et al.*, 1975), besides the one in pancreas (Baló and Banga, 1949), the problem appears complicated by the many possible origins of the elastase activity of the serum. The availability of a simple and reliable method for the quantification of serum elastase activity (Robert *et al.*, 1974; Bellon *et al.*, 1978) rendered feasible serial clinical studies. The above experiments show that elastase activity, as determined by this test, does undergo a significant increase in the sera of heavily burnt patients. It is noteworthy that the increase is relatively slow, peak levels being reached only about four days after the accident. The serum elastase levels then decreased towards normal levels, which were reached in about 16 to 20 days post-burn. The comparable evolution with time of elastase and α_1-antitrypsin levels may suggest that part or all of the serum enzyme is in a complexed form with this inhibitor. It was shown by Bieth *et al.* (1970) that the affinity of α_2-macroglobulin for pancreatic elastase is higher than that of α_1-antitrypsin. Elastase may keep part of its activity in its complex with α_2-macroglobulin, for both small substrates and macromolecular substrates. It was shown that α_1-antitrypsin and α_2-macroglobulin are split by elastase within the enzyme-inhibitor complexes (Baumstark, 1970; Lo *et al.*, 1976). The α_2-macroglobulin complex of elastase has a higher clearance rate from serum than the α_1-antitrypsin complex of elastase (Katayama and Fujita, 1974).

The comparable time curve of elastase and of α_1-antitrypsin (Figs 1 and 2) suggests the possibility of the participation of α_1-antitrypsin in the elimination from the circulation of elastase as an enzyme-inhibitor complex possibly by the reticuloendothelial system. Katayama and Fujita (1974) have shown that ^{131}I-labelled pancreatic elastase, when injected in the circulation, is distributed in several organs with a sequential increase of the α_2-macroglobulin bound form and the α_1-antitrypsin bound form. As both inhibitors are susceptible to degradation by elastase (Baumstark, 1970; Lo *et al.*, 1976) the tissue bound enzyme may well continue its proteolytic activity even after its clearance from the circulation. This could explain the extensive degradation of lung and aorta

elastin produced by intravenously injected elastase even if the amount of enzyme injected is lower than the combining capacity of the serum inhibitors (Turino *et al.*, 1974).

Finally, the inconstant increase of plasminogen and the absence of increase of antithrombin III in burnt patients' sera shows that the rise of serum elastase and of its inhibitors is not part of a general increase of all serum enzymes and proteins but represents part of a selective alteration of some of the serum proteins. This contention was further confirmed by the study of serum total proteins (Moati *et al.*, 1977), glycoproteins, and immunoglobulins. These results have been described elsewhere (Miskulin *et al.*, 1978).

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