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INHIBITION OF PROTEOLYSIS IN LUNG EXTRACT AFTER ANAPHYLACTIC SHOCK

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The cellular changes which accompany anaphylactic reactions have long been studied by means of histological, biological, and chemical techniques. Investigations into delayed allergic reactions by Wesslén (1) have demonstrated lysis of cells (lymphocytes) derived from sensitised animals following contact with the specific antigen. A similar lytic effect has also been observed even in the case of certain acute anaphylactic reactions (2).

The use of suitable antigen doses can almost invariably give a uniform shock picture leading to death within a few minutes. In guinea pigs, the most suitable experimental animal for such studies, a pronounced bronchospasm occurs which in prolonged shock produces changes resembling these of asthma. Markedly emphysematous lungs are found at autopsy.

Similar symptoms and autopsy findings can be obtained with certain other substances such as peptone and histamine without sensitisation. Recent work has shown that several substances may serve as histamine liberators.

As an explanation of the shock mechanism, the hypothesis has been advanced that the antigen-antibody reaction produces cell damage and a concomitant generation of toxic products which might serve as histamine liberators. In this connection attention has been directed towards activation of proteolytic enzymes occurring at an early stage and it has been possible to demonstrate increased proteolytic activity in organ extracts after sensitisation and shock (3, 4).

The following account summarises the results of investigations into proteolysis in lung extracts from shocked guinea pigs and the effect of enzyme-inhibiting substances *in vitro* and *in vivo* (5).

Proteolysis in shock extracts.—Following exsanguination of shocked guinea pigs by cutting off the apex of the heart and immediate removal of the lungs, an extract was prepared from the lungs by grinding with sand. Fixed amounts of the specific antigen were added to the extract and the mixture was incubated for two hours at 37° C. Amino-N was determined initially and after two hours by *van Slyke's* method. Increase of amino-N was significantly greater in shock extracts than in extract of normal lungs (see table 1).

TABLE 1
Proteolytic activity in lung extracts, digestion for two hours.
Average values for 56 normal and 72 shock extracts.

	Amino-N, mg %			
	T N. mg %	Initially	After 2 hrs.	Difference
Normal extract (1:4)	305 ± 6.6 (27 animals)	8.3 ± 0.24	9.8 ± 0.33	1.5 ± 0.41 (18 %)
Lung weight 4.3 ± 0.25 g.				
Shock extract (1:4)	347 ± 9.5	8.2 ± 0.25	12.7 ± 0.29	4.5 ± 0.38 (55 %)
Lung weight 5.4 ± 0.16 g.				
		Diff. 2.9 ± 0.44	3.0 ± 0.56	

Amount of amino-N per lung pair (mg).

	Initially	After 2 hrs.	Difference
Normal lung	1.43	1.69	0.26
Shock lung	1.77	2.74	0.97

Increase after shock = 0.71 mg amino-N.
Total N in normal lung extract = 52.5 mg N.
Total N in shock lung extract = 75.0 mg N.
Increase in total N after shock = 22.5 mg N (43 %).

It appears that the increased proteolysis in shock extracts is of enzymatic nature since optimal temperature and pH-levels can be demonstrated. The phenomenon is not dependent

upon ATP and appears even in a nitrogen atmosphere. Identical results can be obtained with an ultracentrifuged (cell-free) extract and in a globulin fraction of the extract after salting out or Cohn fractionation. Proteolysis depends upon the specific antigen. Extracts of lungs from sensitised animals perfused with the specific antigen give identical results while extracts of sensitised but not perfused lungs give negative results. Addition of antigen to blood or serum is without effect in this respect.

Activation.—Proteolysis in shock extracts is activated by cysteine (1:5,000) and by coenzyme A at a very low concentration (1:100,000). Lung extracts obtained from normal animals are not activated by coenzyme A.

Inhibition by thiol compounds in vitro.—The increased proteolysis in shock extracts is inhibited by cysteine in high concentration (1:500). Several organic SH-compounds were tested and it was found that certain simple thio-alcohols inhibited proteolysis at very much lower concentrations. Compounds with four or five carbon atoms and a branched chain gave the best inhibition. Proteolysis could be inhibited by 50 per cent with a butyl compound in a concentration of 1:150,000. Some aromatic SH-compounds gave a similar inhibition at still lower concentration (1:500,000–1:1,000,000). Several compounds were prepared and tested with results which indicated a certain structural specificity (table 2).

The significance of a methyl group in the p-position can be seen from the table. Active compounds were lipophilic and the introduction of hydrophilic groups reduced the effect, a result which indicates that the reaction is intimately associated with lipid-rich cell elements (cell surfaces). Support for this hypothesis was also obtained with proteolysis experiments following Cohn-fractionating as mentioned previously in which a lipoprotein fraction potentiated the effect of other fractions.

Inhibition in vivo.—These aliphatic and aromatic compounds which were most effective in inhibiting proteolysis in shock extracts were also tested *in vivo* and were shown to have the property of preventing shock. Mortality was reduced from

85 per cent to 20 per cent in some experimental series. These substances, however, did not inhibit histamine shock and therefore, they are not histamine antagonistic. Therapeutic doses were approximately 1/10 L.D.₅₀. In acute experiments toxicity, was insignificant while an anaemia was produced, in chronic experiments. Toxicity can be reduced by "masking" the free SH-group with a methyl group (methyl ether). Der-methylation can be accomplished by liver slices *in vitro*.

TABLE 2

Inhibition of proteolysis in shock extracts expressed in per cent after the addition of various aromatic thiols. Amino-N determinations by van Slyke's method.

Compound added	Concentration in organ extract, m mol	Inhibition per cent
Benzol-thiol	0.018	0
	0.091	45
o-methyl-benzol-thiol	0.032	0
	0.16	40
m-methyl-benzol-thiol	0.032	30
p-methyl-benzol-thiol	0.0040	35
	0.0081	50
p-butyl-benzol-thiol	1.2	30
1 ¹ -thiol, 1,4-dimethylbenzol	0.29	15
p-chlor-benzol-thiol	0.0034	0
p-brom-benzol-thiol	0.0035	5
	0.011	25
p-nitro-benzol-thiol	0.32	0
	1.5	75
naphthalinethiol-1	0.031	0
naphthalinethiol-2	0.012	0
	0.062	35
1-methyl, 2-methylenethiolnaphthaline	0.026	0
o-thiobenzoic acid (thiosalicylic acid)	0.13	0
benzol-2-thio-ethane acid	12	61 (colori- metric method)

Serum inhibitors.—A factor in normal guinea pig serum, "serum inhibitor", will also inhibit proteolysis in shock organs. During the sensitisation phase the activity of this factor decreased and it was absent from shock serum. It is thermolabile

(60° C, 1/2-hour) and since it can be inactivated by thiol antagonists such as o-iodosobenzoate, it is probably of a thiol nature. Serum inhibitor was also present in human, pig, and horse serum but not in rat serum. Fractions of these sera have been prepared and preliminary experiments have shown that the inhibiting factor is found in the globulin fraction. Properdin prepared by the method originally described did not have an inhibiting effect.

The results of these investigations can be summarised as follows:

In extracts prepared from shock organs there is an increased proteolytic activity.

An inhibiting factor is present in normal serum but absent from shock serum.

Organic sulphur compounds at low concentrations inhibit proteolysis in vitro and shock in vivo.

It is possible that decrease of serum inhibitor in shock depends upon its being blocked by antigen (as in the case in complement fixation). The thiol compounds which were used may have their effect through reactivation of serum inhibitor or by direct inhibition of the increased proteolysis within the cells. From experiments carried out up to the present time it appears that the simultaneous addition of serum and a strong activator (coenzyme A) which has a free thiol group results in inhibition of the system. This observation is evidence for the great significance of serum inhibitor in anaphylactic shock. Additional experiments are being carried out to investigate these questions.

SUMMARY

It had previously been demonstrated that lung extracts from guinea pigs after anaphylactic shock (shock lungs) displayed increased proteolysis in comparison with normal extracts. Similar proteolysis, of enzymatic nature and dependent upon the specific antigen, can also be obtained with a globulin fraction of lung extract. The process can be activated or inhi-

bited by the common enzyme activators and inhibitors respectively. Coenzyme A in low concentrations also has an activating effect. Inhibition can be obtained with thiol alcohols in low concentration as well as certain aromatic SH-compounds in extremely low concentrations.

These substances which are not histamine-antagonistic, have also been demonstrated to inhibit shock *in vivo*.

Proteolysis in shock extracts can also be inhibited by a serum factor from normal animals which is absent from the serum of sensitised and shocked animals. This serum inhibitor is inactivated at 60° C and by thiol antagonists.

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