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Proteolytic Enzymes of Monocytic and Polymorphonuclear Pleural Exudates.

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During inflammation an increase in the permeability of the blood vessels permits the passage of erythrocytes, leucocytes, serum proteins and fibrinogen. The digestion and absorption of these elements is accomplished by the proteolytic enzymes of the exudate. The simultaneous presence of substances which inhibit proteolysis suggests a study of their behavior, as well as of the rôle of activators and accelerators of enzyme action during inflammation.

The present contribution is an extension of Opie's observations[1] on the proteolytic enzymes of monocytic and P.M.N. exudates. This material was obtained by injecting mineral oil[2] or an aleuronat-starch mixture[3] into the pleural cavity of rabbits. After separation of the cells from the liquid portion of the exudate, they were washed with saline and extracted in distilled water. Proteolytic activity was then measured during autolysis or during digestion of gelatin by Northrop's[4] formol titration method.

It was observed that whereas monocytes contain only one proteinase, pepsin, which is active from pH 2.0 to 5.0, the optimum being at 3.0, the P.M.N. have pepsin, cathepsin and trypsin with optima at pH 3.0, 5.4, and 8.0, respectively. The serous portions of the exudates (S.F.) also differ in that the monocytic type contains a substance inhibiting peptic digestion by the leucocytes, while the P.M.N. enhances this activity. The S.F. of a P.M.N. exudate inhibits the tryptic activity of the corresponding cells, while cells of a monocytic exudate inhibit the tryptic activity of their S.F.

There is also an inhibitory mechanism which concerns the leucocytes themselves. This was first observed by Willstätter, Bamann and Rohdewald,[5] who showed the presence of mutually antagonistic extractable or "lyo"-enzyme and bound or "desmo"-enzyme in the

[1] Opie, E. L., *Physiol. Rev.*, 1922, **2**, 552.

[2] Lucke, B., Strumia, M., Mudd, S., McCutcheon, M., and Mudd, E. B. H., *J. Immun.*, 1933, **24**, 455.

[3] Gay, F. P., and Clark, A. R., *Arch. Path.*, 1926, **1**, 847.

[4] Northrop, J. H., *J. Gen. Physiol.*, 1926, **9**, 767.

[5] Willstätter, R., Bamann, E., and Rohdewald, M., *Z. f. Physiol. Chem.*, 1932, **204**, 181.

W.B.C. This was confirmed and their presence in both monocytes and P.M.N. cells of inflammatory exudates was demonstrated.

When gelatin was digested by the S.F. of either a P.M.N. or monocytic exudate, there was a decrease in carboxylic groups as evidenced by negative formol titration values between pH 4.5 and 5.5 (or 6.0). A similar phenomenon was observed during the digestion of a dipeptid, leucyl-glycine by the S.F. of the monocytic type. (Table I.) In view of the work of several authors[6, 7, 8]

TABLE I.

Hydrolysis of gelatin and leucyl-glycine by supernatant fluids of inflammatory exudates.

Initial pH	Corrected Formol Titration Values		
	Monocytic		P.M.N.
	Gelatin	Leucyl-glycine	Gelatin
3.0	0.02	—0.03	0.29
5.0	—0.74		—0.99
5.5		—1.61	
6.0	—0.44		0.14
8.0	0.54		3.44
8.5		2.90	

this is suggestive of resynthesis of the protein-split products present in the exudate due to a reversal of catheptic action. Whether a similar process may, under proper conditions, go on *in vivo*, during an inflammatory process, is under investigation. Should this phenomenon occur, it might help to explain some phases of the problem of delayed resolution.

A delay in separation of an exudate into its constituents (cells and S.F.) causes an inactivation of the trypsin inhibitor or a release or activation of trypsin. It also makes conditions unfavorable for resynthesis. Gay and Clark[3] showed that a S.F. loses its bactericidal power if it is permitted to stay in contact with its cells for several hours before being tested. The question naturally arises, do conditions which decrease bactericidal action also favor resynthesis in an inflammatory exudate, and hence delayed resolution? This remains to be determined.

[6] Wasteneys, H., and Borsook, H., *Physiol. Rev.*, 1930, **10**, 110.

[7] Voegtlin, C., Maver, M. E., and Johnson, J. M., *J. Pharm. Exp. Ther.*, 1933, **48**, 241.

[8] Blagowestschenski, A. W., and Jeremejew, G. W., *Biochem. Z.*, 1934, **270**, 66.