TABLE 1

VARIATION IN THE DN-ASE INHIBITOR ACTIVITY WITH CELL TYPE IN VARIOUS DISEASES

Tissue	Inhibition/10^4 cells (%)	Principal cell types (%)						
		Polymorphonuclear leucocytes	Lymphocytes	Metamyelocytes	Myelocytes	Pro-myelocytes	Myeloblasts	Normoblasts
Normal blood	37.4	59	39					
Lymphosarcoma and Hodgkin's, blood	32.4	62	38					
Chronic myelocytic leukemia, blood	31.9*	74	16	3	2	1		
	10.8†	27	0	17	32	21	4	23
Normal bone marrow	14.3	46		13	9	2		
Lymphosarcoma and Hodgkin's, marrow	14.8	44		13	21	1	1	15
Chronic myelocytic leukemia, marrow	6.8	35		12	31	11	8	3
Acute myelocytic leukemia, blood	4.2	16	3	5	4	71		
Acute myelocytic leukemia, marrow	2.7	11		4	13	27	42	
Chronic lymphocytic leukemia, blood	12.1	11	88					
Chronic lymphocytic leukemia, marrow	8.1	10	83	1	1			
Acute lymphocytic leukemia, blood	0‡	3	97 (and/or blasts)					
Acute lymphocytic leukemia, marrow	0‡	0	100 (and/or blasts)					

* Chronic myelocytic leukemia under treatment.
† Chronic myelocytic leukemia in relapse.
‡ Excess enzyme present.

morphonuclear leucocytes and lymphocytes of the blood contain the highest concentration of inhibitor—namely, 37.4%/10^4 cells. Extracts of the less mature cells from the normal marrow exhibit a concentration of one third this value.

Consistent with these findings, the blood and marrow from patients with lymphosarcoma and Hodgkin's disease which are morphologically normal have normal quantities of inhibitor activity.

The more primitive cells of the blood and marrow from leukemic patients show inhibitor activities between 12.1% and 0% per 10^4 cells. In chronic myelocytic leukemia, when, as a consequence of treatment, the peripheral blood cells were qualitatively normal, the inhibitor content was 31.9%. In contrast, the inhibitor activity of the more immature blood cells of leukemic patients in relapse averaged 10.8%/10^4 cells. These data indicate that the absence of inhibitor is not a manifestation of malignancy per se. The progressive decrease in inhibitor activity is paralleled by an increase in the number and degree of immaturity of the cells (and a decrease in the number of mature cells) from which the extracts were prepared. This suggests that a close relationship exists between the degree of immaturity of the cells studied and their content of DN-ase inhibitor.

The existence of DNA in a highly polymerized form in the cell nucleus and particularly in the chromosomal apparatus is well known. Its synthesis and degradation, therefore, must be of prime importance in cellular division and growth. DN-ase, an enzyme capable of depolymerizing DNA, has been detected in many tissues and is probably a constituent of all cells. Highly polymerized DNA is resistant to the action of phosphatases, but may be easily split after depolymerization by DN-ase.

The presence of an inhibitor of DN-ase in cells indicates a possible regulatory mechanism to control the breakdown of DNA. The association of DNA with the chromosomes and hence with cell division, and the present demonstration of a correlation between the inhibitor content and cell maturity, suggest that the inhibitor is intimately associated with the control of cell division and multiplication. Complete data covering the above studies are to be reported (*5*).

References

1. ZAMENHOF, S., and CHARGAFF, E. *J. Biol. Chem.*, **180**, 727 (1949).
2. DABROWSKA, W., COOPER, E. J., and LASKOWSKI, M. *Ibid.*, **177**, 991 (1949).
3. COOPER, E. J., TRAUTMAN, M. L., and LASKOWSKI, M. *Proc. Soc. Exptl. Biol. Med.*, **73**, 219 (1950).
4. HENSTELL, H. H., and FREEDMAN, R. I. *Cancer Research* (in press).
5. HENSTELL, H. H., FREEDMAN, R. I., and GINSBURG, B. *Ibid.* (in press).

Manuscript received November 23, 1951.

Action of Chymotrypsin α and Chymotrypsin B upon Several Protein Substrates[1]

John A. Ambrose[2] and M. Laskowski

Department of Biochemistry, Marquette University School of Medicine, Milwaukee, Wisconsin

It is well known that native proteins are more resistant to proteolytic digestion than denatured proteins. The generally accepted explanation is that this is due to the protective arrangements of the nonspecific groups on the molecule of the substrate. An attempt was made to investigate a reversed case—namely, the influence of the nonspecific groups of the enzyme molecule on the rate of proteolytic digestion. Crystalline chymotrypsin α (*1*) and crystalline chymotrypsin B (*2*) were used. Both enzymes are known to have identical specificity toward synthetic substrates (*3*) but different isoelectric points (*4*). Although it has previously been shown that chymotrypsin B digests casein with a somewhat slower rate than chymotrypsin α (*5*), it was interesting to ascertain whether this difference in the rate of digestion persists with other protein substrates.

The following crystalline proteins were prepared and used as substrates: egg albumin (*6*), edestin (*6*),

[1] Aided by a research grant from the National Institutes of Health, U. S. Public Health Service.

[2] Some of the data included in this report were taken from a thesis submitted by John A. Ambrose to the Graduate School of Marquette University in partial fulfillment of the requirements for the M.Sc. degree. Present address: Department of Chemistry, Oregon State College, Corvallis.

TABLE 1*

Substrate	Percentage
Egg albumin, heat-denatured	46
Egg albumin, urea-denatured	75
Edestin, heat-denatured	86
Edestin, urea-denatured	79
Chymotrypsinogen α, undenatured	48
Hemoglobin, urea-denatured	91
Lysozyme, " "	101

* Figures indicate percentage of digestion by chymotrypsin B, when digestion by chymotrypsin α is 100.

chymotrypsinogen α (*1*), dog hemoglobin (*7*) and lysozyme (*8*). In order to secure a faster rate of reaction some of the substrates were denatured either by heating (30 min at 100° C) or by urea (*9*). The insoluble precipitate was centrifuged off and discarded, and the soluble portion was used. The concentration of substrate was expressed in optical density at 280 mμ and in most cases was adjusted to $E_{280} = 3.0$. The proteolytic activity was determined spectrophotometrically according to Kunitz (*10*).

The results of experiments with different substrates at pH 7.6 are shown in Table 1. Digestion by chymotrypsin α was faster than by chymotrypsin B except in the case of urea-denatured lysozyme, in which both enzymes gave the same results. The observed differences in the rate of digestion by the two chymotrypsins persisted through a fairly wide range of pH values, as shown in Fig. 1. Confirming previous results with casein as substrate (*11*), no significant differences in the optimum pH of the two chymotrypsins were observed with other substrates. Also in agreement with previous observations on casein (*5*), it was found that the end points of the reaction with the denatured substrates were the same for both chymotrypsins (Table 2).

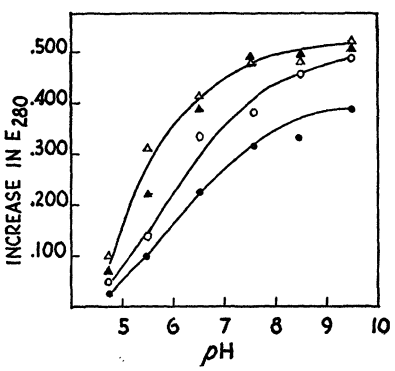

FIG. 1. –○–, Chymotrypsin α, urea-denatured egg albumin; –●–, Chymotrypsin B, urea-denatured egg albumin. –△–, Chymotrypsin α, urea-denatured lysozyme; –▲–, Chymotrypsin B, urea-denatured lysozyme. 500 γ of enzyme and the concentration of substrate $E_{280} = 3.0$ in all tubes. Incubation time, 20 min. All figures corrected for blanks. Buffers 4.5 and 5.5 acetate, 6.5 and 7.6 phosphate, 8.5 and 9.5 borate.

TABLE 2

END POINTS OF DIGESTION BY THE TWO CHYMOTRYPSINS

Substrate*	Chymotrypsin α E_{280}	Chymotrypsin B E_{280}
Egg albumin, urea-denatured	0.660	0.606
Hemoglobin, " "	.473	.492
Lysozyme, " "	.643	.650
Lysozyme, native, fresh	0.495	0.157

* Concentration of substrates adjusted to give the value of $E_{280} = 3.0$, 1000 γ of enzyme, incubation 3 hr, at 37° C, 0.1 *M* phosphate, pH 7.6. Corrected for the controls.

The action of the two chymotrypsins was also compared on two samples of native lysozyme: one freshly prepared in this laboratory, the other obtained from Armour and Co. (Fig. 2). The freshly prepared lysozyme was more resistant to the action of both chymotrypsins and also showed the greatest difference in susceptibility to digestion by the two enzymes; chymotrypsin B accounted for only about 25% of the digestion obtained with chymotrypsin α.

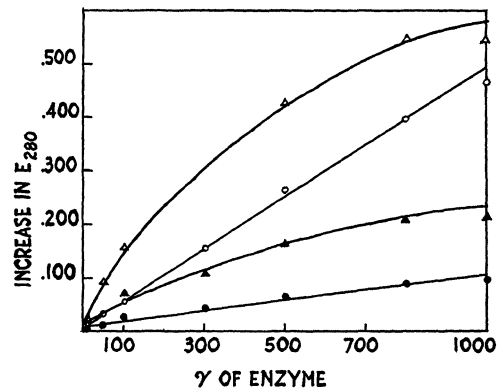

FIG. 2. –○–, Chymotrypsin α, native lysozyme freshly prepared; –●–, Chymotrypsin B, native lysozyme freshly prepared. –△–, Chymotrypsin α, native lysozyme, Armour and Co.; –▲–, Chymotrypsin B, native lysozyme, Armour and Co. Concentration of substrate $E_{280} = 16.4$, pH 7.6, phosphate buffer in all tubes. Incubation time, 20 min. All figures corrected for blanks.

According to Lindenstrom-Lang (*12*), the digestion of native proteins is preceded by denaturation. The experiment with lysozyme supports this interpretation. Furthermore, it suggests that chymotrypsin B differs from chymotrypsin α mainly in its denaturing ability.

References

1. KUNITZ, M., and NORTHRUP, J. H. *J. Gen. Physiol.*, **18**, 433 (1935); **19**, 991 (1936).
2. SMITH, E. L., BROWN, D. M., and LASKOWSKI, M. *J. Biol. Chem.*, **191**, 639 (1951).
3. FRUTON, J. S. *Ibid.*, **173**, 109 (1948).
4. KUBACKI, V., BROWN, K. D., and LASKOWSKI, M. *Ibid.*, **180**, 73 (1949).
5. BROWN, K. D., SHUPE, R. E., and LASKOWSKI, M. *Ibid.*, **173**, 99 (1948).
6. HAWK, P. B., OSER, B. L., and SUMMERSON, W. H. *Practical Physiological Chemistry*, 12th ed. Philadelphia: Blakiston (1947).
7. DRABKIN, D. L. *J. Biol. Chem.*, **164**, 703 (1946).
8. ALDERTON, G., and FEVOLD, H. L. *Ibid.*, 1.
9. ANSON, J. L., and MIRSKY, A. E. *J. Gen. Physiol.*, **17**, 151 (1934).
10. KUNITZ, M. *Ibid.*, **30**, 291 (1947).
11. KEITH, C. K., KAZENKO, A., and LASKOWSKI, M. *J. Biol. Chem.*, **170**, 227 (1947).
12. LINDERSTROM-LANG, K. Rept. at XII Intern. Congr. Pure Applied Chem., New York (Sept. 1951).

Manuscript received November 7, 1951.