have 3 elements (*11*): (1) the number of the individual skeletons; (2) the arrangement in space of the skeletons—i.e., the molecular pattern of the structure; (3) specifications of the manner in which the various complements of R_1, R_2, . . . side chains are inserted into the sites of each skeleton. If this situation materializes, there may well be general principles regarding the sets of points with which the skeletons —and also water clusters—are associated (*11*). Molecular and water cluster patterns associated with points of cristobalite and tridymite networks, for example, would explain many facts regarding the space groups of crystalline proteins and the nature of various protein intergrowths (*12*). We also notice that a supposed difficulty (*2*) in the relation between, for example, the myoglobin and the hemoglobin of horse is resolved in this system of ideas. The myoglobin is, seemingly, not a precursor of the hemoglobin: how, then, can an apparent structural relation between them be explained? The difficulty disappears when we see that the myoglobin would have a certain system of skeletons characteristically substituted, and the hemoglobin the same set four times repeated, also characteristically but differently substituted.

In the light of these results, the time seems ripe for the application to intensity maps of protein crystals, as they become available, of certain of the techniques prepared for this purpose (*13*). It has been shown that a structure comprising a unit D repeated by translation at a set of points has the transform $T = T_\delta T_D$. In such a case we may make tentative assumptions regarding δ and D and construct new intensity maps entry by entry, the map $|T|^2/|T_D|^2$ when D is "given," the map $|T|^2/|T_\delta|^2$ when δ is "given." The transform of the first is, then, the vector map of the molecular pattern; the transform of the second is the vector map of the unit D. The transforms of a variety of structural types and of various kinds of molecular pattern, which have already been recorded (*13*), may prove useful in this connection. With the present viewpoint, the shapes of protein structures (such as the hemoglobin structure with mol wt $\sim$ 66,700) are functions of all three elements cited above. From these shapes the shape of the unit is not deducible. A possible starting point, however, is the assumption of a globulite type of skeleton, in which there is no gross difference in dimensions in various directions. The testing of this hypothesis from intensity maps of protein crystals is in progress.

References

1. WRINCH, D. *Nature*, **139**, 972 (1937).
2. TRISTRAM, G. R. *In Haemoglobin*. New York: Interscience, 109 (1949).
3. PORTER, R. R., and SANGER, F. *Ibid.*, 121.
4. SCHMID, K. *Nature*, **163**, 481 (1948).
5. CARLISLE, C. H., and SCOULOUDI, H. *Proc. Roy. Soc. (London)*, **207**, A, 496 (1951).
6. CROWFOOT, D. *Proc. Roy Soc. (London)*, **164**, A, 580 (1938).
7. CHIBNALL, A. C. *J. Intern. Leather Trades' Chemists*, **30**, 1 (1946).
8. SANGER, F. *Biochem. J.*, **39**, 507 (1945).
9. LOW, B. *Cold Spring Harbor Symposia Quant. Biol.*, **14**, 77 (1949).
10. WRINCH, D. *Wallerstein Lab. Communs.*, **11**, 175 (1948).
11. ———. *Phil. Mag.*, **26**, 313 (1938).
12. ———. *Am. Mineral.*, **33**, 781 (1948).
13. ———. *Fourier Transforms and Structure Factors*. Monogr. No. 2, Am. Soc. X-Ray and Electron Diffraction (1946).

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An Inhibitor of Desoxyribonuclease in Human White Blood Cells and Bone Marrow Cells and its Relationship to Cellular Maturity[1]

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Inhibitors of the enzyme desoxyribonuclease (DN-ase) have been reported. Zamenhof and Chargaff (*1*) have noted an inhibitor in yeast that is specific for yeast DN-ase. Laskowski *et al.* (*2*) have reported a similar, but not identical, inhibitor in the crop gland of the pigeon; and, in another communication (*3*), an inhibitor in various normal and cancerous tissues (human), as well as in the tissues of the normal rat, has been noted. No significant variations in the amount of inhibitor per gram weight of tissue were demonstrated. No studies have been reported on human blood or bone marrow cells, with the exception of one human marrow (*3*).

In the present work, the DN-ase inhibitor content of human white blood cells and bone marrow cells, both from normal subjects and from patients with leukemia, was determined. It was found that the inhibitor activity present in the cells varies with the degree of cellular immaturity. The inhibitor activity of mature human white blood cells averaged 37.4%/10^4 cells. The inhibitor activity of the primitive blast cells from acute leukemia was close to zero. Cells of intermediate degrees of maturity were found to have intermediate amounts of inhibitor.

A viscosity method was used for the determination of the enzyme inhibitor. The DN-ase activity was measured by the time required for the enzyme to reduce to one half the relative viscosity of a solution of desoxyribonucleic acid (DNA). The inhibitor activity of a given cell extract was measured by the amount of enzyme inactivated and is expressed as the percentage of the enzyme inactivated by the extract of 10^4 cells. Cell extracts of whole blood and of bone marrow were prepared by dilution (1/100) with water. The inhibitory activity of whole blood was found to reside solely in the white blood cells. Neither hemolyzed red cells nor plasma had appreciable inhibitor activity. The technique is to be described elsewhere (*4*).

The concentration of the desoxyribonuclease inhibitor in the various types of cell preparations is summarized in Table 1. The normal mature poly-

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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).

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

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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*),

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[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.