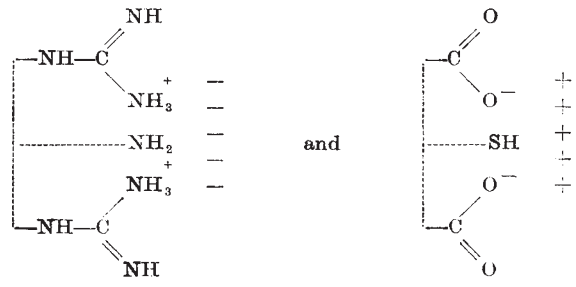
Potentiation of Chemical Reactivities at Protein Surfaces

The ability of toxicants which function as alkylating agents to react at specific sites on protein surfaces may be caused by differences in the reactivities of the functional groups of the side-chains as well as molecular geometry. Several mechanisms by which these reactivities might be modified are suggested by the results of some recent investigations on the kinetics of alkylation of amino-acids, peptides and proteins by the fungicide 2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine[1]. These studies show that only dissociated amino- and sulphydryl-groups can take part in the reactions, while the NH_3^+ and SH groups are inert. In free amino-acids the latter groups predominate under physiological conditions, so that only small fractions of their potential reactivities are utilized. It is suggested that any structural features in protein molecules giving rise to micro-environments which behave as though they are more acidic or more basic than the surrounding medium can suppress or increase the ionization of the functional groups contained in them, thus giving rise to regions of low and high reactivity on the protein surface. It can be seen that the gains from such situations might be considerable when it is taken into account that only 0·25 per cent of the reaction potential of the amino-group of glycine is utilized at *p*H 7·0.

Two ways by which this might be achieved are suggested by the reaction-rate and ionization constants obtained on small molecules[1,2]. The first of these involves isolation of functional groups from surrounding carboxyl groups. For example, it has been found that asparagine is six times more reactive than aspartic acid, indicating that the second carboxyl group suppresses reactivity by increasing the *pK′* values of the amino-group (Table 1). On separating the amino- and carboxyl-groups by peptide linkages as in triglycine, the *pK′* is reduced to 7·91 and the reactivity relative to aspartic acid is increased to 43. The highest degree of potentiation obtainable through isolating an amino-group from surrounding carboxyl groups is represented by the methyl ester of glycine, in which the acid function is suppressed altogether. This compound is 88 times more reactive than aspartic acid at *p*H 7·0. However, the ultimate in reactivity has not been achieved, since *p*-aminobenzoic acid is capable of combining with the *s*-triazine 230 times more rapidly than with aspartic acid. In this case more than 99 per cent of the reaction potential is utilized.

Since 50 per cent of an amino- or sulphydryl-group will be present in reactive form when the *pK′* of the ionizing group is equal to the *p*H of the medium, a second mechanism must be found by which *pK′* values can be reduced to 7 or lower in order to utilize the maximum amount of latent reactivity under physiological conditions. It is believed that this can be achieved if the ionizing group is situated in a region rich in positively charged centres having very high *pK′* values, such as the guanidino-groups of the arginine residue. These would tend to repel protons and attract negatively charged ions, and so create a micro-environment with a higher *p*H than the main body of the solution through the formation of a localized Helmholtz double layer. A larger proportion of the functional group could then ionize to produce an effect equivalent to lower *pK′* value. Thus the reactivities of functional groups located in environments such as :



could vary considerably.

An inspection of the *pK′* values compiled by Cohn and Edsall[2] reveals that this can occur to some extent even when the interacting groups are the same. Thus the *pK′* values of the two amino-groups of cystine are reported to be 7·48 and 9·02, compared with values of 9·0–9·7 for most of the other amino-acids. When proximity to basic groups is coupled with isolation from carboxyl groups, an even greater degree of ionization can occur. Thus the *pK′* values reported for cystinyl-di-di-glycine are 6·01 and 6·87 respectively, indicating that 55–90 per cent of the amino-groups are present in reactive form at *p*H 7·0.

These examples include only primary amino-groups. However, it is believed that the reactivities of the functional groups of lysine, cysteine and tyrosine residues with electrophilic toxicants might be suppressed or potentiated, depending upon whether they are located in anionic, non-polar or cationic micro-environments. The probability that effects such as these are operative in large molecules is suggested by the recent finding that bovine serum albumin reacts about 100 times more rapidly with 2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine than would be anticipated from the velocity coefficients of its component amino-acids.

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Table 1. Effect of *pK′* on the Reactivity of Primary Amino-Groups at *p*H 7·0 relative to Aspartic Acid

Compound	*pK′*	Relative reactivity
Aspartic acid	9·60	1
Asparagine	8·80	6
Glycine	9·60	7
Triglycine	7·91	43
O-Methyl glycine	7·73	88
p-Aminobenzoic acid	4·92	230

[1] Burchfield, H. P., and Storrs, Eleanor E., *Contrib. from Boyce Thompson Inst.*, **18**, 395 (1956).
[2] Cohn, Edwin J., and Edsall, John T., "Proteins, Amino Acids, and Peptides as Ions and Dipolar Ions", 85 (Amer. Chem. Soc. Mon. Series. Reinhold Pub. Corp., New York, 1943).

Isolation of Nucleic Acids from *Micrococcus lysodeikticus*

We have found a simple method for the preparation of a mixture of ribonucleic acid and deoxyribonucleic acid and the preparation of purified deoxyribonucleic acid in a highly polymerized state from cells of *M. lysodeikticus*. In arriving at the techniques, we have taken advantage of the fact that above *p*H 8·0 the activity of the ribonuclease system in *M. lysodeikticus* is negligible. Moreover, in the absence of orthophosphate, polynucleotide phosphorylase (polyase) is inactive toward ribonucleic acid. Therefore, for maximum yields of ribonucleic acid the lysis