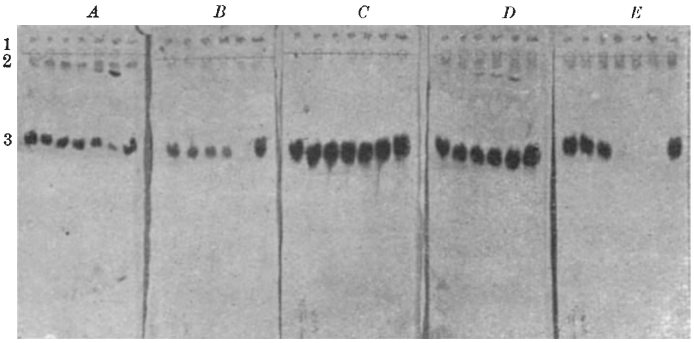

Fig. 3. Solvent system : *n*-butanol–pyridine–water (45 : 25 : 40). Spraying reagent : silver nitrate (modified method).

A, Glucose + selenite (1×10^{-3} *M*) + pantothenate ; *B*, glucose + arsenite ; *C*, glucose + selenite + arsenite ; *D*, glucose + selenite ; *E*, glucose + selenite + cysteine.

1, Starting line ; 2, unidentified metabolite ; 3, glucose

bolism of glucose and inhibited completely the metabolism of lactose.

Investigations on the nature of the intermediate metabolite will be the subject of a further communication.

Thus experiments using inhibitors show that enzymes catalysing the first stage of lactose metabolism of *E. coli* (1) contain active sulphydryl groups ; and (2) are more sensitive to the influence of all the inhibitors employed by us than enzymes catalysing glycolysis. This work emphasizes the importance of paper chromatography for studies of metabolism.

J. Opieńska-Blauth
I. Madecka-Borkowska
T. Borkowski

Z Zakładu Chemii Fizjologicznej,
Wydz. Lek. Akademii Medycznej,
Lublin.

Optimum *p*H and Iodine Absorption of an Enzyme Molecule

In a previous communication it has been shown by Basu and Nandi[1] that the extent of inactivation consequent on the unfolding of the protein chain of an enzyme molecule could be detected by iodimetric titration of the SH-groups uncovered by the unfolding of the coiled-up molecules. In this connexion it has been noted that increase in iodine absorption occurs in cases where the enzyme has been partially deactivated, although no denatured, insoluble protein was formed. It may be concluded, therefore, that denaturation leading to insolubilization takes place only when the unfolding of the protein coil has proceeded to an appreciable extent, whereas deactivation makes its appearance much earlier and with a lesser degree of uncoiling. These observations suggest that the variation in the extent of enzyme activity at different *p*H's may, to some extent, depend on the extent of coiling or uncoiling of the enzyme molecule. In order to test this point, pepsin solution was adjusted to different *p*H's by the addition of the necessary amount of hydrochloric acid or sodium hydroxide as the case may be, and titrated with *N*/2,000 iodine solution below 15° C., using starch as indicator. The results are shown in Table 1.

Table 1. Iodine Absorption by Pepsin at Different *p*H's (*p*H adjusted by the addition of 0·1 *N* hydrochloric acid or 0·1 *N* sodium hydroxide to 5 per cent pepsin solution of initial *p*H 5·8)

*p*H	Iodine (gm.)/gm. enzyme × 10^4
2·20	0·3241
3·00	0·3831
3·95	0·4715
5·80	0·6365
7·85	7·6718
8·50	0·7366
9·55	0·9724

It may be observed from this table that at about *p*H 2 the pepsin has got the lowest iodine absorption value, a *p*H at which the activity of this enzyme is maximum. Now it has been shown[1] that minimum iodine absorption value is associated with maximum tightness of coiling, and so the optimum *p*H is associated with maximum coiling of the pepsin molecule. As the *p*H is increased, the iodine absorption value increases, which is also parallel to increased uncoiling or diminished activity.

When we consider the case of trypsin, an exactly similar conclusion is reached. From Table 2 it is to be noted that iodine absorption is minimum at about *p*H 8, which also corresponds to maximum activity range, whereas at lower *p*H values the iodine absorption is higher, corresponding to lesser activity of trypsin.

Table 2. Iodine Absorption by Trypsin at Different *p*H's (*p*H adjusted by the addition of 0·1 *N* hydrochloric acid or 0·1 *N* sodium hydroxide to 3·2 per cent trypsin solution of initial *p*H 4·8)

*p*H	Iodine (gm.)/gm. enzyme × 10^4
1·1	2·65
1·9	2·27
3·5	1·88
5·4	1·80
6·7	1·49
8·0	1·33
8·2	1·41
8·5	1·41
10·0	1·72

From these two sets of results, it is therefore evident that, at least in the case of pepsin and trypsin, the *p*H optima is associated with maximum compactness of the coiled-up structure of the protein molecule. It is difficult to account for this variation in the extent of folding of the enzyme molecule at different *p*H's, but in all probability it may be ascribed to the formation of salt with either NH_2- or COOH-groups (whichever is predominant) of the molecule, electrolytic dissociation of the salts, and mutual repulsion between the similarly charged COO^-- or NH_3^+-groups on the same molecule with the consequent unfolding of the protein molecule.

Sadhan Basu*
Satyendra Prosad Nandi

Indian Association for the Cultivation of Science,
Calcutta 32.

* Present address :

Department of Chemistry,
Indiana University,
Bloomington, Indiana.
Nov. 26.

[1] Basu and Nandi, *Enzymologia*, **14**, No. 6 (1951).