LXXVIII. THE SOLUBILITY OF PROTEINS AND PROTEOSES IN ALDEHYDES AND OTHER ORGANIC SOLVENTS.

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ALTHOUGH the action of aqueous solutions of aldehydes upon proteins has been extensively studied, the behaviour of aldehydes in the anhydrous state does not seem to have been investigated in detail, except for the single observation of Mathewson [1906] that gliadin appeared to be somewhat soluble in impure benzaldehyde. Apart from its chemical interest such an investigation might lead to improved methods of separation and purification of proteins and proteoses. The present methods of fractionation depend largely on the use of inorganic salts, and there is a considerable field for the application of organic solvents in protein chemistry.

Observations were made in the first place with benzaldehyde. It was found that, whilst gelatin, egg-albumin, gliadin, and proteoses (Witte's peptone) were precipitated from their solutions in anhydrous phenol by paraldehyde, the addition of benzaldehyde had no effect. Egg-albumin was, furthermore, slightly soluble in benzaldehyde alone, and was reprecipitated on the addition of alcohol. Gelatin, gliadin and caseinogen, however, appeared to be insoluble in benzaldehyde. Glycine and alanine were similarly insoluble, but proteoses dissolved in benzaldehyde with great readiness at ordinary room temperature, only a small residue being left undissolved. The proteoses were precipitated by the addition of various organic solvents (alcohol, acetone, chloroform, paraldehyde, mesityl oxide), or even by warming the benzaldehyde alone to 37°; on cooling the proteoses re-dissolved.

Benzaldehyde is thus characteristic (1) in exerting a selective solvent action upon intermediate hydrolytic products of proteins, amino-acids and proteins themselves being almost insoluble; (2) in being efficacious as a solvent for proteoses within only a very restricted range of temperature (approx. room temperature).

Similar experiments were carried out with many other aldehydes and derivatives, and the results are as follows:

Table I. *Solubility of proteoses.*

<i>m</i> -Toluylaldehyde ...	Sol. at room temp.	Insol. at 37°
Benzaldehyde cyanohydrin		
Salicylaldehyde ...	Slightly sol. at room temp.	Readily sol. at 37°
Anisaldehyde ...	"	"
Acetaldehyde ...	Insol.	"
Oenanthal ...	"	"
Chloral ...	"	"
Chloral hydrate ...	Sol. just above M.P. (57°)	
Butylchloral hydrate ...	"	"
Chloral alcoholate ...	Insol.	
Phenylacetaldehyde ...	"	
Cinnamaldehyde ...	"	
Citral ...	"	
Furfuraldehyde ...	"	
Acetone ...	"	
Mesityl oxide ...	"	
Acetophenone ...	Sol. just above M.P. (20°)	Insol. on warming
Benzophenone ...	Insol.	

A consideration of these results shows that proteoses are only soluble in aldehydes of the aromatic type, *e.g.* compounds in which the —CHO group is directly attached to the benzene nucleus.

In other aldehydes, *e.g.* aliphatic and heterocyclic, the proteoses are insoluble.

Substitution of groups in the benzene nucleus of the aromatic aldehydes, although not unfavourable to the solubility of proteoses, sometimes had the interesting result of reversing the effect of temperature (*cf.* salicylaldehyde). It is noteworthy that proteoses were also soluble in acetophenone, but not in acetone or benzophenone.

It is quite possible that this apparent solubility is in reality determined by chemical factors, as previous treatment of proteoses with formaldehyde and nitrous acid destroyed their solubility in benzaldehyde. However, even in the case of aldehydes which exert no solvent action, chemical reactions apparently take place, *e.g.* proteoses and gelatin become yellow when warmed with phenylacetaldehyde.

Whilst proteoses were insoluble in chloral, they were soluble in fused chloral hydrate and butylchloral hydrate, but not in chloral alcoholate, in which one of the OH groups is replaced by an ethoxy group. In addition to proteoses, gelatin, albumin, gliadin, and lecithin were also soluble in chloral hydrate, and these substances were precipitated therefrom by paraldehyde, but not by alcohol. It is remarkable that the addition of the elements of water to chloral, forming a dihydric alcohol, in which the two OH groups are attached to the same carbon atom, should lead to the production of solvent action upon the foregoing colloids, as alcohol, glycerol, and chlorohydrin are without such solvent power.

The effect of adding various aldehydes to an ethereal solution of lecithin has also been studied. In certain cases precipitation of the lipin took place, but with some aldehydes the solution remained clear, indicating that in such aldehydes the lecithin was soluble. The results in general are similar to those

obtained with the proteoses, but a survey of the following table will indicate a few exceptions.

Table II. *Ethereal solution of lecithin.*

Substance added	Observation
Benzaldehyde	No effect
<i>m</i> -Toluyaldehyde	"
Salicylaldehyde	"
Anisaldehyde	Ppt.
Acetophenone	"
Acetaldehyde	"
Paraldehyde	"
Phenylacetaldehyde	"
Cinnamaldehyde	"
Oenanthal	No effect

The application of aldehydes as fractionating solvents may thus be serviceable in the separation and purification of proteoses and lipins, as was suggested in the case of paraldehyde [Cooper, 1924].

The influence of the factor of chemical structure has also been considered in connection with the action of aldehydes and ketones on amino-acids.

The action of formaldehyde on amino-acids is well known, and is, of course, the basis of Sørensen's method. Using the same technique, the action of formaldehyde, acetaldehyde, and acetone on amino-acids has been compared quantitatively. It was found that acetaldehyde reacted much less readily than formaldehyde with glycine and alanine, whilst in the case of acetone there was practically no chemical action at all. It was therefore of interest to study the distribution of acetone between water and certain proteins, especially as acetone has been employed as a protein precipitant [Piettre and Vila, 1920]. In fact, acetone has quite a general precipitating action on various colloidal solutions, *e.g.* proteins, starch, glycogen, dextrin, lipins.

The following tables give the quantitative results of experiments on the distribution of (a) acetone between water and gelatin, (b) acetone between water and egg-albumin (heat coagulum) at room temperature.

The estimation was carried out according to Messinger's iodometric method. Preliminary experiments showed that equilibrium was attained within 7 days.

Table III.

(a) *Gelatin.*

Acetone concentration in water phase	Distribution coeff.	(amount of acetone in 1 g. protein) (amount of acetone in 1 g. water)
(I) 0.2 %		0
2.0		2
8.0		0
(II) 0.25		0
0.50		0
1.00		0
2.00		0
4.00		1.7
8.00		1.2
20.00		0

(b) *Egg-albumin. (Heat-coagulum.)*

2 %	2.5
8	0.0

A point of interest, difficult to understand, is the phenomenon of a maximum shown with respect to the distribution coefficient; below and above a certain critical range of acetone concentration there is no evidence at all of any absorption of acetone by the protein. Although these results differ from the observations made on the distribution of phenol between water and proteins, in which case the partition-law was found to hold [Cooper, 1912], there is very little evidence that acetone reacts chemically with amino-acids or proteins.

Accordingly, as in the case of alcohol, the action of acetone as a protein-precipitant is probably physico-chemical.

The fact that the behaviour of aldehydes towards proteoses depends so much upon chemical structure and temperature rendered it of great interest to investigate the influence of these factors in the case of other solvents.

It is well known that proteins are soluble in molten phenol and cresols, and the phenols therefore afforded a convenient group in which to study the effect of structural differences. Observations have also been made in the case of certain amines.

The tests were carried out with proteoses, gelatin, and egg-albumin at 40° (except when the substance had a m.p. above 40° in which case the experiment was carried out at a temperature just above the m.p.), with the following organic substances.

Phenol and derivatives.

Phenol, cresols, carvacrol, *p*-chlorophenol, *o*-nitrophenol, α -naphthol, hexahydrophenol, anisol, guaiacol, salol, methyl and amyl salicylates, benzyl alcohol.

The main results of the observations are as follows:

1. The solvent action of the phenols diminishes as the homologous series is progressively ascended.
2. The introduction of a chlorine atom into phenol diminishes solvent power, but to a less degree than a methyl group.
3. Solvent action is diminished by introduction of the $-\text{COOCH}_3$ group, and abolished by that of $-\text{COOC}_5\text{H}_{11}$, $-\text{COOC}_6\text{H}_5$, nitro and methoxy groups; and by hydrogenation of the benzene nucleus (hexahydrophenol).
4. α -Naphthol and benzyl alcohol have no solvent action on proteoses and gelatin, although Mathewson [1906] found that gliadin was soluble in benzyl alcohol.

Amines and derivatives.

The observations were made at room temperature (whenever possible) and at 40°, unless a higher temperature was necessary to melt the substance.

Amines and their derivatives belonging to the aliphatic, aromatic and heterocyclic types were employed. The following results were obtained:

(1) Aniline, *o*-toluidine, *m*-xylidine, methylaniline, chloroaniline, anisidine and phenylhydrazine dissolved proteoses on warming but not at room temperature.

(2) Dimethylaniline and ethylaniline, however, dissolved proteoses at room temperature, but not on warming, showing that the introduction of two methyl groups or one ethyl into the —NH_2 radicle had the peculiar result of reversing the temperature effect (cf. benzaldehyde and salicylaldehyde).

(3) Proteoses were slightly soluble in formanilide (46°), but insoluble in benzylamine, dichloroaniline, phenylethylamine, diphenylamine, α -naphthylamine, azobenzene, urethane, pyridine, and camphylamine.

Proteoses are thus insoluble in amines of the aliphatic and heterocyclic types, as was also observed in the case of the aldehydes.

SUMMARY.

The results as a whole indicate that the solubility of proteins, proteoses, and lipins in organic substances, *e.g.* aldehydes, phenols, amines, is readily influenced by the chemical structure of the solvent and by temperature, and such solvents may thus be of service in biochemistry for the selective extraction or purification of the colloidal constituents of protoplasm.

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