LXXXIII. OBSERVATIONS ON THE SOLUBILITY OF INSULIN.

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It is of the greatest importance for the isolation and purification of insulin to know its solubility in different organic solvents. Our knowledge as to this has been up till now very limited. Insulin is freely soluble in water. We know, further, that it is insoluble in ethyl ether, toluene and neutral ethyl alcohol. Doisy, Somogyi and Shaffer [1922] find that insulin is certainly soluble in water, but that it is incompletely precipitated from its solution at p_H about 5 to 6. They find, also, that it is soluble in "alcohol" except at its isoelectric point.

The following experiments on the solubility of insulin have been performed with "crude insulin" prepared partly according to Collip [1922] and partly according to Shaffer. About 20–25 mg. contained one "rabbit-unit" and 0.04–0.02 mg. produced characteristic convulsions in mice.

The solubility experiments were made in the following way. It might be expected that the salt of insulin would have a different solubility from the free compound. For this reason we have made the experiments in two different series: acid and alkaline extractions.

Extractions at acid reaction. 10 mg. insulin were dissolved in 1 cc. water and 1 cc. acid buffer solution (equal parts of *N* acetic acid and *N* Na-acetate, p_H about 4.75) was added. The solution was equally distributed (0.2 cc.) in ten small Petri dishes and evaporated to dryness in a vacuum exsiccator over sulphuric acid. The activity of the insulin was not hereby diminished. 2–3 cc. of the organic solvent were now added. The solvent was filtered from the residuum and again evaporated in a dish. 2 cc. of physiological salt solution were added and 0.5 cc. of this solution was injected into each mouse. The animals were placed in a thermostat at 28° and the occurrence of convulsions was noted. The convulsions demonstrated the solubility of the insulin in the solvent used. The activity of the residuum from the extractions was often controlled in the same way in order to show that the insulin had not been destroyed during the operations.

The extraction at alkaline reaction was made in the same way only with the difference that an alkaline buffer was used (sodium hydroxide-borate, p_H 10) instead of the acid.

With this method we tried some different organic liquids until we found in absolute methyl alcohol the first water-free solvent.

Extraction at acid reaction.

Solvent	Extract	Residuum
Tetrachloromethane	- - - -	+ + + +
Ethyl acetate	- - - -	+ + - -
Light petroleum	- - - -	+ - - -
Methyl alcohol	+ + (+)	+ + -

The plus sign indicates that convulsions occurred, the minus sign that they failed to appear. With tetrachloromethane, ethyl acetate and light petroleum no effect on the animals could be observed. This means that, inasmuch as the quantity of insulin used was many times greater than that which produces convulsions, the insulin is insoluble or only very slightly soluble in the solvents mentioned. The methyl alcohol on the contrary dissolves the insulin, although the extraction is incomplete.

Extraction at alkaline reaction.

Solvent	Extract	Residuum
Tetrachloromethane	- - -	+ + +
Ethyl acetate	- - -	
Ethyl ether	- - -	
Ethyl alcohol	- - -	
Chloroform	- - -	
Acetone	- - -	
Light petroleum B.P. below 50°	- - -	
B.P. 60-80°	- - -	
Methyl alcohol	+ + (+)	+ + +

Here, too, we obtain the same results. Methyl alcohol alone is able to extract the insulin.

The solubility experiments with methyl alcohol were repeated on a larger scale. Neutral, anhydrous methyl alcohol dissolves the insulin only with difficulty. Acid methyl alcohol dissolves it much more easily. The pure alcohol was acidified by passing in dry HCl. 200 cc. of this liquid, containing 6.6 millimols HCl dissolved 500 mg. crude insulin only leaving a slight residue.

The insulin may be precipitated from the methyl alcoholic solution by reagents which mix with methyl alcohol, but in which the insulin itself is insoluble. This procedure was used in order to detect other solvents for insulin. A saturated solution of insulin in pure, neutral methyl alcohol was prepared. The solution was distributed in a series of test-tubes and several volumes of the different fluids were added to each of the tubes. A precipitation occurred with the following fluids: ethyl acetate, ethyl isobutyl, and amyl alcohol, chloroform, tetrachloromethane, acetone, light petroleum, ethyl ether, benzene, xylene and pyridine. Whether the precipitates contained insulin was tested by activity experiments on mice, positive results being obtained with those from ethyl acetate, ethyl, isobutyl, and amyl alcohol, ethyl ether, light petroleum, acetone and tetrachloromethane. These experiments thus show the insolubility of the insulin in these solvents.

By the addition of glacial acetic acid, phenol, formamide and glycerol to the methyl alcoholic solution no precipitations were obtained. The solubility in glycerol can be explained through the high water content of this solvent.

But anhydrous glacial acetic acid, phenol and especially formamide were found capable of dissolving considerable amounts of insulin. It could be precipitated from these solutions, for instance, by ethyl ether, and this precipitate was very active.

These facts concerning the solubility of the insulin may be of value for further research on the nature of this hormone. They do not say much about its chemical nature, but I will remark that methyl alcohol, phenol and above all formamide are solvents for albumoses. Other facts also favour the belief that insulin belongs to this class:

1. Crude insulin gives a decidedly purple colour by the biuret test, and an orange colour by the xanthoproteic test.
2. It gives a precipitate with nitric acid, which disappears on heating.
3. It is not precipitated on boiling.
4. It is precipitated from watery solution by half saturation with ammonium sulphate.
5. It is very easily destroyed by trypsin.
6. It is impossible to demonstrate any dialysis of insulin through parchment membranes. It cannot be ultrafiltered through 3 % collodion filters.
7. It is freely soluble in pure, salt-free water.

I cannot find any valid reason for the suggestion that insulin is a globulin.

SUMMARY.

1. Insulin is insoluble or very slightly soluble in tetrachloromethane, ethyl acetate, ethyl alcohol, isobutyl alcohol, amyl alcohol, chloroform, acetone, light petroleum, ethyl ether, benzene, xylene and pyridine. Easily soluble in methyl alcohol, glacial acetic acid, phenol and formamide.
2. Several facts argue in favour of its being an albumose.

REFERENCES.

- Collip (1922). *J. Biol. Chem.* 55; *Proc.* xl.
Doisy, Somogyi and Shaffer (1922). *J. Biol. Chem.* 55; *Proc.* xxxi.