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Attenuation of Insulin by Interfacial Adsorption.*

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In a large series of experiments carried out on a group of 8 rabbits (average weight about 3 kilos) it was found that the physiological behavior and physical properties of crystalline insulin† are markedly altered by interfacial adsorption, so that a prolonged action of the insulin is acquired. The change is apparently that of denaturation such as proteins undergo at interfaces. Experiments have been largely of an exploratory nature to determine the most desirable mode of procedure but the results thus far obtained definitely indicate the effect which such adsorption has on insulin.

Aqueous solutions of crystalline insulin (U 40) having a pH of 2.5, were emulsified with equal volumes of Merck's Blue Label chloroform by means of an electrically driven shaking device. While other water insoluble volatile organic liquids can be used and have been used in these and other similar experiments, chloroform was chosen because of the purity of the material available, the ease with which it forms stable emulsions with protein solutions, and the ease with which it can again be removed by subsequent evaporation under reduced pressure.

The procedure used in emulsification can naturally be varied as to the length of time of shaking and of subsequent standing before recovery of the insulin solution. The optimum conditions need still to be determined. In the experiments which here illustrate the effects of emulsification, shaking was continued for 18 hours and the insulin solution recovered immediately by completely evaporating the chloroform at 45° under reduced pressure.

Following evaporation of the chloroform the insulin solution was strongly opalescent although its pH was practically unchanged. Centrifugation usually leaves an opalescent supernatant liquid over the insoluble residue which is thrown down.

The comparative physiological effect, expressed in milligrams percent of blood sugar in rabbits, of untreated insulin and that recovered

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in both the supernatant liquid and the insoluble residue of insulin treated by interfacial adsorption, is shown in Table I, where the prolonged action of the treated insulin may be observed. The insulin in each case was injected subcutaneously.

TABLE I.

Hours	0	1	2	3	4	5	6	7	8	10	12	13	18	22	25	30
A	89	31	30	33	52	82	93	93								
B	82	63	56	59		65		74			78					
C	83	42	36	41	42		51		55			56	80	78	80	83
D	87	78	71	77	77	74	75	68		80	78					

A represents the standard dose of crystalline insulin required to bring the blood sugar of a rabbit to the convulsive level. B is a dose of supernatant fluid representing 3 times the original volume of untreated insulin normally used as in A. C represents the insoluble fraction of 30 units of crystalline insulin after denaturation. D represents 5 times a normal dose of insulin contained in a mixture of the supernatant liquid and insoluble residue of another sample of treated insulin.

Variations in the methods of procedure are being tried to note whether the apparent decrease in activity per unit of insulin can be prevented while retaining its prolonged activity after injection.

As will be shown elsewhere, this method has also been used to bring about the attenuation of bacterial toxin.

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A Colorimetric Assay for Male Sex Hormones in Urine.

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Zimmerman[1] described a color reaction for the R-CH_2-CO-R group of the sex hormones using meta dinitrobenzene as his reagent. Although the reaction is not specific the author after some preliminary trials has been able to adapt it so that it can be used as an index to the male sex hormone content of urine and as a guide for capon assays of urinary extracts where more complete characterization of the extract is desired.

Capon assays were performed using the alcoholic inunction technique described by Fussgänger[2] and elaborated by Dessau.[3] The

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1 Zimmerman, W., *Z. f. Physiol. Chem.*, 1935, **233**, 257.

2 Fussgänger, R., *Medicine in its Chemical Aspects*, 1934, **2**, 185.

3 Dessau, F., *Acta Brev. Neer.*, 1935, **5**, 139.