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The reactivation of inactivated insulin in vitro and in vivo.

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Studies previously reported[1, 2] have established the fact that trypsin can inactivate insulin both *in vitro* and *in vivo*. It has also been ascertained that dissociation of insulin[3] from its inactivate combination with trypsin is possible *in vitro* by proper adjustment of the hydrogen ion concentration of the substance in solution, *i. e.*, by shifting the pH to the acid side of 4.6. It became desirable to ascertain whether or not dissociation of insulin from trypsin could be effected *in vivo*. It is obvious that the hydrogen ion concentration of the body fluids could not be altered to the point necessary for the dissociation of insulin. Other means were therefore tried, and the following results obtained:

1. Upon the addition, *in vitro,* of such substances as pepsin, safranin and cryogenin (M-Benzaminosemicarbazide) to the solutions of inactivated insulin (trypsulin), liberation of the insulin takes place. This is evidenced by the result of injection of these mixtures into suitable test animals.

2. These agents (pepsin, safranin and cryogenin) can cause dissociation of insulin from trypsin directly *in vivo*. Injection of adequate amounts of these substances into animals (subcutaneously or intravenously) made just prior to the parenteral administration of inactivated insulin, cause liberation of the insulin and the production of its physiological effects.

The accompanying tables illustrate the effect on the blood sugar of one of the reactivating substances used:

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[1] Epstein, Albert A., Rosenthal, Nathan, and others, *Am. J. Physiol.*, 1924, lxx, 225.

[2] Epstein, Albert A., Rosenthal, Nathan, and others, *Am. J. Physiol.*, 1925, lxxi, 316.

[3] Epstein, Albert A., Rosenthal, Nathan, and others, *Am. J. Physiol.*, 1924, lxx, 225.

TABLE I.

Showing the effect on the blood sugar of inactive insulin and of reactivated Insulin.

Animal No. 67. Rabbit, weight 1.5 kgm.

Blood sugar examination	Inactivated Insulin* Insulin + Trypsin	Reactivated Insulin Insulin + Trypsin preceded by Cryogenin
	Per cent	Per cent
Before injection	0.125	0.129
15 min. later	0.131	0.125
30 min. later	0.120	0.070
45 min. later	0.130	0.080
60 min. later	0.134	0.063
75 min. later	0.135	0.070 (Convulsions)
Animal No. 68. Rabbit, weight 1.4 kgm.		
Before injection	0.126	0.130
15 min. later	0.115	0.120
30 min. later	0.135	0.105
45 min. later	0.140	0.090
60 min. later	.....	0.078
75 min. later	0.143	0.095

TABLE II.

Showing the effect on the blood sugar of insulin, insulin and the reactivating substance, and of the reactivating substance alone.

Animal No. 65. Rabbit, weight 1.5 kgm.

Blood sugar examination	Insulin†	Insulin and the Reactivating Subst. (Cryogenin)	Reactivating Subst. (Cryogenin)
	Per cent	Per cent	Per cent
Before injection	0.135	0.122	0.140
15 min. later	0.133	0.140	0.155
30 min. later	0.115	0.090	0.145
45 min. later	0.107	0.110	0.145
60 min. later	0.106	0.100	.....
75 min. later	0.112	0.120	.....
90 min. later	0.080	.....	
105 min. later	.....	.....	
120 min. later‡	.....	0.125	

*All the injections were made intravenously.

†One unit of insulin per kgm. body weight was used.

‡Animals were observed for 4 hours more, and when no convulsions developed they were fed.