HYPOGLYCEMIC ACTION OF IMMOBILIZED INSULIN IN EXPERIMENTAL DIABETES

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Known long-acting insulin preparations (suspensions of amorphous and crystalline insulin) in large scale production act up to 28 h but they are not very effective during the first few hours after administration, and because of this additional injections of water-soluble insulin are required. There is thus a need for a long-acting preparation which will be effective with effect from an hour after administration.

The writers have studied the hypoglycemic action of insulin bound with erythrocytes.

EXPERIMENTAL METHOD

A preparation of bound insulin was obtained by covalent immobilization of crystalline insulin (activity 25 U/mg, from the Endocrine Preparations Factory, Moscow) on human erythrocytes by the method described in [2]. Activity of the resulting preparation was determined by bioassay on mice [1]. The hypoglycemic action of the preparation was studied on animals with normoglycemia (intact rabbits) and on rats with experimental diabetes. Diabetes was induced by means of alloxan, injected intraperitoneally in a dose of 65-80 mg/kg body

TABLE 1. Dynamics of Blood Sugar of Rabbits Receiving Insulin Preparations

Preparation	Blood sugar, mM									
	before injection	time after injection, h								
		1	2	4	6	24	25	28	32	40
Protamine-zinc-insulin	5,5±0,5	5,1±0,4	4,8±0,5	3,8±0,3	3,7±0,8	3,5±0,3	4,1±0,4	5,5±0,5		
P		>0,5	>0,5	<0,05	<0,05	<0,05	<0,05	>0,5		
Immobilized insulin	5,0±0,5	3,0±0,2	2,2±1,8	1,7±1,6	1,9±1,8	2,6±2,0	2,3±1,9	2,5±2,0	2,7±2,0	3,7±3,0
P		<0,01	<0,01	<0,01	<0,01	<0,05	<0,01	<0,05	<0,05	<0,05

Legend. P relative to sample before injection, n = 15.

TABLE 2. Dynamics of Blood Sugar of Rats (in mM) Receiving Insulin Preparations

Preparation	Before injection	Time after injection, days		
		1	2	3
Not receiving preparation	22,3±1,20	18,3±1,45 (22)	All animals died	—
Insulin-Lente	16,6±1,70	17,3±1,52	All animals died	—
P	<0,05	<0,05		
Immobilized insulin	19,5±1,80	7,4±0,64 (63)	7,9±0,78 (60)	7,9±0,81 (60)
P	<0,02	<0,05	<0,01	<0,01

Legend. P relative to control (without preparation), n = 20. Percentage decrease shown in parentheses (sugar level before injection of preparation taken as 100%).

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TABLE 3. Stability of Immobilized Insulin Preparation

Preparation	Activity, U/g dry weight	Insulin content, mg/g preparation	Duration of action	
			rabbits (normal)	rats (diabetes)
After preparation	420	21	40 h	3 days
After sterilization and keeping	420	21	40 h	3 days

weight daily for 5 days [4] or by a single intraperitoneal injection of streptozotocin,* in equivalent dose [3]. Animals in which the blood sugar was not below 16.6 mM were considered to be diabetic. The insulin preparation, in the form of aqueous suspension, was injected subcutaneously in a dose of 2.5 U/kg body weight into a rabbit or 1.0 U/100 g body weight per rat. The duration of the hypoglycemic effect was indicated by the time during which the blood sugar remained at least 27% (intact animals) or 50-60% (diabetic animals) below the initial level. Blood sugar was determined at intervals by the glucose oxidase method. For comparison of the action of the immobilized insulin with commercial preparations, Soviet protamine-zinc-insulin and Insulin-Lente (India) were used.

EXPERIMENTAL RESULTS

Starting with the first hour, for 40 h the preparation of immobilized insulin kept the blood sugar level of the rabbits low, much longer than protamine-zinc-insulin, the action of which lasted only for 6 h (Table 1).

Immobilized insulin caused a lasting decrease in the blood sugar of the diabetic rats in the course of 3 days whereas control animals died as early as on the 2nd day. Insulin-Lente had a weak effect after 1 day (Table 2).

The preparation of immobilized insulin tested thus gives an earlier and more prolonged hypoglycemic action in experimental animals (both intact and with induced diabetes), after a single subcutaneous injection, than other known insulin preparations.

Testing the preparation of immobilized insulin for stability (preservation of its biological activity and duration of action) after lyophilization and its preservation in sealed flasks at 4°C for 60 days showed (Table 3) that it remains effective. The stability of the preparation was unchanged also after sterilization by γ-rays in a dose of 2.5 Mrad (Table 3). The preparation studied thus has a lasting hypoglycemic action and can be used in the treatment of several diseases characterized by a hyperglycemic state (diabetes, surgical sepsis, extensive infected wounds, burn toxemia).

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*Alternative name streptozocin (translator).