9276

Studies on Alum-Precipitated Insulin.

L. ROSENTHAL AND J. KAMLET.

From the Department of the Laboratories of Israel Zion Hospital, Brooklyn, N. Y.

The precipitation of active biological principles, such as diphtheria toxoid, by alums suggested to us the possibility of preparing an alum-precipitated insulin. After trial of several preliminary procedures, we adopted the following technic:

To 100 cc. of ordinary insulin U40 is added 10 cc. of a 50% (by weight) solution of $NaAl(SO_4)_2.12\ H_2O$ in sterile distilled water. A heavy turbidity forms instantly and, upon mixing, a copious precipitation occurs. After standing for 24 hours in the refrigerator at 5°-10°C., the mixture is centrifuged at 3500 r.p.m. for 30 minutes. The clear supernatant fluid is now decanted from the sediment. Bioassay of this fluid, performed in the usual manner on rabbits, shows that it contains only 5-10% of the original insulin unitage.

To this supernatant fluid is now added a further portion of 10 cc. of 50% soda-alum solution. After remaining for one week in the refrigerator at 5°C., a second crop of precipitate is obtained. This crop is separated by centrifuging from the supernatant fluid which now proves insulin-free by bioassay.

The precipitate obtained by the action of alum on insulin solutions, hereinafter referred to as alum precipitated insulin, is a white amorphous powder, insoluble in alcohol, ether and petroleum ether and slightly soluble in neutral (pH 7) distilled water. It is readily soluble in undiluted serum, but only slightly soluble in serum diluted 1:5 with saline. In solutions of acidity greater than pH 2.5, it dissolves completely. In solutions of pH 2.5 to 7, it forms insoluble suspensions, the minimum solubility being obtained in solutions of pH 6.

On dissolving separately the 2 precipitates in solutions of pH 1 to pH 2 and assaying the resultant clear solutions on rabbits, it is found that the first precipitate contained 90-95% of the total original insulin unitage. The second precipitate contained the remaining 5-10%. Thus, we find that a quantitative recovery of the active principle from insulin solution may be effected by fractional alum precipitation.

Chemical analysis of the precipitate showed a concentration of 0.10 to 0.12 γ of aluminum and 4.0 to 7.0 γ of non-protein nitrogen per unit of insulin.

For experimental and clinical trial, the precipitate of 100 cc. of insulin U-40 was suspended in 50 cc. of sterile distilled water containing 0.1 cc. N/10 HCl per liter, to which has been added 0.2% of phenol. This suspension now assays 80 units of alum-precipitated insulin per cc.

Hypoglycemic activity of the suspension was studied in the following manner: A series of 44 rabbits weighing between 1.72 and 2.28 kg., which had been starved for 24 hours, were injected subcutaneously with a suspension of 3 units of the alum-precipitated insulin. In view of the relative uniformity of our results, Table I is limited to the blood sugar levels observed in series of 6 rabbits.

TABLE I.

Animal No.	8	11	14	18	24	30	38
				Blood Glucose.			
Fasting	168	148	111	122	129	234	184
hr.							
1	158	149	108	118	126	240	172
2	152	154	107	119	124	220	170
3	148	139	101	111	122	194	166
4	140	138	101	108	121	186	154
5	128	137	88	102	108	172	120
6	129	135	86	99	104	170	111
7	111	132	74	94	101	162	98
8	108	120	70	80	94	140	88
9	101	111	68	62	84	131	74
10	98	101	62	54	62	128	70
11	98	108	50	55	59	124	64
12	110	120	52	53	60	126	64
13	114	124	56	68	62	128	68
15	119	128	59	84	64	134	79
17	128	123	72	90	69	168	101
19	162	129	88	92	88	170	138
21	158	140	98	97	111	172	164
23	164	144	108	101	120	168	158
25	165	158	112	124	134	184	160
26	163	156	113	125	130	170	166

These results show that 3 units of alum-precipitated insulin cause a blood glucose depression in rabbits of 62 to 110 mg. % in 7 to 11½ hours, with recovery to the preprandial glycemia in 15 to 24 hours. Hypoglycemic tetany (insulin shock) was evident in no case.

Three days after recovery from the alum-precipitated insulin, each of the animals in the above series received injections of 3 units of ordinary insulin. The maximum depression of blood sugar was 74 to 122 mg. % in 1 to 2 hours, with insulin shock in all 6 cases.

It is not within the scope of this paper to discuss the therapeutic action of alum-precipitated insulin, which is still under clinical in-

vestigation. However, a few typical data of its hypoglycemic effect on human diabetics are here presented.

Five patients with diabetes who had been attending the hospital dispensary for a number of years and whose diet and insulin requirements had been established previously, received in one morning injection, a unitage of alum-precipitated insulin equivalent to the total number of units of ordinary insulin previously administered in 3 or 4 daily injections. The patients were kept on their usual diet in the course of these observations. Table II illustrates their blood glucose fluctuations during the day.

TABLE II.

Patient Injected	♀ Aet 45 150 U	♂ Aet 29 100 U	♂ Aet 52 160 U	♀ Aet 52 160 U	♀ Aet 29 100 U
			Blood Glucose Concentration.		
Time hr.					
1	264	212	328	312	234
2	278	244	310	308	212
3	256	210	288	290	208
4	221	186	248	288	196
5	186	178	231	246	208
6	174	172	234	240	198
7	148	130	230	227	182
8	138	122	212	181	140
9	122	104	186	148	121
10	118	105	142	130	111
24	246	196	322	302	212
25	258	210	329	308	215
26	270	208	312	318	220

This table indicates that alum-precipitated insulin injected into human diabetics causes a maximum blood glucose depression in 7½ to 12½ hours, with recovery to the preprandial level in 15 to 30 hours.

We may assume, therefore, that alum-precipitated insulin has, in comparison with ordinary insulin, a delayed and prolonged hypoglycemic action on experimental animals, as well as human diabetics.