Adsorption of Insulin to Glass.*† (25148)

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Several investigators have shown that I^{131} insulin is adsorbed to glassware(1,2). The reversibility of this phenomenon has been demonstrated(3). If non-iodinated insulin behaves in a similar manner, those working with small amounts of insulin, such as are used in micro-insulin assays designed to measure circulating insulin, must take precautions to avoid errors owing to losses and contamination because of adsorption and desorption. This report describes experiments demonstrating the adsorption of non-iodinated insulin to glass and its desorption from glass.

Materials. Crystalline zinc insulin was used. Acidified saline was made by adding 1 ml concentrated HCl to 2 l 0.9% NaCl. All glassware used was washed and then rinsed with 5 N or stronger NaOH to destroy any insulin adsorbed to it. It was rinsed in distilled water to remove the alkali. Powdered Pyrex glass which had been through a 200 mesh screen was placed on a medium porosity sintered glass funnel. The funnel containing the powdered glass and a stirring rod was allowed to stand overnight in a solution of 5 N NaOH or concentrated HNO_3. They were then washed with distilled water and dried in oven at 120°C. *Determinations of hypoglycemic activity* were carried out in 2 groups of hypophysectomized mice. The North Carolina mice have been inbred since 1941 at the State Laboratory of Hygiene, Raleigh, N.C. The other group used were mice of the A stock. Hypophysectomy and postoperative treatment were performed according to the method of Lostroh and Jordan(4). The morning of the experiment the hypophysectomized mice were placed in individual cages without food or water. About ½ hr later the first blood sample was taken and appropriate gelatin solution was injected intraperitoneally in volume of 0.25 ml. The mouse was returned to its individual cage. At 60 min. after injection the second blood sample was taken. Blood was collected from the cut tip of tail in tared capillary tubes. The weighed sample of blood (5 to 10 mg) was rinsed out of the tube into 0.5 ml of water and proteins were precipitated according to the method of Somogyi(5). After centrifugation a measured volume of the supernatant was used for blood sugar determination, which was done either by the method of Park & Johnson (6) or a modification of the glucose oxidase method(7). In the hypoglycemic convulsion experiments mice which had been starved for 20 hr were placed in groups so that each group had a similar distribution of mice of varying weights. Solutions were injected intraperitoneally and the mice kept at 37°C.

Results. Table I summarizes 4 experiments in which 1 ml acidified saline containing crystalline insulin was diluted to 1000 ml with acidified saline or acidified saline containing 5% U.S.P. gelatin in 1000 ml glass stoppered graduated cylinders. The solutions were mixed by inverting the cylinders several times. The cylinders were allowed to stand as noted in Table I. The contents were poured out and the vessel allowed to drain for short time, after which it was weighed. The increase over the dry weight of vessel was used as a measure of solution which did not drain. The cylinders used in experiments B, C, and D were then filled with 1000 ml of distilled water and tipped several times. The water wash was discarded and the vessel again weighed. The amount of insulin in the solution which did not drain was calculated. Fifty ml of 5% U.S.P. gelatin in acidified saline was then placed in each of the 4 cylinders, which were stoppered and placed horizontally

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TABLE I. Hypoglycemic Activity Recovered from Walls of Graduated Cylinders.

Exp.	A	B	C	D
Units crystalline zinc insulin placed in cylinder	2	25	25	25
Diluent	acidified saline	acidified saline	acidified saline	5% gelatin
Time and temp. of storage	30 min. room temp.	35 min. room temp.	6 days 5°C	6 days 5°C
No. times emptied*	1	2	2	2
No. hypophysectomized mice used in exp.	15	18	18	18
Mice	N.C.	A	A	A
% fall in blood sugar				
Gelatin from cylinder	18.2	14.8	11.4	−1.6
Control gelatin	−3.3	−2.1	0.4	0.4
P†	.02	.04	.001	.45
Estimate of max amt insulin which could have been inj. into mouse owing to non-drainage (microunits)‡	20	.5	.1	1.8

* In exp. B, C, and D, 1000 ml distilled water were added to cylinder after first emptying. The cylinder was tipped 40 times and emptied a second time.

† Exp. C and D were done as a 3 × 3 Latin Square. These probabilities are the result of carrying out 2 independent comparisons available within two degrees of freedom for differences between 3 treatments.

‡ These estimates are based upon weight of dry cylinder and weight of cylinder after drainage of various solutions. Our hypophysectomized mice do not give significant hypoglycemic responses until doses of the order of 250 microunits/mouse are given.

on a metal lathe and turned slowly for one-half hour. The gelatin solutions from the cylinders were compared with samples of the same gelatin solutions which had not been in the cylinders with respect to their hypoglycemic effects in hypophysectomized mice. In every case where the initial dilution of the insulin was made with acidified saline not containing gelatin (experiments A, B, and C) significant hypoglycemic activity appeared in the final gelatin wash. When the amount of insulin which could have appeared in this wash owing to lack of drainage of solution from the cylinders during the experiment was calculated, it was always considerably less than the amount which causes hypoglycemia in hypophysectomized mice. When the initial dilution of insulin was made in the presence of 5% gelatin (D), significant amounts of hypoglycemic activity did not appear in the final gelatin wash. When the per cent fall in blood sugar elicited by the gelatin from the cylinder in Exp. C of Table I (11.4) was compared with that of Exp. D (−1.6), the probability of the difference being due to chance variation was less than 0.001. This indicates 5% gelatin inhibits adsorption to glass of the hypoglycemic material.

Table II summarizes data from 2 experiments in which insulin in acidified saline was stirred with 5 g of powdered glass in a sintered glass funnel. The solutions were allowed to remain in contact with the glass from 1 to 2 hours with occasional stirring, then sucked through the filter into graduated cylinders. An acidified saline wash was placed on the glass and after thorough stirring sucked into a graduated cylinder. Ten ml of 5% U.S.P. gelatin in acidified saline was then stirred with the glass and allowed to remain in contact with it for 24-48 hours in a refrigerator. After the glass-gelatin mixture was liquefied by warming, it was centrifuged since it was too viscous for filtration. The supernatant gelatin was injected into mice as indicated in Table II. On the basis of volumes of solution recovered a calculation was made of maximal amount of insulin which could have remained on the glass at each step of experiment if no adsorption had occurred. In both experiments the gelatin solution which had been in contact with powdered glass caused hypoglycemic convulsions while insulin added to gelatin in amounts greater than would have been expected owing to lack of drainage of solutions from the powdered glass

TABLE II. Hypoglycemic Convulsions Produced with Insulin Eluted from Powdered Glass.

10 ml insulin sol. in contact with glass			10 ml acidified saline wash		10 ml 5% U.S.P. gelatin			Control insulin in 5% gelatin		
						Gelatin from glass				
Conc., μg/ml	ml recovered	μg left on glass	ml recovered	μg left on glass	Max not draining, μg/ml	ml	Mice in convulsions	μg/ml	ml	Mice in convulsions
30	9.4	18	10.2	1	0.1	1.0	3/3	4	1.0	0/3
						.3	1/3			
35	9.0	35	10.2	2.5	0.25	.25	15/17	4.5	.25	10/10
						.10	2/10	4.5	.10	3/10
								.5	.25	0/17

did not cause convulsions. Evidence that the convulsions were hypoglycemic was obtained by administering 10% glucose intraperitoneally to mice in convulsions and terminating the convulsions. Three mice bled during convulsions had blood sugar values below 45 mg %. To obtain some idea of quantity of insulin eluted from the glass by the gelatin, a solution of 4.5 μg/ml insulin diluted with 5% U.S.P. gelatin in acidified saline was administered in the same dose ratio as the gelatin from glass in the second experiment of Table II. The results are similar to those obtained when gelatin from the glass was used. This suggests that the gelatin from the glass contained several μg/ml. The difference in insulin sensitivity of the mice used in the 2 experiments of Table II is probably owing to size and strain difference.

Conclusions. Our results indicate that material having hypoglycemic activity is adsorbed to glass from a solution of insulin and may be eluted from it with a solution of 5% U.S.P. gelatin. Since adsorption of proteins to glass is a well known phenomenon and since the hypoglycemic activity of insulin has never been dissociated from the intact molecule, it is safe to assume that insulin is adsorbed to glass and may be eluted from it. Under proper conditions a 5% solution of gelatin will inhibit adsorption of insulin to glass.

Summary. By elution with gelatin solutions a material having hypoglycemic activity in mice can be recovered from laboratory glassware which has previously been exposed to insulin. Gelatin solutions can prevent adsorption of insulin to glass.

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Choriocarcinoma of Women Maintained in Serial Passage in Hamster and Rat. (25149)

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The heterologous transplantation of malignant tumors of man was initially reported by Greene(1). Toolan(2) and Sommers *et al.* (3) described methods for increasing the frequency of success of such heterologous tumor grafts through prior suppression of the immune response of the animal host by x-irradiation and by cortisone administration. Pierce *et al.* described the biological behavior of human testicular tumors carried in the hamster