Effect of Glucagon on Insulin Hypoglycemia. (20934)

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The hyperglycemic-glycogenolytic factor of the pancreas has been under investigation since 1924 when Kimball and Murlin(1) first proposed the name glucagon for this substance. Parenteral administration of glucagon causes liver glycolysis and hyperglycemia in man and animals(2,3). Since many insulin preparations contain small amounts of hyperglycemic activity, it is important to know what, if any, effect this factor has on the hypoglycemic action of insulin. It has been shown that, under certain experimental conditions, glucagon can counteract the hypoglycemia of insulin. De Duve and his coworkers(4) demonstrated that, when hyperglycemic factor was added to a glucose solution being infused into rabbits which had previously received a large dose of insulin (30 units), the amount of glucose necessary per unit of time to maintain a normal blood sugar level was decreased. Similar observations were made by Olsen and Klein(5) and by Sutherland and Cori(2). In a review of glucagon, de Duve has remarked that this ability of glucagon to counteract insulin hypoglycemia might be expected to decrease the clinical effectiveness of subcutaneously injected insulin and to alter the biological assay of insulin(6). Both these possibilities are of importance to physicians, patients and investigators. In order to ascertain how much, if any, effect the presence of glucagon activity in insulin preparations has on the hypoglycemic reaction, rabbits and mice were treated with glucagon-free insulin and with the same insulin plus various amounts of glucagon.

Methods and materials. Rabbits weighing from 2.5 to 4.6 kg were maintained on rabbit pellets and carrots in air-conditioned quarters, and were starved for 18 hours before each test. A group of 12 rabbits was used for each series of tests. No animal was used more frequently than every other day and usually there was a week between tests. Each solution was tested twice in each rabbit, making a total of 24 injections of insulin and 24 injections of insulin plus 1 or 10% glucagon. The injections were all made subcutaneously in a volume of 0.2 to 0.5 ml. Blood samples were taken at 10 and one minutes before and at ¼, ½, 1, 1½, 2, 3, and 4 hours after the injection. The method of Hagedorn and Jensen(7) was used for blood sugar determinations. White, male mice of a standard strain, weighing from 17 to 21 g, were used for the mouse convulsion assay and larger mice (30-40 g) of the same strain were used for blood sugar studies. All mice were maintained in air-conditioned quarters on a diet of Fox Chow and were starved for 17 hours before the test. Insulin samples were assayed by the standard mouse convulsion method(8). In larger mice the blood sugar was determined on 0.05 ml samples of blood taken before and at 15, 30, and 60 minutes after the injection of insulin, glucagon, or insulin plus glucagon. Blood sugar was determined by the method of Haslewood and Strookman(9). The glucagon-free crystalline insulin used for all experiments had a potency of 24.4 units per mg (lot 208-108B-238). In order to be certain that this material was devoid of glucagon activity, a sample was incubated with cysteine, a procedure which destroys insulin but has no effect on glucagon. When the cysteine-incubated sample was administered intravenously to anesthetized cats in doses of 30 to 600 μg per kg there was no increase in blood sugar. Two lots of crystalline glucagon were employed; lot 208-158B-117A in the mouse experiments and lot 208-158B-30 in the rabbit tests. Both lots were prepared according to the method developed by Staub(10). The glucagon stock solution was prepared by dissolving the crystals in water with a few drops of dilute NaOH. The final pH of the clear solution should not exceed 10.5. Under these conditions it can be stored in the refrigerator for at least 2 weeks without loss of biological activity. Glucagon solutions were prepared for injection by diluting this stock solution

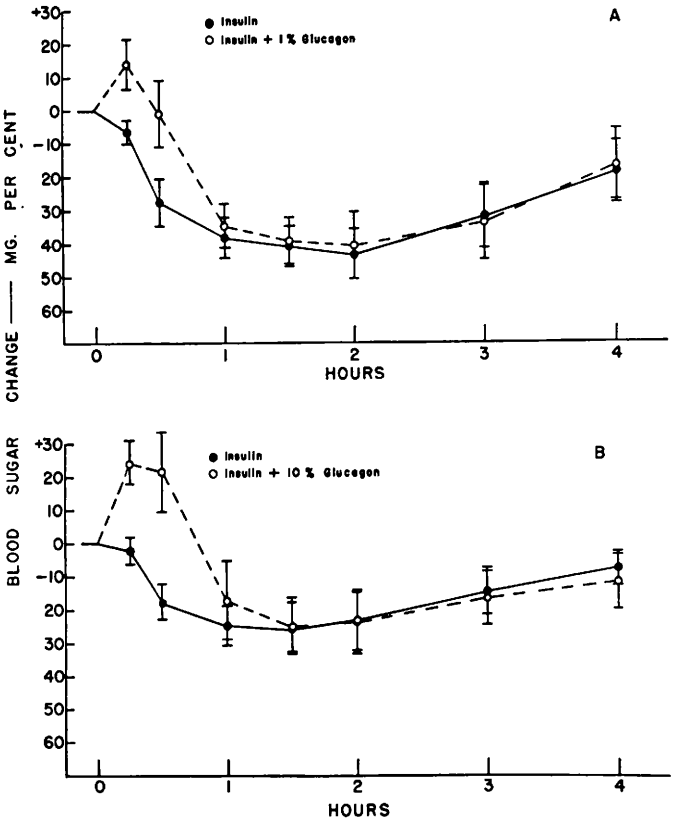

FIG. 1. Blood sugar response in rabbits treated with insulin and with insulin plus glucagon. Each point represents the mean of 24 tests and the bars indicate the 95% confidence limits. (A) At zero time 0.3 unit/kg of insulin S.C. (B) At zero time 0.1 unit/kg of insulin S.C.

with the diluent used to prepare Insulin Injection, U.S.P.

Results. 1. *Rabbit blood sugar.* A. *Glucagon-free insulin plus 1% glucagon.* The rabbits received 0.3 units of insulin per kg of body weight in each test. The mean of 24 tests with each solution is plotted in Fig. 1A; the vertical bars represent the 95% confidence limits for each point. There is a difference in the 2 curves only during the first hour after the injection. During the period from one hour to 4 hours after the injection the curves are identical. The presence of glucagon in the insulin solution produced a hyperglycemia that reached its peak at 15 minutes and had given way entirely to the insulin action by one hour. Since the point of maximum insulin hypoglycemia did not occur until about 2 hours after the injection, the glucagon action did not alter the degree of hypoglycemia produced by the insulin.

B. *Glucagon-free insulin plus 10% glucagon.* In the second series of animals the dose of insulin was reduced from 0.3 unit per kg to 0.1 unit per kg. This reduction in insulin dose assured that the hypoglycemia would not be maximal; thus, any glucagon effect should not be masked by an excess of insulin. When the ratio of glucagon to insulin was 1:100 the dose of glucagon administered was 0.14 μg per kg. In the second experiment the ratio was increased to 1:10 but the insulin dose was decreased by a factor of 3; thus, the amount of glucagon administered was increased only to 0.45 μg per kg. The results of this test are plotted in Fig. 1B. With the higher concentration of glucagon in the insulin solution, the hyperglycemia was greater and its duration was increased slightly. However, after this initial period the blood

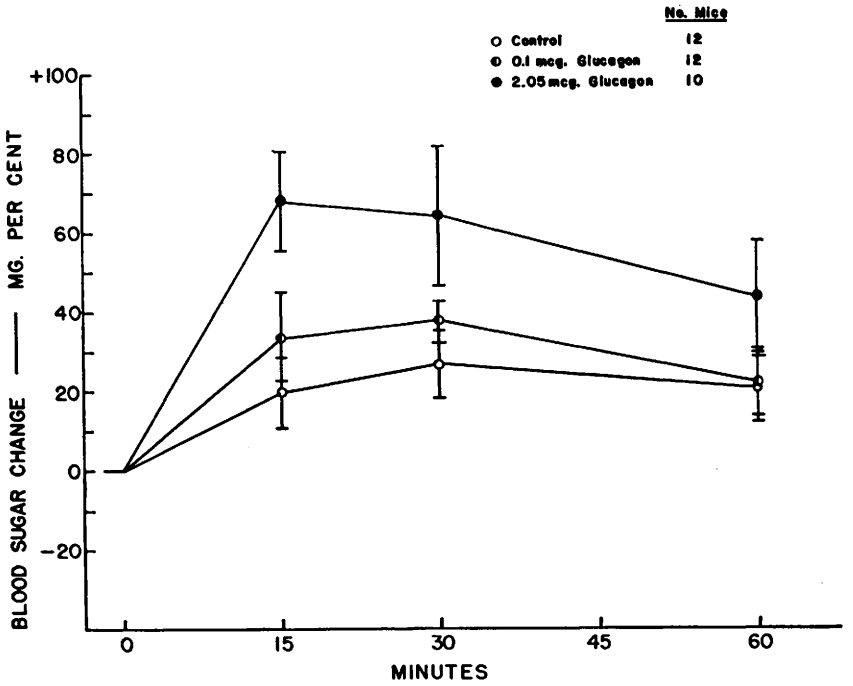

FIG. 2. Blood sugar response of mice injected subcutaneously with glucagon. Each point is a mean value and the bars indicate 95% confidence limits.

sugar curve was again the same as that observed following the administration of glucagon-free insulin.

2. *Mouse convulsion assay of insulin.* The mouse assay of insulin is based on the fact that low blood sugar levels will cause convulsions(8). In this assay the doses of standard and unknown solutions are adjusted so that convulsions are produced in approximately one-half of the group of mice within 1½ hours. From the figures so obtained the potency of the unknown may be calculated. An average dose of 4.1 μg of insulin was required to produce convulsions in 50% of a test group of mice. The minimal amount of glucagon necessary to produce hyperglycemia in mice has not been determined, but Fig. 2 shows the blood sugar response in control mice injected with the solvent used for all solutions, and in mice that received 0.1 μg and 2.05 μg of glucagon. The blood sugar increase in the control animals can probably be attributed to the increased temperature to which all mice were subjected immediately after the injection. The hyperglycemia produced by 0.1 μg of glucagon was not significantly greater than that of the control mice. It would therefore be necessary for any insulin solution to contain more than 2.5% glucagon in order for the glucagon to have any effect on mouse blood sugar and consequently on the mouse convulsion test. The blood sugar response to 2.05 μg of glucagon was significantly greater than the control values. This represents a glucagon concentration of 50% of the concentration of insulin used for the mouse convulsion assay.

The results of assays of solutions of insulin

TABLE I. Production of Convulsions in Mice Treated with Insulin and Glucagon.

Treatment	No. mice	No. with convulsions	% convulsions
I†	200	97	48.5
I + 1.5%* G†	190	102	53.7
I	80	43	53.8
I + 3.5%* G	60	36	60.0
I	728	403	55.4
I + 100%* G	728	401	55.1

* Calculated on the basis of wt.
† I = Insulin; G = Glucagon.

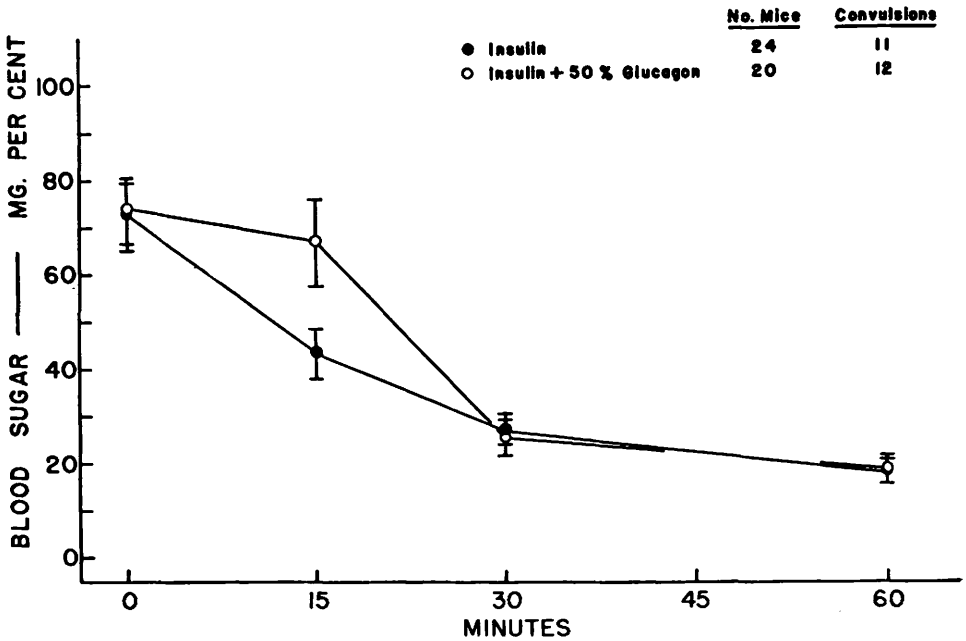

FIG. 3. Blood sugar response of mice to insulin and insulin plus glucagon. Each point is a mean value and the bars represent 95% confidence limits.

to which 1.5, 3.5, and 100% by weight of glucagon were added are presented in Table I. Little, if any, effect would have been expected from the low concentrations of glucagon since, even in the absence of insulin, they caused little or no hyperglycemia. However, even when the ratio of glucagon to insulin was 1:1 there was no change in the production of convulsions.

3. *Mouse blood sugar.* The lack of influence of glucagon on insulin convulsions in mice prompted a study of the effect of glucagon and insulin on the blood sugar of mice. Fig. 3 presents the results of these experiments. The degree of hypoglycemia produced by insulin plus 50% glucagon by weight was the same as that produced by insulin alone. When glucagon was present in the solution, the blood sugar did not fall as rapidly as when insulin alone was injected, but the level reached at 30 minutes was the same. In mice, the convulsions produced by insulin occur between 30 and 90 minutes; therefore, the duration of the glucagon hyperglycemia is too brief to alter the mouse assay of insulin.

Discussion. It has been suggested that even the small amounts of hyperglycemic activity existing in commercial insulin preparations might reduce the effectiveness of the insulin by as much as 10%, and that this hyperglycemia could modify the results of the standard mouse convulsion test in such a way that this loss of effectiveness would not be apparent. The work that has been presented here shows clearly that the presence of comparatively large amounts of glucagon in insulin solutions does not alter the degree of hypoglycemia in starved rabbits or mice, nor does it have any effect on the standard mouse convulsion assay.

The animals used in these experiments were all starved for 17-18 hours and may be presumed to have had low stores of liver glycogen. Since the degree of hyperglycemia produced by glucagon is dependent on the supply of liver glycogen, it is possible that the initial blood sugar increase in fed animals after the administration of insulin containing glucagon would be enhanced. However, even in well-fed rabbits, the peak of the response to subcutaneously injected glucagon occurs within 30 minutes after the injection and then falls rapidly toward normal, hence it is not likely that the glucagon in insulin would alter

the degree of hypoglycemia even in the presence of a large liver glycogen store. Experiments to clarify this point are currently in progress.

Summary. Experiments in rabbits and mice have demonstrated that the presence of glucagon in insulin solutions in ratios of from 1:100 to 1:1 did not influence the degree of hypoglycemia produced by the insulin, and consequently did not alter the potency of the insulin preparation as determined by the mouse convulsion assay.

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Composition of Rat Seminal Vesicles and Effects of Testosterone Propionate on Lipid Distribution.* (20935)

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Moore, Hughes, and Gallagher(1) have pointed out the usefulness of rat seminal vesicles in investigations dealing with the physiological effects of male sex hormone. Furthermore, biochemical and histochemical studies have been carried out dealing with the action of this hormone on various aspects of the metabolic processes of the gland such as protein synthesis(2,3), respiratory metabolism(4), fructose production(4), and the activities of various enzymes(5). According to Roberts and Szego(6), there is little information available relative to the lipid metabolism of the male reproductive target organs. Mann(7) has described 3 types of cells in the epithelium of the seminal vesicles of the bull. The most numerous, type A, is columnar and borders on the lumen. The cells of B type are located basally and are characterized by the presence of lipid. The C type is the least numerous and occurs among the A cells. The B type described by Mann(7) is probably the lipid-containing cell mentioned by Limon(8). It is of interest that Limon(8) reported the absence of lipid in the seminal vesicle of a bull calf of 3 months of age. Akutsu(9) observed the presence of lipid material in the epithelium of rat seminal vesicles.

Data are presented dealing with changes in chemical composition, including dry matter, total N, and total lipid, of rat seminal vesicles during sexual development. In addition, observations are included on sudanophilia and phospholipid distribution in this organ in intact as well as castrates and hormone-treated castrates.

Materials and methods. Rats of the Holtzman-Rolfsmeyer strain were used and were fed Purina Laboratory chow supplemented

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