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Glucagon Antibodies and Their Use for Immunoassay for Glucagon.* (25338)

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Clarification of the physiologic status of glucagon may well depend upon development of a practical and specific method for its detection in body fluids. Bioassay technics of "glucagon-like" activity have recently been developed(1,2,3) but are fraught with many serious objections(4). It seems that an immunoassay for glucagon, similar to those being developed for insulin(5,6,7), would provide greater specificity. The present report deals with efforts to develop an immunoassay for glucagon.

Methods. Female rabbits were given monthly subcutaneous injections of crystalline "insulin-free" beef-pork glucagon (Lilly†) suspended in Freund's adjuvant. A control group received either Freund's adjuvant alone, Freund's adjuvant plus insulin, or no injections at all. At intervals of several weeks specimens of serum were obtained and tested for development of non-precipitating antibodies to glucagon by means of Berson-Yalow radiochromatographic and electrophoretic technics used by these authors to detect insulin antibodies(8). The Berson-Yalow procedure(8) was adapted without major change, except that glucagon-I^{131} was substituted for insulin-I^{131}. In general 0.01 μg of glucagon-I^{131} was added to 1 cc of rabbit serum and incubated with shaking for 2 hours at 37° C. Ten to 50 μl were applied to cathodal end of Whatman No. 3 mm filter paper strip and chromatographed in barbital buffer at pH 8.6 for one hour. In some instances this was followed by 16 hours of electrophoresis. After heat drying for 1 hour at 120°C, staining with naphthalene blue black, and destaining in methyl alcohol and 10% acetic acid for 10 minutes, the strips were divided and segments counted in well-type scintillation

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† Supplied through generosity of Dr. W. R. Kirtley, Eli Lilly Co. Indianapolis, Ind., and considered to be very pure(4) with at most no more than 1% insulin contamination.

counter or placed intact in an actigraph and counted with TGC-2 Geiger-Muller tube. In the latter case a count rate tracing was obtained with Texas Instruments Rectiriter Recorder. In addition the Skom-Talmadge technic for detection of non-precipitating insulin antibodies(9) by precipitation of radio-insulin-globulin complex with antiglobulin was adapted unchanged to study glucagon antibodies, except that glucagon-I^{131} and anti-rabbit globulin were used. Glucagon-I^{131} was iodinated by modification of Pressman-Eisen technic and yielded specific activities at time of use, from 13.5 to 14.1 $\mu c/\mu g$. By this method damage to the fragile glucagon molecule as determined by criteria of Berson, Yalow, and Volk(10) was restricted to 4-8% at time of use.

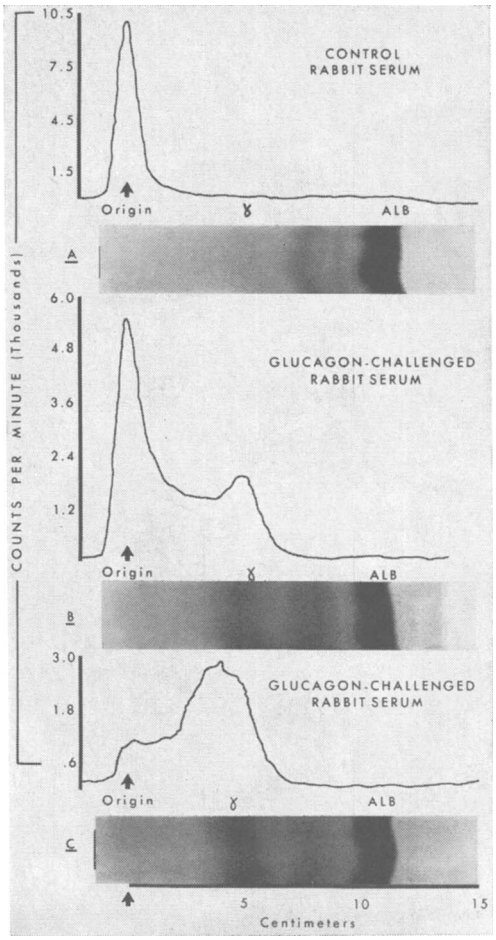

FIG. 1. A. Incubation of glucagon-I^{131} with sera from all 3 control groups of rabbits characteristically resulted in above paper radioelectrophoretic pattern in which radioactivity is concentrated at origin of strip. B and C. Incubation of glucagon-I^{131} with sera from glucagon-challenged rabbits gave distinctly contrasting radioelectrophoretic patterns, in which radioactivity migrates in varying degrees with the gamma globulin fraction. Note, too, the remarkable increase in gamma globulin which occurs in glucagon-immunized rabbits.

Results. Control rabbits: According to Berson, Yalow, and Volk(10) glucagon-I^{131}, like insulin-I^{131}, binds firmly to filter paper at point of application (origin). Fig. 1A demonstrates that when 0.1 μg of glucagon-I^{131} is incubated with serum and subsequently subjected to paper electrophoresis most radioactivity remains at the origin. The small amount of radioactivity which migrates with serum proteins represents, according to Berson and co-workers(10), damaged glucagon-I^{131}. After its incubation with serum of rabbits from each of 3 control groups, the migration of 0.01 μg of glucagon-I^{131} averaged 6.5% (4.8-8.8%), an average of 93.5% (91.2-95.2%) remaining bound to origin of strip.

Glucagon-challenged rabbits. Monthly injections of glucagon in Freund's adjuvant resulted in progressive change from above pattern (Figs. 1B, C). After incubation with serum from rabbits challenged with glucagon for 3 months or more, an average of 73.7% (45.9-87.3%) of radioactivity of 0.01 μg of glucagon-I^{131} migrated with the gamma globulin fraction of the serum. An average of only 26.3% (12.7-54.1%) remained at the origin. This was assumed to be the consequence of binding of glucagon-I^{131} to a non-precipitating antibody-like substance similar to the Berson-Yalow insulin antibody.

The Skom-Talmadge technic of precipitation of antigen-antibody complex with rabbit antiglobulin gave results which were virtually identical to the Berson-Yalow radiochromatographic technic.

Cross reactions between insulin-I^{131} and glucagon antibodies or between glucagon-I^{131} and insulin antibodies did not occur.

Kinetic studies patterned after those by which Berson and Yalow characterized the reaction between insulin and its antibody are in progress. Thus far, it appears that the glucagon-antibody complex is rapidly formed and

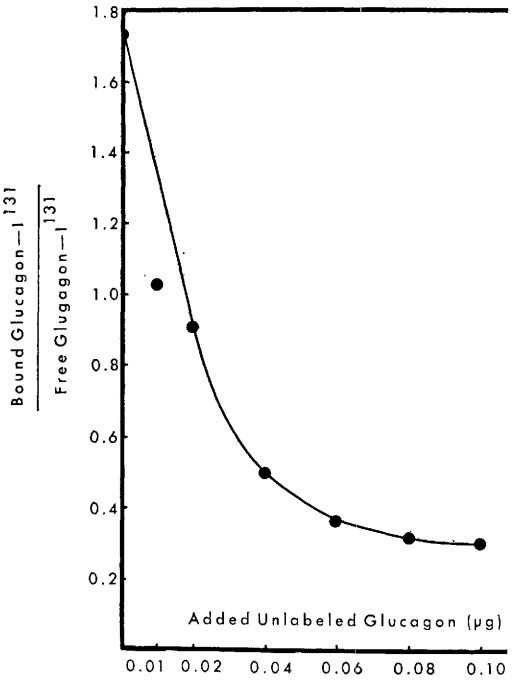

FIG. 2. Addition of increasing amounts of unlabeled glucagon to a mixture of dilute (1:40) antiglucagon serum and glucagon-I^{131} causes a progressive decrease in the ratio of bound to free glucagon-I^{131}. The above curve could serve as standard for an immunoassay for glucagon within a range of 0-0.06 μg/cc.

dissociates very rapidly at any time up to 2 hours after its formation.

Basis of immunoassay for glucagon. By preincubating unlabeled glucagon in dilute antiglucagon serum it has been possible to reduce the percentage of glucagon-I^{131} bound to globulin. In Fig. 2 the quantity of unlabeled glucagon added, is plotted against the ratio of bound to free glucagon-I^{131} displaced from antibody. It is clear that within a range of 0.01 to 0.06 μg increasing amounts of non-radioactive glucagon displace proportionately increasing amounts of radioactive glucagon from antibody. This would indicate that standard curves for various concentrations of beef-pork glucagon can be constructed and that an immunoassay, at least for beef-pork glucagon, is possible.

Discussion. Our data reveal for the first time that specific non-precipitating antibodies to beef-pork glucagon are readily produced in the rabbit. The antigenicity of glucagon was somewhat unexpected in view of its low molecular weight. Furthermore, there was reason to believe that the amino acid sequences of beef and pork glucagon might be identical (personal communication), suggesting that identity in other species might also exist.

The practical implication of this work lies in the demonstration that non-radioactive glucagon competes with radioactive glucagon for glucagon antibody in proportion to its concentration, thereby establishing the basis for a relatively simple immunoassay for glucagon.

Summary. Non-precipitating antibodies to glucagon have been produced in rabbits and detected by both Berson-Yalow and Skom-Talmadge technics. A basis for an immunoassay for glucagon appears, thereby, to have been established.

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