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PRELIMINARY COMMUNICATIONS

The Inactivation of the Biological Properties of Insulin by 131 I Labelling or Irradation

By

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Summary. The various biological properties of crystalline insulin labelled with ^{131}I and crystalline insulin submitted to irradiation were studied. The properties investigated were the hypoglycemic, convulsive and antigenic activities, and the effect on the glucose uptake of the diaphragm and the epididymal fat pad of the rat. It was observed that the ^{131}I-insulin rapidly lost its hypoglycemic activity and its insulin effect *in vitro*, whereas the antigenic and the convulsive activities were retained longer. The irradiated insulin behaved in the same manner. The influence of the radioactive labelling was also studied on the reaction between insulin and anti-insulin antibodies.

Résumé. Nous avons étudié le comportement des différentes activités biologiques de l'insuline, à savoir l'activité hypoglycémiante, convulsivante et antigénique, de même que l'action sur l'utilisation du glucose, en employant de l'insuline cristallisée marquée par l'iode radioactif ou bien de l'insuline soumise à l'irradiation de ^{131}I. On a observé que l'insuline marquée par ^{131}I perd rapidement, jusqu' à la suppression complète, l'activité hypoglycémiante et l'activité sur l'utilisation du glucose, tandis que les propriétés antigénique et convulsivante se montrent plus résistantes vis-à-vis de l'irradiation. L'insuline soumise à l'irradiation de ^{131}I se comporte de même. On a étudié en même temps l'interférence de la radioactivité sur l'équilibre de la réaction entre l'insuline et les anticorps anti-insuliniques.

Zusammenfassung. Es wurden verschiedene biologische Eigenschaften von kristallinem, mit ^{131}I markiertem Insulin und von kristallinem, einer Bestrahlung ausgesetztem Insulin untersucht. Dabei handelte es sich um die hypoglykämische, konvulsive und antigene Wirkung sowie die Insulinwirkung auf die Glukoseaufnahme des Rattenzwerchfells und des epididymalen Fettgewebes. Es wurde beobachtet, daß das ^{131}I-Insulin seine hypoglykämische Wirkung und seinen „in vitro"-Insulineffekt rascher verliert, während die antigene und die konvulsive Eigenschaft länger bestehen bleiben. Das einer Bestrahlung ausgesetzte Insulin verhielt sich gleichartig. Schließlich wurde auch der Einfluß der Radioaktivität auf die Reaktion zwischen Insulin und Insulinantikörpern untersucht.

The different biological properties of crystalline, pork insulin before and after labelling with ^{131}I were studied. The hypoglycemic activity was determined by its hypoglycemic effect in the rabbit[6], convulsive activity by the convulsion test according to DEBARBIERI[5], antigenic activity by the immunological method of ARQUILLA and RODARI[1] and glucose uptake *in vitro* by the rat diaphragm and the rat epididymal fat pad techniques. The specific activity of the hormone, kept at 4°C and at pH 3.5 was 2 mC/mg.

The labelled insulin showed over a period of 4 days a complete loss of its hypoglycemic activity, and a decrease in both its antigenic activity, and its capacity to stimulate glucose uptake *in vitro*, whereas the convulsive property was scarcely influenced (Fig. 1). Similar results were obtained when the crystalline insulin was submitted only to irradiation of intensity between 0.04 — 10 mC/mg or only to iodination of the hormone. It is obvious that irradiation and iodination affect the different biological properties of insulin to different extents.

The same quantities of crystalline, pork insulin and insulin extracted from human plasma with acid-alcohol[4] were irradiated at 4°C and pH 7.4. It was observed that although both preparations of insulin exhibited a similar reduction in their stimulating activity on the glucose uptake of the rat diaphragm and epididymal fat pad, the extracted insulin showed a faster and a more considerable decrease of its antigenic and hypoglycemic activity *in vivo*, as determined by the method of BASTENIE[3] (Fig. 2).

Rabbit antibodies against pork insulin were irradiated in either the free form or bound to crystalline

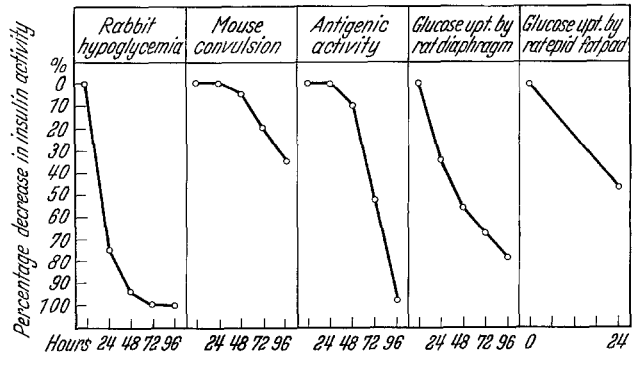

Fig. 1. Effect of labelling with ^{131}I on various properties of crystalline pork insulin. Crystalline pork ^{131}I-insulin: 1 mg/ml - spec. act. 2 mC/mg, pH 3.5, Temp. 4°C

pork insulin. Subsequently, the antibodies capable of binding with crystalline pork insulin were measured by the hemoagglutination technique[2], before, and 24, 48, 72 and 96 hours after the irradiation. The mixture during this time was kept at 4°C and at pH 7.4 (Fig.3). The free antibodies lost their affinity for insulin 72 hours after the irradiation. The antibodies bound to the crystalline insulin, or to the extracted insulin became free 24 hours after the irradiation and lost their immunological properties in the same way as the initially free antibodies. During preliminary work it was observed that both crystalline insulin and extracted insulin maintained their antigenic property 24 hours after the irradiation. It is therefore possible to put forward the hypothesis that the irradiation breaks the bonds between insulin and antibody.

All of these observations suggest the possibility that in the radioimmunological assay crystalline insulin and insulin extracted from plasma behave differently. The radioimmunoassays are based on the displacement by unlabelled insulin added in the mixture

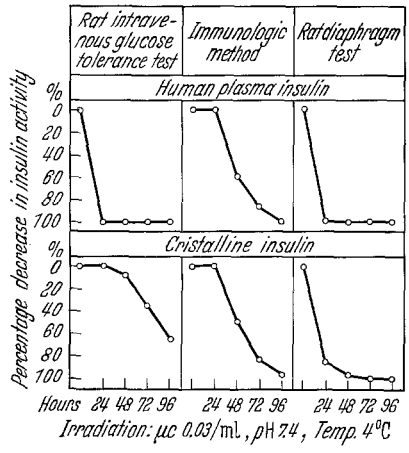

Fig. 2. Effect of irradiation on the activity of crystalline, pork insulin and insulin extracted from human plasma

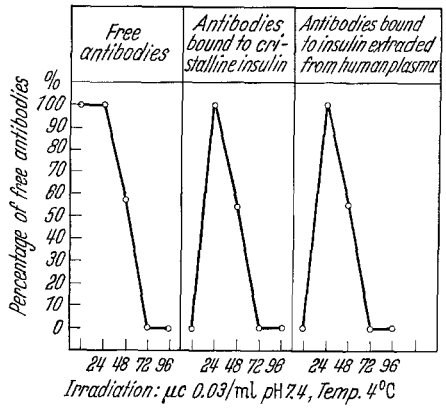

Fig. 3. Effect of irradiation on the immunologic activity of the free antibodies and the antibodies bound to crystalline, pork insulin or insulin extracted from human plasma

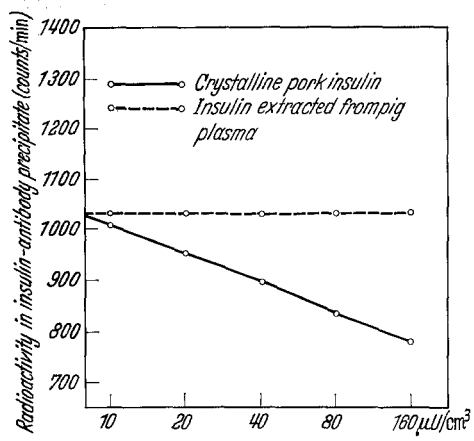

Fig. 4. Different influence of equal doses of crystalline, pork insulin and of insulin extracted from plasma on the radioactivity of insulin antibody precipitate (method of HALES and RANDLE)

of the ^{131}I-insulin bound to the antibody. The amount of labelled insulin displaced is related to the quantity of unlabelled insulin to be tested. It is obvious that, under the influence of the irradiation, the crystalline insulin used as the standard and the plasma insulin may show a different decrease in their biological properties. It is also possible that the ability of the unlabeled insulin to displace the ^{131}I-insulin bound to antibodies is not affected by the irradiation in the same way as the other biological properties of the hormone.

Therefore, equal amounts of crystalline insulin obtained from the pig pancreas and of insulin extracted from pig plasma were compared in the immunoassay of HALES and RANDLE[7]. The insulin concentration of the extract was previously measured by the hypoglycemic response of the hepatectomized rabbit kept under continuous intravenous glucose perfusion which indicated an insulin concentration of 6 mU/ml of pig plasma.

It was observed (Fig. 4) that a linear relationship existed between the logarithm of 5 doses (10, 20, 40, 80, 160 microunits) of crystalline insulin and the radioactivity of the precipitate, whereas the relationship failed for insulin extracted from pig plasma. In fact, the values of the radioactive precipitate for insulin extracted from pig plasma remained equal to the zero value for the crystalline insulin.

Such behaviour suggests that radiation also affects the capacity of unlabelled insulin to displace ^{131}I-insulin from the insulin-antibody complex and that the extracted plasma insulin completely loses this property.

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