A Comparison of the Behavior of Insulin and Insulin Labeled with I^{131} in Serum

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The use of insulin labeled with iodine (I^{131}) has facilitated study of the factors influencing insulin metabolism.[1, 2, 3] The rate of disappearance of intravenously administered insulin I^{131} has been studied by two methods. In the first of these, the radioactivity of the precipitate obtained by the addition of trichloroacetic acid to the plasma has been measured. This is the generally accepted method of study. In the second method, recently introduced by Berson and his associates,[3] electrophoresis of the serum has been employed to separate insulin I^{131} from the plasma for measurement. By this technic, they identified a major fraction as unaltered insulin I^{131} and, in addition, a minor fraction of insulin I^{131} which had a different physical behavior. Employing the two methods, these workers studied rates of disappearance of insulin I^{131} given by intravenous injection to rabbits. The rate of disappearance of the radioactivity of the TCA precipitate was much slower than the rate of disappearance of the major radioactive fraction of insulin I^{131} as measured by electrophoresis. The persistence in the circulation of the minor fraction of insulin I^{131} appeared to account for the difference in behavior of insulin I^{131} when studied by these two technics.

Since changes in the structure of insulin might affect its biologic activity, it appeared worthwhile to determine the rate of disappearance of administered unlabeled insulin by means of a bio-assay. The studies of the disappearance of insulin I^{131} from the blood as calculated from measurements of radioactivity have been repeated and compared with a study of the disappearance of unlabeled insulin as determined by biological assay.

METHODS

Insulin I^{131}. The radioactive insulin used was obtained from a commercial source.* Prior to iodination the insulin† was stated to have a biologic activity of 27 units per mg. The specific radioactivity at the time of use varied between 2.4 and 8.2 microcuries of I^{131} per microgram of insulin. Both dialysed insulin and non-dialysed insulin were used. Dialysis was carried out against running water for periods of twelve to forty hours; this increased the TCA precipitable radioactivity of the original insulin sample from 88 per cent to 97 per cent. The insulin molecule was stated to have one atom of iodine per mole assuming its molecular weight to be 6,000.

Trichloroacetic Acid Precipitation: Insulin I^{131} was injected intravenously into rabbits in doses from 0.08 to 0.15 units of insulin per kg. body weight. This gave an approximate radioactivity of 20 microcuries per kg. body weight. Blood samples were taken from the central artery of the ear at ten-minute intervals from ten to seventy minutes following injection. In some experiments, samples were taken for periods up to 250 minutes. The precipitable radioactivity was obtained by adding one ml. of 20 per cent trichloroacetic acid to 0.05 ml. of plasma. After centrifugation the precipitate was washed twice with 20 per cent trichloroacetic acid, recentrifuged and the supernatant poured off. The precipitate was dissolved in 30 per cent KOH with heat. This solution was poured into a metal planchet; in order to insure transference of all the radioactivity the centrifuge tube was washed several times with water containing a detergent and the washings were added to the planchet. The solution was evaporated to dryness, following which the radioactivity was measured by means of a shielded Geiger-Muller tube and scaling unit.

Paper electrophoresis: Paper electrophoresis with Whatman No. 3 MM filter paper was carried out in 0.05 molar barbital buffer (*p*H 8.6) at a constant voltage of 115 volts. Runs lasted for eight to ten hours.

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*Abbott Laboratories.

†Eli Lilly crystalline zinc insulin.

When the radioactivity at the point of application, or origin, was studied, however, no current was applied but the plasma proteins were separated from the insulin on the filter paper strips by hydrodynamic flow as previously described.[3] After electrophoresis the filter paper strips were heated for thirty minutes at 100° C. and stained with bromphenol blue followed by washing with 5 per cent acetic acid. The various protein fractions were identified and cut separately from the strips. The radioactivity of these and of the origin was then determined.

Biologic Assay: A biologic assay of insulin was carried out, using the rat hemidiaphragm technic modified from Stadie and Zapp.[4] The rats used in the assay procedure were of the Sprague-Dawley strain. The animals were fasted for a period of sixteen to twenty hours preceding the experiment. Only male rats were used, their weights varying from 150 to 200 gm. Each rat was killed by stunning and decapitation, and the diaphragm was quickly removed and placed in a salt solution buffered with bicarbonate and containing 250 mg. per cent glucose.[5] The thick crura and central tendon were excised and the diaphragm was bisected. The hemidiaphragms were trimmed to similar weights. After removal of excess fluid by laying on filter paper, the hemidiaphragms were weighed on a Roller-Smith balance. The average weight of the hemidiaphragms was 172 mg. with an average difference between pairs of 2.6 mg. Glucagon-free insulin* was freshly diluted in the glucose-salt solution immediately prior to use in concentrations from 2.5 to 80 milliunits per ml. One hemidiaphragm was placed in 0.4 ml. of glucose-salt solution containing insulin and shaken gently for two minutes at 37° C. The hemidiaphragm was then washed in glucose-salt solution and blotted lightly with filter paper. The other hemidiaphragm was similarly treated with the exception of the exposure to insulin. Both hemidiaphragms were placed in separate bottles containing 1 ml. of the glucose-salt solution. Since it is difficult to maintain the bicarbonate buffer at a physiological *p*H, the solution was gassed prior to use with 90 per cent oxygen and 10 per cent carbon dioxide. In addition, the bottles were gassed again for several minutes after receiving the hemidiaphragms and were then tightly stoppered. They were incubated at 37° C. for one hour with gentle rocking.

Analyses of the glucose concentration of the incubating fluid were determined in duplicate by a modified glucose-oxidase method[6, 7] before and after incubation. The glucose uptake of each hemidiaphragm was expressed in mg. of glucose per gm. of diaphragm per hour. The increase in glucose uptake of the hemidiaphragm exposed to insulin above that of the control was plotted against the concentration of the insulin.

Assay of the insulin content in the sera of the rabbits was carried out before and after the intravenous injection of crystalline zinc insulin. Doses ranging from three to fifty units per kg. body weight were used, and sera were collected at intervals up to 140 minutes following injection. The insulin content of the sera was determined by the rat hemidiaphragm technic as described above. One hemidiaphragm was placed in 0.4 ml. of serum in a small test tube and incubated at 37° C. for two minutes with gentle shaking. It was then washed in 5 ml. of glucose-salt solution for five minutes and lightly blotted on filter paper. The hemidiaphragm was then placed in glucose-salt solution as previously described. The control hemidiaphragm was similarly handled with the exception of the exposure to serum. The results were expressed as the increase of glucose uptake of the hemidiaphragm treated with serum above that of the control in mg. of glucose per gm. of diaphragm per hour. For each of the sera duplicate or occasionally triplicate diaphragm assays were made.

The blood sugars of the rabbits during the experiments were determined by the anthrone method.[8]

RESULTS

With electrophoresis or hydrodynamic flow, the main fraction of insulin I^{131} remains adsorbed to the filter paper at the point of application. The adsorption to the paper is unaffected by the presence of plasma proteins in normal concentrations. Following intravenous administration of insulin I^{131}, the disappearance of the radioactivity from the serum is rapid for the first twenty-five to thirty-five minutes, presumably due to distribution of insulin in the available body space. Figure 1 shows the electrophoretic pattern of the serum of a rabbit thirty-one minutes after intravenous injection of insulin I^{131}. The strip has been scanned for its protein fractions, and the amount of radioactivity with the various fractions has been measured. The major radioactive fraction remains adsorbed at the origin, and variable amounts of radioactivity migrate with the plasma proteins. Since the strip has been stained and washed, only protein-bound radioactivity remains, and a part equivalent to 28 per cent of the precipitable radioactivity migrates with the proteins. The major radioactive fraction adsorbed at the origin disappears uniformly between thirty and seventy minutes whereas the radioactive frac-

*Kindly supplied by Eli Lilly and Company, Indianapolis, Indiana.

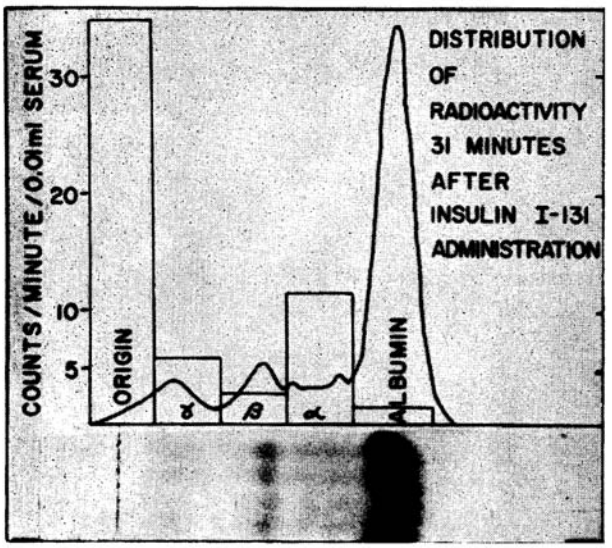


FIG. 1. Paper electrophoresis of serum after intravenous administration of insulin I^{131}. Vertical columns represent radioactivity along the strip. The point of application is designated "origin."

tion migrating with the proteins shows little change over this period of time.

Since the TCA precipitate includes the material at origin and the radioactivity migrating with the proteins, the persistence of the latter fraction in the serum leads to a slower rate of disappearance for the TCA precipitate than for the fraction at origin alone (figure 2).

The mean half life of insulin I^{131} calculated for the period of thirty to seventy minutes following injection is sixty-seven minutes by the technic of TCA precipitation and twenty-four minutes by electrophoresis. At later periods of time the TCA precipitable radioactivity disappears at progressively slower rates until it corresponds to that of the fraction migrating with the plasma proteins. Over short periods of time the migrating fraction shows no detectable degradation, and the difference in absolute counts per minute between the fraction remaining at origin and the TCA precipitate is constant. In most experiments this constant difference was present throughout the period of study (figure 3). In some experiments a constant difference could only be demonstrated in the later determinations, i.e., after forty minutes, suggesting that in these cases contact with the serum had some effect in producing the migrating component (figure 4).

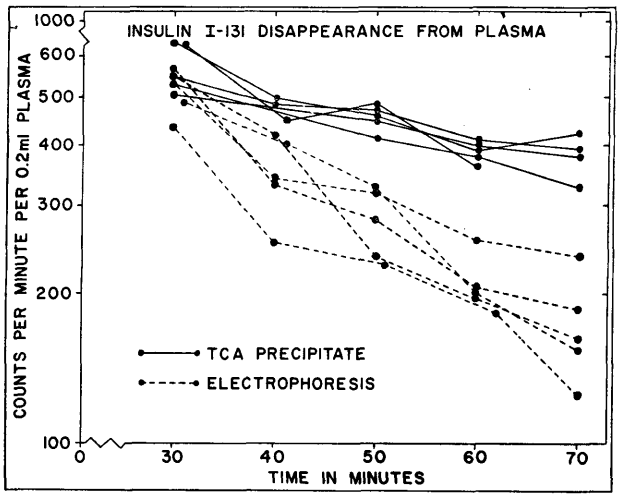
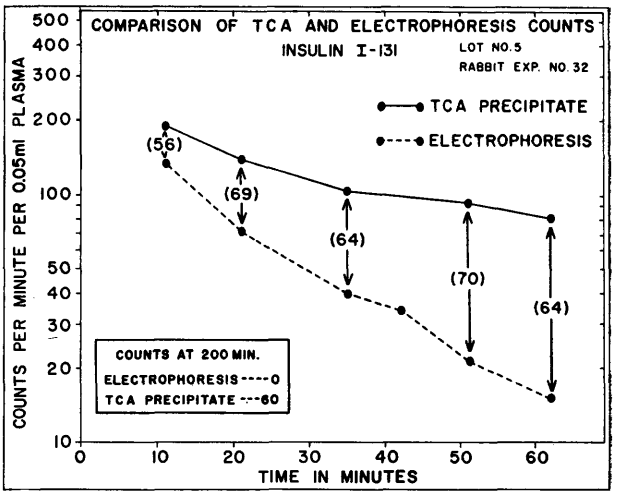
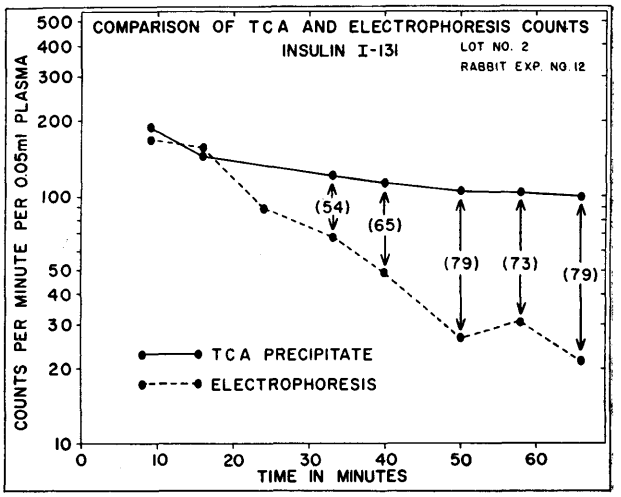


FIG. 2. Comparison of the rates of disappearance of insulin I^{131} from plasma in vivo as measured by trichloroacetic acid precipitation and paper electrophoresis.





FIGS. 3 & 4. Differences are shown by figures in parentheses, in absolute counts per minute, between the trichloroacetic acid precipitate and paper electrophoresis. These differences are equivalent to the radioactivity migrating with the protein fraction.

The disappearance rate of insulin I^{131} is not changed by the simultaneous administration of large doses of insulin; crystalline zinc insulin in doses of 100 units per kg. body weight had no effect on the rate of disappearance of radioactivity from the plasma (figure 5). Berson, Yalow and Volk[9] have demonstrated that the degradation mechanisms are saturated only with doses exceeding 200 units of crystalline zinc insulin per kg. body weight.

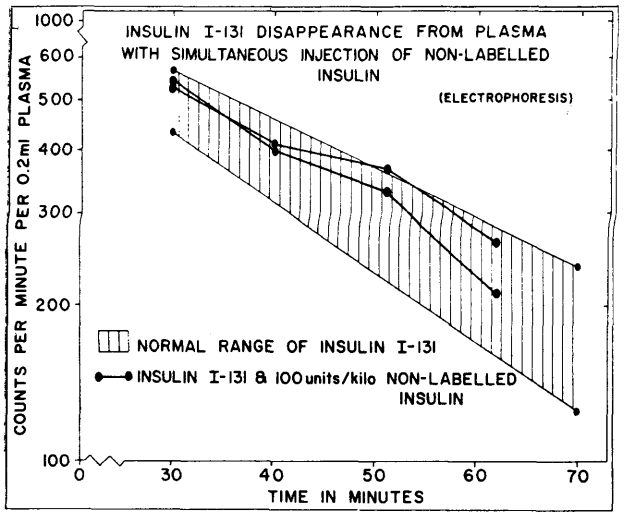


FIG. 5. Comparison of the rate of disappearance of insulin I^{131} alone and with simultaneous injection of large doses of unlabeled insulin.

A theoretical space of distribution for insulin I^{131} has been calculated by extrapolation to zero time of the exponential decay curve for electrophoresis. This is based on the assumption that the disappearance rate is constant from the time of injection. The average space of distribution was 25.8 per cent of body weight. This calculation of a theoretical space of distribution does not imply that the insulin is uniformly distributed throughout this space.

It appeared likely that biologically active insulin I^{131} in serum might be quantitated by its ability to bind itself to the rat hemidiaphragm. A modification of the technic described by Stadie, Haugaard and Vaughan[10] was used. Rat quarter-diaphragms were dipped for two minutes in 0.4 ml. of sera taken at thirty, forty, fifty and sixty minutes from three of the rabbits which had received insulin I^{131}. The diaphragms were then washed for five to six hours in large volumes of normal saline. The radioactivity was determined after digestion of the diaphragms with 30 per cent hot KOH. The radioactivity adsorbed on the muscle was relatively constant up to 250 minutes after injection, in contrast to the decrease in radioactivity of the electrophoretic fraction at origin and TCA precipitate (figure 6). Insulin used in this experiment was dialysed prior to injection. The finding that the radioactivity bound to the diaphragm exceeded that at origin at 250 minutes indicates that excess radioactivity came from either the total TCA precipitable or free radio-iodine liberated during degradation.

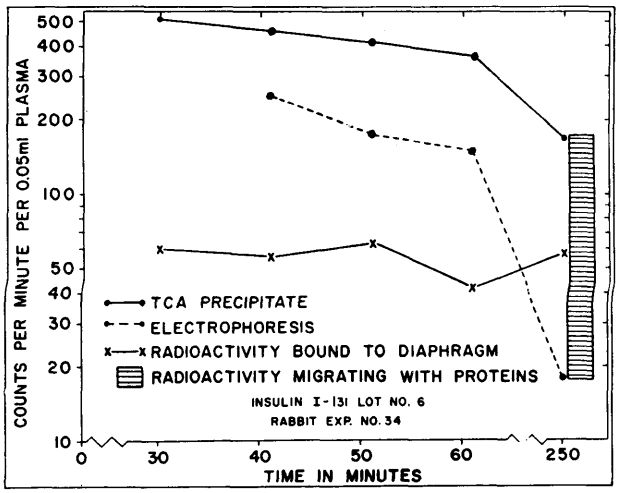


FIG. 6. Comparison of the concentration of radioactive insulin in plasma as measured by trichloroacetic acid precipitation, paper electrophoresis and binding to rat diaphragm.

Insulin I^{131} was mixed with 1/40 molar cysteine in vitro for twenty-four hours in order to inactivate the insulin.[11] It was found that this insulin was bound to the rat diaphragm to the same degree as untreated insulin I^{131}.

In order to assay for biologically active insulin in serum, a log dose response curve of the rat hemidiaphragm to various concentrations of insulin was constructed as outlined in the methods (figure 7). It should be noted that in this procedure, the diaphragms were incubated in the concentrations of insulin for two minutes at 37° C. prior to incubation in the buffered salt solution. There is a curvilinear relationship between the log concentration of insulin and the increase in uptake of glucose in mg. per gram of diaphragm per hour. The slope of the linear portion of the response between five and forty milliunits per ml. is 1.44 with a combined standard deviation about means of 0.54, giving an index of precision of 0.37. At concentrations lower than 2.5 milliunits per ml. it was found that the increase in glucose uptake approached the limits of accuracy for glucose estimation.

In preliminary experiments in which insulin was administered intravenously to rabbits in doses of three units per kg. body weight, it was found that in some

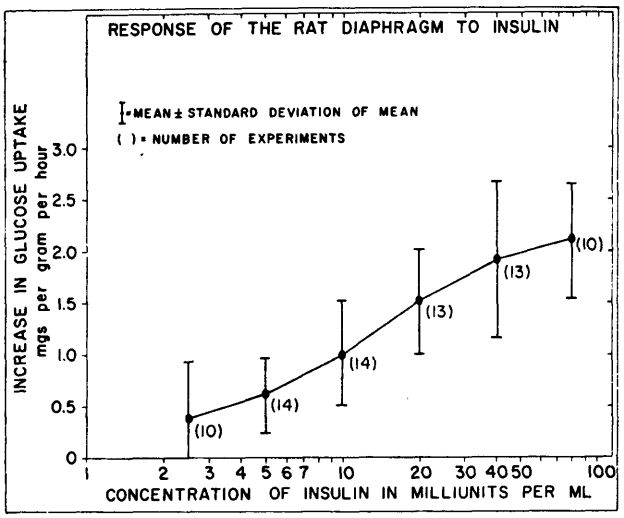


FIG. 7. Log-dose response curve of the rat hemidiaphragm to standard concentrations of insulin.

cases insulin-like activity of the serum at sixty minutes was below the sensitivity of the assay procedure. At fifty units per kg. body weight, insulin-like activity remained above the upper limits of the dose response curve between thirty and sixty minutes after injection.

Seven normal rabbits were given an intravenous injection of fifteen units of crystalline zinc insulin per kg. body weight. The effect of sera obtained from the rabbits on the glucose uptake of the rat hemidiaphragm is shown in figure 8. The rat hemidiaphragms were incubated in the sera obtained from the rabbits for two minutes at

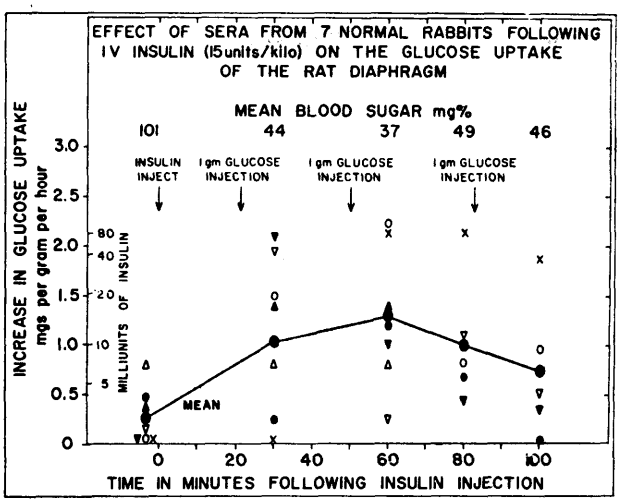


FIG. 8. The effect of the sera from each rabbit on the glucose uptake of the diaphragm is represented by a different symbol. The mean value for each time interval is shown by the closed circle.

37° C. as stated in the methods. The results are expressed as the absolute gain of glucose in mg. per gram hour of the treated hemidiaphragms over the controls. The comparable concentration of insulin producing the same effect in the bio-assay is shown on a scale alongside the glucose uptake. The results were expressed in this manner to avoid the inference that insulin alone was being measured in these sera. The sera obtained from the rabbits before injection of the insulin increased the glucose uptake of the diaphragms to an average of 0.25 mg. per gram hour. This is equivalent to an insulin effect of less than 2.5 mu. per ml. of serum. At thirty and sixty minutes following injection, there is considerable individual variation in the glucose uptake with an average gain of 1.01 and 1.29 mg. per gram per hour respectively. Equivalent effects are produced by insulin in concentrations of ten and fifteen milliunits per ml. At eighty minutes the response is more uniform, and there is a decrease in the average glucose uptake to 1.05 mg. per gram hour, equivalent to an insulin effect of eleven milliunits per ml. The average response in glucose uptake decreased further at 100 minutes to 0.75 mg. per gram hour, equivalent to an insulin effect of 6.0 mu. per ml. In the sera taken at 140 minutes from three of these rabbits the insulin-like effect had returned to the pre-injection level. In these experiments the rabbits received intravenous glucose at twenty, forty, and sixty minutes in order to prevent hypoglycemia. However, the blood sugar figures were low and although no hypoglycemic signs occurred, this may be a factor influencing the results.

From the previously determined space of distribution and the rates of disappearance for TCA precipitation and electrophoresis, a calculation has been made of the theoretical concentrations of insulin present in the serum from 0 to 140 minutes after the injection of insulin in the dose of fifteen units per kg. body weight. These concentrations have been compared with the insulin-like activity recovered by bio-assay (figure 9). The line representing the TCA concentrations is outside the standard deviations about the mean of the bio-assay levels, except at sixty minutes. There is little correlation between the electrophoretic and the bio-assay concentrations at thirty minutes. Between sixty and 140 minutes the concentrations derived from the electrophoresis results are within the standard deviations for the levels obtained by bio-assay.

The biologic activity of one lot of radioactive insulin was measured by its effect on the rat hemidiaphragm, using the mean of three diaphragms. At a concentration of forty milliunits per ml., the activity was 65 per cent

of that of the glucagon-free insulin standard at this concentration.

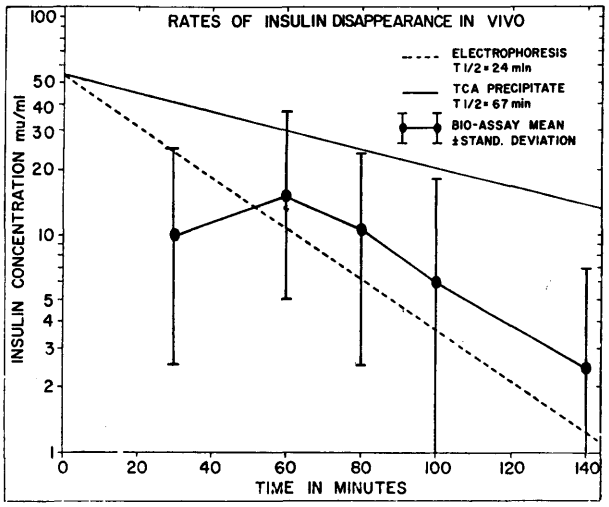


FIG. 9. Rates of insulin disappearance in vivo comparing trichloroacetic acid precipitation, paper electrophoresis and the bio-assay, at a dose level of fifteen units of crystalline zinc insulin per kg. body weight.

DISCUSSION

The study of insulin metabolism with insulin I^{131} is based on the assumptions that the radioactive insulin is unaltered by the labeling process and that it is representative of endogenous insulin. An accepted method of measuring radioactive insulin in biological fluids has been the precipitation of radioactivity by the addition of trichloroacetic acid. Recently, however, Berson and his associates have found that a variable portion of insulin is physically altered by the labeling process.[3] This is demonstrated by the behavior of insulin I^{131} when studied by electrophoresis. Under these conditions, unaltered insulin remains adsorbed to the paper strip at the point of application, whereas the altered insulin moves at a rate approximately equal to that of albumin whether or not plasma proteins are present. This altered fraction may represent as much as 25 per cent of the total radioactivity. The technics of TCA precipitation and electrophoresis were compared in a study of the rates of disappearance of insulin I^{131} from the plasma of rabbits. The radioactive insulin was administered intravenously. The biological half life of insulin I^{131} as determined by TCA precipitation was found to be much longer than that determined by measurement of the radioactivity adsorbed at the point of application in the electrophoretic technic. The prolonged half life of the radioactivity of the TCA precipitate was shown to be due to the persistence in the circulation of a physically altered fraction of the insulin I^{131}. Similar physical alterations in the insulin molecule have been produced by external irradiation with X rays in doses comparable to those given by I^{131}.[11] Chemical analysis of the insulin irradiated by either X ray or I^{131} has demonstrated that the disulfide bonds of insulin are separated and reduced to the sulfhydryl form during irradiation.[12]

In the studies reported here, rates of disappearance of insulin I^{131} were also measured in the rabbit following intravenous administration. The mean biological half life of the TCA precipitate from the plasma was sixty-seven minutes, whereas that of the radioactivity adsorbed at origin in the electrophoretic technic was twenty-four minutes. The difference found between the absolute counts of the radioactivity measured in these two methods was relatively constant throughout the period of study (figure 3). The radioactive fraction was found to migrate with the plasma proteins, when studied by electrophoresis (figures 1, 6), and persisted, with no measurable biological decay, after disappearance of the unaltered insulin fraction from the plasma (figure 3). It was this recirculating altered fraction which accounted for the prolonged biological half life of the TCA precipitate.

Bio-assay of the insulin-like activity of sera obtained from rabbits, following intravenous injection of unlabeled insulin, was made using the rat hemidiaphragm technic. There was a wide individual variation in the level of insulin found at thirty and sixty minutes. This may have been due to the presence of insulin antagonists in some sera samples. From sixty to 140 minutes following injection of insulin there was a gradual decrease in the mean level of insulin in the serum with a return to the pre-injection level at about 140 minutes.

The volume of distribution of insulin I^{131} and the rates of disappearance of insulin I^{131}, as determined by TCA precipitation and electrophoresis, were used in the calculation of the theoretical concentration of insulin present in the serum. From sixty to 140 minutes following injection of insulin there was good correlation between the levels of insulin measured by the bio-assay and the theoretical concentration of insulin I^{131} as calculated from the previous electrophoretic study of sera. At 140 minutes the insulin level had returned to the pre-injection level as measured by bio-assay, and the theoretical concentration of insulin as determined by the electrophoretic technic was similar to that of the bio-assay. At this time the insulin concentration calculated by the TCA precipitation technic was persistently high and showed poor correlation with the bio-assay of un-

labeled insulin. The rate of disappearance of insulin I^{131} from the plasma as measured by the technic of TCA precipitation is much slower than the disappearance of unaltered insulin as determined by bio-assay. This implies that the residual recirculating radioactivity, equivalent to the altered insulin fraction, is biologically inactive.

The observation that a standard solution of insulin I^{131} had less effect on the glucose uptake of the diaphragm than an equivalent concentration of unlabeled insulin also suggests that biological activity is diminished by the labeling process. However, further comparisons of the labeled and unlabeled insulin at various concentrations will be necessary to confirm this finding.

Thus, biological inactivation of insulin I^{131} may occur without the separation of the radioactive label. This places a limitation on the use of insulin I^{131} as a reliable tracer of insulin metabolism. Some of these limitations are overcome, however, by the use of the electrophoretic technic or by more refined method of iodination with I^{131} [13, 14]

SUMMARY

Insulin, labeled with radioactive iodine (I^{131}), was given to normal rabbits by intravenous injection. The rate of disappearance of the radioactivity from the plasma was studied by two technics. In the first, the radioactivity in the plasma was precipitated with trichloroacetic acid; whereas in the second, the insulin I^{131} was separated from the plasma by electrophoresis. The rate of disappearance of the radioactivity was found to be much slower by the technic of trichloroacetic acid precipitation than by that of plasma electrophoresis. The biological half life of the radioactivity in the first method was sixty-seven minutes, whereas in the second it was twenty-four minutes. The longer biological half life of the radioactivity precipitated by trichloroacetic acid was due to persistence of a portion of the radioactivity migrating with the plasma proteins.

The rate of disappearance of unlabeled insulin from the plasma of rabbits was also studied by means of a bio-assay. This rate of disappearance showed good correlation with that of the disappearance of radioactive insulin found by electrophoresis. Conversely, the rate of disappearance as determined by bio-assay did not correlate with that found by trichloroacetic acid precipitation. The theoretical levels of insulin predicted by the electrophoretic technic from sixty to 140 minutes following administration of insulin were similar to the levels of insulin found by bio-assay. These results do not support the assumption that the trichloroacetic acid precipitate of radioactive insulin in serum represents only intact, biologically active insulin. However, the method for the separation of insulin I^{131} from plasma employing electrophoresis appears to be accurate.

SUMMARIO IN INTERLINGUA

Un Comparation Del Destino De Insulina In Le Sero, Sin E Con Marcage Per I^{131}

Insulina marcate con iodo radioactive (I^{131}) esseva administrate a conilios normal per injection intravenose. Le rapiditate del disparition del radioactivitate ab le plasma esseva studiate per medio de duo technicas. In le prime, le radioactivitate in le plasma esseva precipitate per acido trichloroacetic. In le secunde, le insulina a I^{131} esseva separate ab le plasma per electrophorese. Le valor trovate pro le rapiditate del disparition del radioactivitate per medio del technica a precipitation trichloroacetic esseva multo plus alte que illo trovate per medio del technica a electrophorese del plasma. Le medie vita biologic del radioactivitate secundo le prime methodo esseva sexanta-septe minutas; secundo le secunde, vinti-quatro minutas. Le plus longe medie vita biologic del radioactivitate precipitate per acido trichloroacetic esseva causate per le persistentia de un portion del radioactivitate migrante con le proteinas del plasma.

Le rapiditate del disparition de non-marcate insulina ab le plasma de conilios esseva etiam studiate per medio de un bio-essayo. Le valores assi trovate monstrava un bon grado de correlation con le valores trovate pro le rapiditate del disparition del insulina radioactive per medio del technica electrophoretic. Del altere latere, le rapiditate del disparition determinate per bio-essayage non se monstrava in correlation con le valores determinate per precipitation a acido trichloroacetic. Le nivellos theoric de insulina predicite super le base del methodo electrophoretic pro sexanta a quaranta minutas post le administration de insulina esseva simile al nivellos trovate per bio-essayage. Iste resultatos non supporta le supposition que le precipitato de insulina radioactive effectuate in le sero per acido trichloroacetic representa solmente insulina que es intacte e biologicamente active. Tamen, le methodo de separar insulina a I^{131} ab le plasma per medio de electrophorese es apparentemente accurate.

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DISCUSSION

FRANCIS D. W. LUKENS, M.D., (*Philadelphia*): It is a pleasure to hear this critical comparison of different methods of assaying or appraising the presence of insulin. We have run into a similar problem in Philadelphia, in which an immunologic method of measuring insulin has failed to agree with Dr. Vallance-Owen's bio-assay. The fact that two such methods are being critically compared by the Baltimore group is an encouraging thing for the methods of tomorrow.

Galactose Utilization in Sucklings

More critical evaluation of galactose tolerance revealed that suckling rats can utilize galactose completely when it is present in no larger proportion than 5 per cent of the total diet by dry weight. If fat or protein rather than glucose is present as a high per cent of the diet, the tolerance can be elevated to approximately 10 per cent, the criterion for tolerance being the appearance of galactose in the urine. Young rats fed an artificial mixture of 44 per cent fat, 39 per cent casein, 5 per cent salt mixture, and 12 per cent lactose nevertheless contained 10 per cent less total liver lipid than did rats fed a similar diet except for the substitution of glucose for lactose. Unfortunately no data from the above experiments relate the absolute rather than relative amounts of galactose in the diet to the changes reported.

When comparison is made of the lactose content of rat's milk with that of other animals, it is found that the former contains only about 9 per cent lactose, whereas cow's milk contains about 38 per cent lactose and human milk contains almost 57 per cent. This may indicate that galactose tolerance differs widely among mammalian sucklings. Apparently, suckling as well as adult rats differ from human and bovine species in that they lack the capacity to utilize galactose as a major source of energy (galactosemia occurs only very rarely in human infants). Indeed, it was reported by Scheunert and Sommer that when galactose is withheld from 40-gm. suckling rats, development takes place readily in the presence of glucose. Thus the role of this sugar in development remains obscure. Perhaps other species are more dependent on its presence in the diet than is the rat. It has been reported that milk sugars aid in the absorption of calcium, a finding which is supported by the present studies on mineral deposition but seems to be contradicted by the X-ray studies of P. Handler (J. Nutrition *33*:221, 1947). It is further suggested that galactose is involved in the development of the central nervous system, in which there is intensive activity in many species during the first few postpartum days.

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