The Reaction of Rabbit Anti-beef Insulin Serum with Heterologous and Homologous Insulin Preparations

H. T. Narahara, M.D., and Robert H. Williams, M.D., Seattle

SUMMARY

A method for assaying antibodies to insulin that is based on the protection of insulin-I-131 from degradation by a liver extract is described. Moderately potent antibodies to insulin were obtained in rabbits by the injection of crystalline beef insulin, and a high degree of insulin resistance was demonstrable in some of the animals. The immunized animals did not become diabetic.

The biological activity of various insulin preparations was detected by their ability to lower the blood sugar of rabbits or through the stimulation of glucose uptake by the rat hemidiaphragm. Crystalline beef insulin and crude extracts of beef pancreas were effectively neutralized under the conditions of the tests whereas corresponding pork insulin preparations were incompletely neutralized in the rat hemidiaphragm assay. The insulin activity of sera from beef, pork, sheep and human sources was neutralized by the rabbit antisera.

The insulin activity of rabbit pancreas extracts and rabbit sera exhibited significant individual differences in the extent of neutralization. These observations on rabbit insulin preparations support the concept of Moloney and Coval that insulin of a given animal species can exist either in a "native" form that is not readily neutralized by antiserum produced in the same species, or an "altered" form that can be neutralized.

Antibodies to insulin of a given animal species can neutralize the biological effects of crystalline insulin of other species as well.[1,2] Although the immunized animal does not become diabetic,[1,3] injection of the anti-insulin serum into animals of other species can produce a diabetic syndrome.[1,4] This differential reactivity may depend upon species specific differences in the primary structure of insulin. However, Moloney and Coval[1] have found that an anti-insulin serum can also neutralize certain preparations of insulin from the same species of animal. The present studies extend these observations to anti-insulin sera produced in rabbits. The interaction of these antisera with crystalline insulin, pancreatic extracts, and serum insulin activity of various species has been studied. A convenient method for measuring the relative potency of anti-insulin sera during a course of immunization is described.

From the Department of Medicine, University of Washington School of Medicine, Seattle, Washington. Dr. Narahara's present address is Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri.

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METHODS

Production of antisera to insulin in rabbits. Crystalline beef insulin (0.44 mg.) in Freund's complete adjuvant[5] was injected subcutaneously in the groin and axilla of white male rabbits weighing approximately 3 kg. Additional doses containing 0.22 mg. of insulin were injected once or twice a month; blood was drawn one to two weeks after a sensitizing injection. In a series of rabbits that were immunized for nine to fifteen months, four sera exhibited a fairly good binding capacity of approximately 20 μg. of insulin-I-131 per milliliter, as determined by the method to be described in a later section. The binding capacity was only 2 to 4 μg. per milliliter in the remaining four rabbits. (Nineteen μg. of the crystalline beef insulin employed was equivalent to approximately 0.5 unit.) When a high antibody titer was produced, it usually became evident by the third or fourth month of immunization, and was then maintained at a fairly uniform level. Considerable variations in antibody response to insulin have been reported;[1,3,6] there is evidence that incorporation of tubercle bacilli with the antigen, as was done in these experiments, may enhance the formation of more potent anti-insulin sera.[7]

Hypoglycemic reactions were not a complication of immunization in the present studies. The average fasting blood sugar (generally tested a week or more after injection of antigen) was 61 mg. per 100 ml. in immunized rabbits, and 72 mg. per 100 ml. in control animals injected with Freund's adjuvant alone. Glycosuria was not demonstrable at any time.

Detection of insulin antibodies with a liver extract system. The method used for measuring antisera during the course of immunization was based on the principle that antibodies to insulin can protect the hormone from degradation by a liver homogenate.[8,9] Liver extract was prepared by stirring an acetone powder of beef liver for one hour at 4° C. with 10 ml. of 0.08 M potassium phosphate buffer, *p*H 7.4, per gram of powder. The supernatant fluid remaining after centrifugation for twenty minutes at 9,000 × *g* was used; this extract could be stored for several weeks at −20° C. without significant loss of activity.

Crystalline beef insulin labeled with I-131 at a specific activity of 3 to 6 millicuries per milligram of protein was obtained from Abbott Laboratories. A five-fold to twenty-fold excess of unlabeled insulin was added to a dialyzed aliquot of the labeled material to provide a suitable substrate mixture. In the following presentation, concentrations of insulin-I-131 will refer to the total concentration of labeled and unlabeled insulin, unless otherwise stated.

Insulin-I-131 was incubated at 37° C. with liver extract in 2 ml. of 0.02 M phosphate buffer, *p*H 7.4, and bovine plasma albumin was included at a concentration of 2 mg. per milliliter to diminish adsorption of insulin to glassware.[10,11] The reaction was stopped by the addition of 2 ml. of 20 per cent trichloroacetic acid (TCA) preceded by 1 ml. of a 2 per cent solution of lyophilized human plasma to serve as additional protein carrier. The TCA-soluble and insoluble fractions were processed and counted as described previously.[12] The amount of TCA-soluble radioactive material that appeared during incubation with extract, after correction for the TCA-soluble radioactivity in control samples incubated without liver extract, was taken as a measure of the extent of degradation. When varying amounts of antiserum were added to the insulin-I-131, enough normal (nonimmune) serum was also added to keep the total amount of serum constant in all tubes. The effect of antiserum on the degradation of insulin-I-131 was compared with control incubation mixtures that contained normal serum without antiserum. All incubations were done in duplicate.

Pancreatic extracts. Extracts with insulin activity were obtained from pancreatic tissue with a salt-ethanol mixture, and lipid was removed with petroleum ether, as described by Romans.[13] Some extracts were also prepared with acid alcohol by the method of Somogyi et al.;[14] purification was carried through the stages of ammonium sulfate fractionation and isoelectric precipitation.

RESULTS

Detection of antibodies to insulin with liver extract. Maximal degradation of insulin-I-131 in a ten-minute period was obtained with approximately 0.1 ml. of liver extract per 2 ml. of reaction mixture (figure 1). In the presence of 0.5 ml. of liver extract the amount of TCA-soluble radioactive material produced was directly proportional to the concentration of insulin-I-131, up to a level of around 15 μg. per 2 ml. of reaction mixture. In assays of antisera, the amount of insulin-I-131 used did not exceed 4 μg. per tube. Figure 2 shows that when rabbit anti-insulin serum was added to the reaction mixture before the addition of liver extract, the reaction stopped at a lower level of degradation. The slight tendency for the values with and without antiserum to converge after the first ten minutes of incubation would be compatible with a slow dissociation of insulin from the protecting antibody.[15] Substrate that dissociated rapidly from antibody would presumably have been degraded within the first ten minutes of incubation.

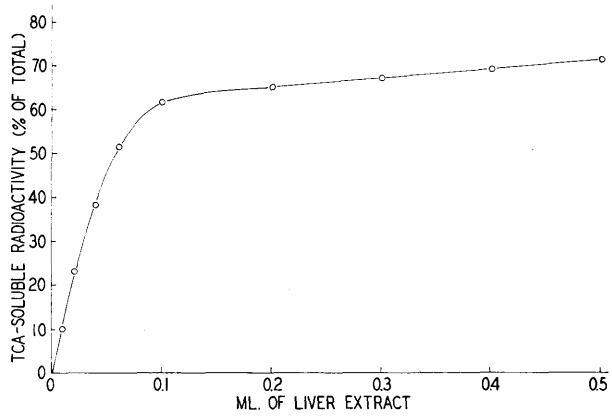

FIG. 1. Extent of degradation of insulin-I-131 with increasing amounts of liver extract. Incubation was for ten minutes with 2 μg. of insulin-I-131 per tube.

When varying small amounts of antiserum were added to insulin-I-131, degradation by liver extract was decreased proportionately. The extent of binding of the labeled substrate by antiserum could be calculated from the degree to which degradation was decreased when compared with reaction mixtures containing only normal (nonimmune) serum. The linear relationship that resulted (figure 3) has permitted the use of the liver extract system as a simple assay of relative antiserum potency. It has been assumed that the affinity of antibody for insulin was not changed appreciably by iodination, in accordance with the report by Berson and Yalow.[15]

Welsh et al.[8] have shown that there is a direct

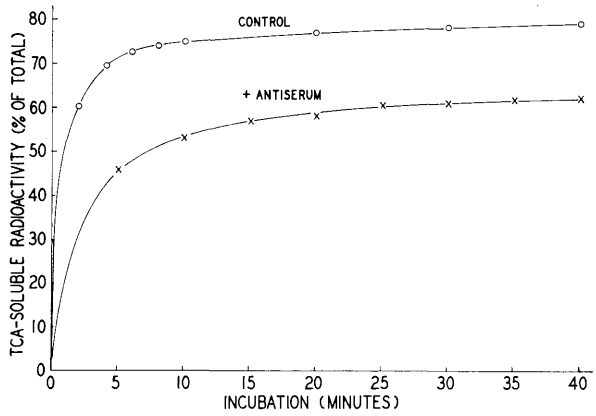

FIG. 2. Effect of antiserum upon the extent of degradation of insulin-I-131 during longer incubation. A mixture containing 0.1 ml. of antiserum and 2 μg. of insulin-I-131 in a volume of 1.5 ml. was kept at 0° C. for thirty minutes, and then warmed to 37°C. for five minutes. Incubation was carried out for varying periods after addition of 0.5 ml. of liver extract.

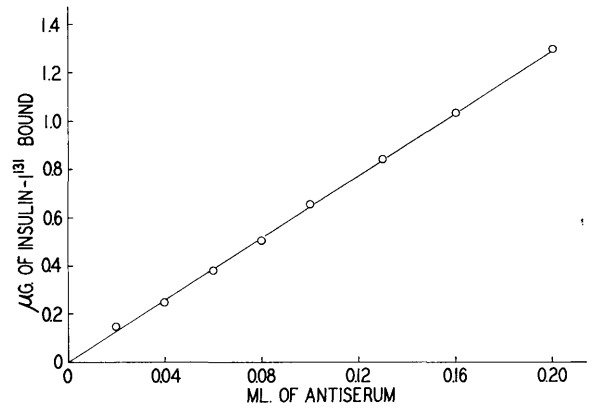

FIG. 3. Relationship of extent of binding of insulin-I-131 to the amount of antiserum added. Total amount of antiserum plus normal rabbit serum in each tube was 0.2 ml. The antiserum and 4 μg. of insulin-I-131 were mixed and left for thirty minutes at 0° C. and five minutes at 37° C. before incubation with 0.5 ml. of liver extract for ten minutes.

correlation between the extent of insulin-I-131 retention in the circulating plasma of insulin-treated diabetics and the degree of protection that the serum affords in vitro against degradation of insulin-I-131 by a rat liver homogenate. Berson and Yalow[15] used a rat liver homogenate system to measure the rate of dissociation of insulin-I-131 from antibody, and found that the results correlated well with measurements made by chromatoelectrophoresis.

Sera from four diabetic patients who required 100 units or more of insulin per day were assayed for their ability to bind insulin-I-131 by the liver extract method, and comparative measurements were made by the technic of Skom and Talmage[16] in which the complex of insulin-I-131 and antibody was precipitated with antiserum to human γ-globulin. The degree of binding varied in a parallel fashion from one serum to another for the two assay procedures, but somewhat less binding was found with the liver extract method (table 1). Patient DE was previously reported to retain a high percentage (72 per cent) of a dose of insulin-I-131 in the circulating plasma one hour after intravenous injection (Case 112 in reference 8).

TABLE 1

Comparison of assays for insulin-I-131 binding in sera of diabetic patients

Patient	Daily insulin requirement	Amount of insulin-I-131 bound (μg. ml. of serum) Liver extract method	Globulin precipitation method
BB	750	15.5	20.0
GS	500-600	3.9	7.0
DE	60-120	1.8	2.8
VL*	100-160	0.2	0.3

*Thirty-eight-year-old male with active acromegaly.

It is likely that more dissociation of insulin-antibody complexes occurred during incubation with the insulin-degrading system than during precipitation with anti-globulin serum. However, the production of anti-γ-globulin serum was laborious, and limited the method to studies on antiserum from a single animal species. Therefore, the liver extract method was used for following antibody titers in rabbits during immunization.

Effect of insulin on blood sugar levels in immunized rabbits. Subcutaneous injection of 1 u. of crystalline beef insulin per kilogram caused no decline of blood sugar levels in four immunized rabbits with high antiserum titers (figure 4A), in keeping with similar findings by Lowell and Franklin.[3] Larger amounts of insulin, 20 to 30 u. per kilogram, did cause a slow fall of blood sugar in immunized rabbits (figure 4B). In the most responsive immunized animal, the insulin-binding capacity of the serum was 18 μg. per milliliter before injection, as measured by the liver extract method, and fell to 1 μg. per milliliter one and a half hours after the injection of 30 u. per kilogram (100 u. total dose). Test doses of 1 u. of crystalline pork or sheep insulin per kilogram also failed to lower the blood sugar of rabbits immunized against beef insulin. The presence of cross reactions among crystalline insulin preparations of these species has been noted earlier in other types of assays.[1,2,17,18]

Crystalline rabbit insulin was not available for neu-

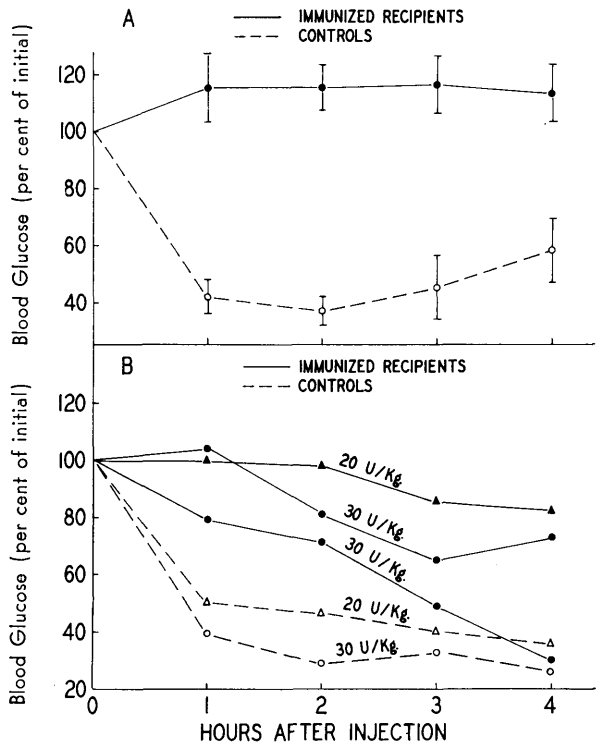

FIG. 4. Response of blood sugar to injection of insulin in immunized rabbits and nonimmunized control animals. Part A: Crystalline beef insulin, 1 U. per kilogram of body weight, was injected subcutaneously. Each value is the mean for four animals, and the vertical bars indicate twice the standard error of the mean. Part B: 20 or 30 U. of crystalline beef insulin per kg. were injected. Each curve represents one immunized rabbit or two control rabbits.

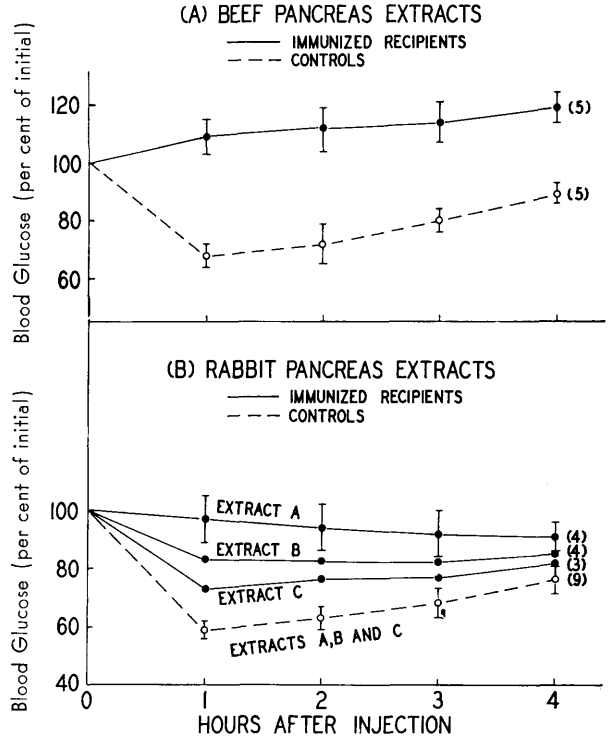

FIG. 5. Effect of pancreatic extracts upon blood sugar of rabbits. Results obtained with two beef pancreas extracts have been averaged together; control results for three rabbit pancreas extracts were also pooled. The same group of immunized animals was used for each extract, and the number of animals used for each curve is indicated in parentheses. The standard error of the mean was approximately ± 14 for the values of Extract B and ± 4 for the values of Extract C.

tralization tests and crude extracts of rabbit pancreas were prepared by extraction with a salt-alcohol mixture as described by Romans.[13] Beef pancreatic extracts prepared by this technic had hypoglycemic activity that was completely antagonized in immunized rabbits (figure 5A). In contrast, different preparations obtained from rabbit pancreas were neutralized to varying extents (figure 5B). All extracts were tested within one to five days after preparation.

Studies with the rat hemidiaphragm assay system. In order to obtain a better evaluation of the ability of rabbit anti-insulin serum to neutralize different types of insulin preparations, additional studies were carried out with the rat hemidiaphragm assay for insulin.[19] The average basal glucose uptake in the absence of insulin in forty-eight hemidiaphragms, with 300 mg. of glucose per 100 ml. of medium, was 10.6 ± 0.3 mg. per 100 ml. of medium per 10 mg. dry weight of muscle in ninety minutes (mean ± standard error of the mean). The average value for the absolute difference between the basal uptake of right and left hemidiaphragms was 1.2 ± 0.3, with a range of 0 to 3.6. A difference of 4 was regarded as significant. Occasional muscle pairs, for reasons that were not evident, gave aberrant results wherein the deviation from the average value was more than six times the standard error of the mean; these aberrant results were not included in the final averages. This exclusion did not alter the qualitative nature of the effects reported here.

Table 2 shows that antiserum at an appropriate concentration could inhibit the biological activity of crystalline beef insulin. Whenever antiserum was used, it was added to both the basal and the insulin-treated diaphragm in order to offset any effect of the antiserum itself upon glucose uptake. Large batches of pooled antiserum were used to permit more uniform comparisons within each series of experiments. Crystalline pork insulin was not as readily inhibited as beef insulin. This finding is in agreement with the observation of Yalow and Berson[20] that antibodies to beef insulin produced

in guinea pigs reacted more strongly with beef insulin than with pork insulin, when tested by the chromatoelectrophoretic technic.

Preliminary tests had shown that 0.8 mU. of beef insulin per milliliter was the highest concentration that could be neutralized by 0.05 ml. of the pooled antiserum. Yet, the addition of twice as much insulin produced only a rather small effect (table 2). These observations resemble the well-known Ehrlich phenomenon encountered in the neutralization of diphtheria toxin with antitoxin, which has been ascribed to the ability of antigen and antibody to combine in various molecular ratios.

It is of interest that the experiments in table 2 were performed with a batch of antiserum that bound 6.5 μg. of insulin-I-131 per milliliter in the liver extract assay system. This value corresponds to a binding of 8.5 mU. per 0.05 ml. of antiserum, which is obviously greater than the neutralization equivalent found in table 2. A similar type of discrepancy between different kinds of assay was noted by Moloney and Aprile[21] during studies with a horse antiserum to beef insulin that was able to flocculate with insulin. They found that at the equivalence zone 1 ml. of antiserum flocculated with 5 units of insulin, and some nonflocculating neutralizing antibodies were thought to be present as well. In contrast, a mouse convulsion test revealed that 1 ml. of horse antiserum neutralized only 0.8 unit of insulin.

The results of studies on several types of pancreatic extracts are shown in table 3. An exact measurement of the insulin activity was not carried out for each extract, but an amount of extract was used that was less active than 0.5 mU. per milliliter of crystalline beef insulin. A quantity of antiserum that was sufficient to neutralize 1 mU. of crystalline beef insulin per milliliter of medium was able to antagonize completely the biologic activity of two extracts of fresh beef pancreas prepared by the salt-alcohol method. An extract of pork pancreas, similarly prepared, was only partially neutralized. It will be recalled that crystalline pork insulin was also less readily neutralized than crystalline beef insulin (table 2).

In the case of rabbit pancreas extracts, different batches varied in behavior in a manner that suggested that factors other than species alone could influence the degree of neutralization. There was moderately effective neutralization of salt-alcohol extract A, but insignificant neutralization of extract B (table 3). This difference in reactivity shows a trend similar to the results obtained with these extracts in the blood glucose response test (figure 5B). Rabbit pancreas extract C was not examined in the rat hemidiaphragm system. One acid-alcohol extract of rabbit pancreas, lot D, was neutralized well by antiserum. However, extract E, also prepared with acid alcohol, was incompletely neutralized even after repeated freezing and thawing of the extract. The factors responsible for the observed differences in immunologic reactivity remain unresolved.

The ability of rabbit anti-insulin serum to neutralize the insulin activity of normal sera from rabbits and other animal species was also investigated with the rat hemidiaphragm assay. Under the conditions employed, the insulin-like activity of beef, pork, sheep and normal human sera were effectively neutralized when tested

TABLE 2

Effect of anti-beef insulin serum on crystalline insulin in the rat hemidiaphragm assay

Insulin Species	Insulin Amount (mU./ml.)	Antiserum (ml./ml.)	Insulin effect* (mean ± standard error)
Beef	0.5	0	13.6±1.1 (4)
	0.8	0.05	1.6±0.4 (7)
	1.6	0.05	4.1±0.3 (8)
Pork	0.5	0	13.7±0.8 (7)
	0.4	0.05	1.9±0.5 (9)
	0.8	0.05	5.7±0.3 (27)

*Glucose uptake is expressed as milligrams of glucose per 100 ml. of medium per 10 mg. dry weight of muscle per ninety minutes. The insulin effect is the glucose uptake of the hemidiaphragm exposed to insulin minus the uptake of the hemidiaphragm not exposed to insulin. The number of determinations is shown in parentheses. When antiserum was used, it was added to both hemidiaphragms.

TABLE 3

Effect of anti-beef insulin serum on activity of pancreatic extracts in rat hemidiaphragm assay

Pancreatic extract	Antiserum (ml./ml.)	Insulin effect*
Beef, 2 lots	0	11.8±0.8 (12)
	0.05	0.5±0.7 (12)
Pork	0	7.9±1.5 (9)
	0.05	4.2±1.1 (9)
Rabbit, lot A†	0	10.2±0.9 (5)
	0.05	4.4±0.6 (6)
Rabbit, lot B	0	9.3±1.1 (12)
	0.05	8.4±0.6 (12)
Rabbit, lot D	0	7.9±0.5 (9)
	0.10	1.4±0.7 (10)
Rabbit, lot E	0	9.6±0.5 (5)
	0.10	4.3±0.5 (6)

*Expressed as in table 2.
†Prepared from frozen glands; other extracts were from fresh glands.

against the same batch of pooled antiserum (table 4). Two normal rabbit sera (*a* and *b*), on the other hand, were incompletely neutralized by this antiserum. Serum *b* was frozen and thawed five times and retested; this time there was more complete neutralization (serum *b'* in table 4). The biological activity of a sample of heparinized normal rabbit plasma also showed complete neutralization of its insulin-like activity by antiserum. Thus, as in the case of rabbit pancreatic extracts, a variation was found in the extent to which different samples of rabbit serum insulin could be neutralized, but the nature of these variations in immunologic reactivity has not been elucidated.

TABLE 4

Effect of rabbit anti-beef insulin serum on the insulin-like activity of normal sera in rat hemidiaphragm assay

Serum*	Antiserum (ml./ml.)	Insulin effect†
Beef	0	4.9±0.3 (11)
	0.1	0.7±0.3 (15)
Beef	0	8.8±0.8 (8)
	0.1	0.2±0.8 (10)
Pork	0	7.5±0.8 (5)
	0.1	1.6±0.4 (10)
Pork	0	5.3±0.3 (6)
	0.1	2.0±0.4 (6)
Pork	0	8.6±0.2 (6)
	0.1	0.6±0.3 (5)
Sheep	0	8.7±0.7 (6)
	0.1	1.4±0.2 (5)
Human	0	7.5±0.7 (8)
	0.1	1.1±0.5 (7)
Rabbit, lot A	0	11.8±0.6 (6)
	0.1	4.6±0.9 (6)
Rabbit, lot B	0	6.2±0.8 (8)
	0.1	3.6±0.7 (7)
lot B; frozen and thawed	0	6.0±0.8 (5)
	0.1	0.1±0.8 (6)
Rabbit, lot C	0	7.4±1.1 (5)
	0.1	1.1±0.8 (5)
Rabbit, lot D; frozen 11 mo.	0	9.7±0.7 (5)
	0.1	1.1±0.4 (6)

*0.25 ml. of normal serum was used per milliliter of medium. Unless otherwise stated, fresh sera were refrigerated for one to four days before use.
†Expressed as in table 2.

DISCUSSION

Some of the insulin in the body appears to exist in a form that has many of the characteristics of crystalline insulin, and has been called "typical"[22] and "uncomplexed" or "free."[23] This type of endogenous insulin stimulates glucose uptake by the isolated rat diaphragm, is neutralized by appropriate antisera to insulin, is secreted in larger amounts after a glucose load, and disappears from the circulation soon after pancreatectomy (cf. reference 24). A second form of endogenous insulin, called "atypical" and "complexed" or "bound," has been postulated to occur in combination with a basic protein in the pancreas[25] and in the circulating plasma.[23] This second form of insulin is detectable through its ability to stimulate the oxidation of glucose-1-C-14 by adipose tissue, but it does not seem to enhance the glucose uptake of rat diaphragm, and apparently it is not neutralized well by antibodies to insulin.[22,26]

The existence of a "typical" form of endogenous insulin that can be neutralized by heterologous antiserum has been found in all of the species of animals examined, including the rabbit. Moloney and Coval,[1] Wright,[4] and Kitagawa et al.[27] have made the important observation that antisera to beef insulin produced in guinea pigs can elicit a diabetic syndrome in animal species other than the guinea pig. In addition, the neutralization of the biological activity of beef serum insulin[28] as well as of crystalline insulin from various species[2] by antisera produced in the guinea pig has been demonstrated with the rat hemidiaphragm bioassay. The results of the present studies on the neutralization of insulin activity in beef, pork, sheep and human serum by insulin antibodies produced in rabbits supports the generalization that in each species there is a "typical" component of endogenous insulin.

On the other hand, experimental animals can form potent antibodies to insulin without becoming diabetic themselves.[1,3,6] This finding is in accord with the well-known fact that animals do not ordinarily produce antibodies that react with normal constituents of their own plasma.

Moloney and Coval[1] have discovered a noteworthy variability in the reactivity of anti-insulin sera with preparations of insulin from homologous species. These workers found that antiserum from guinea pigs immunized against beef insulin could actually neutralize one out of several extracts of guinea pig pancreas; this particular extract had been produced from tissue that had been stored in the cold for several months, whereas the others were fresh extracts. They also found that a sheep that had been immunized against crystalline beef insulin showed no hypoglycemic response to crystalline sheep insulin, presumably because the insulin was neutralized by homologous antibodies. Furthermore, Molo-

ney and Aprile[21] demonstrated that anti-beef insulin serum produced in a horse was able to flocculate and neutralize the insulin in extracts of horse pancreas. On the basis of these observations, Moloney and Coval[1] have suggested that two forms of insulin can be distinguished by studying their immunologic reactivity with homologous antisera. They have called the form of endogenous insulin that does not react with homologous antiserum "native," and have suggested that this type can be converted, for example, by certain storage or purification procedures, into an "altered" form that does react. The results of the present experiments support their conclusion, and extend the investigation to antisera produced in rabbits. The exact nature of the changes involved, and the factors that bring about conversion from the "native" to the "altered" state require further investigation.

The data available at present certainly do not warrant extensive speculation, but it may be of interest to consider a few concepts that might help to correlate some of these diverse findings. It is possible that the difference between the "native typical" and the "atypical" forms of insulin could be caused by masking, in the "atypical" form, of an antigenic site that is accessible to antibodies in the "typical" form. Species specificity of this antigenic site might help to explain why homologous antisera do not react well with the "native typical" form of insulin. By further extension of this line of reasoning, the conversion of a "native typical" to an "altered typical" form of insulin might involve the unmasking of a second antigenic site that is not species specific. Such a change would permit homologous antisera to react with the insulin. If the nonspecific site were ordinarily inaccessible as an antigen in plasma insulin but freely accessible in crystalline insulin, this masking might explain how an animal could produce antibodies against the site even though the site was actually present in a normal constituent of the immunized animal's plasma.

SUMMARIO IN INTERLINGUA

Le Reaction de Sero Conilian Anti Insulina Bovin con Hetero- e Homologe Preparatos de Insulina

Es describite un methodo pro le essayage de anticorpore anti insulina, basate in le protection de insulina a I-131 contra su degradation con le uso de un extracto hepatic. Moderatemente potente anticorpores anti insulina esseva obtenite in conilios per le injection de crystallin insulina bovin, e un alte grado de resistentia contra insulina esseva demonstrate in certes del animales. Le immunisate animales non deveniva diabetic.

Le activitate biologic de varie preparatos de insulina esseva detegite per lor capacitate de reducer le sucro sanguinee de conilios o de stimular le acceptation de glucosa per le hemidiaphragma del ratto. Crystallin insulina bovin e crude extractos de pancreas bovin esseva neutralisate efficacemente sub le conditiones del tests, durante que correspondente preparatos de insulina porcin esseva neutralisate incompletemente in le essayo a hemidiaphragma de ratto. Le activitate insulinic de seros ab fontes bovin, porcin, ovin, e human esseva neutralisate per le antiseros de conilio.

Le activitate insulinic de extractos de pancreas de conilio e de seros de conilio exhibiva significative differentias individual in le grado de neutralisation. Iste observationes in preparatos de insulina de conilio supporta le concepto de Moloney e Coval que le insulina de un specie animal particular pote exister in un forma "native" que non es facilemente neutralisate per antisero producite in le mesme specie o in un forma "alterate" que non pote esser neutralisate.

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Recent Trends in Diabetes Mortality

The mortality from diabetes declined slightly, although not continuously, in the past decade. The age-adjusted death rate decreased from 14.3 per 100,000 in 1950 to 14.0 in 1953, and further to 13.3 in 1954. In the ensuing five years, the death rate from diabetes showed little change, but provisional data indicated a pronounced rise in 1960, with a return to the earlier level in 1961.

In interpreting the recorded mortality from diabetes, it should be recognized that the deaths ascribed to that condition represent only a fraction of the deaths occurring among persons with the disease. This is particularly the case at ages forty-five and over, where at least half of the deaths of diabetics are ascribed to associated complications, notably those of the circulatory system.

Significant departures from the over-all trend of diabetes mortality become evident when the data are analyzed by color and sex. White females recorded a fairly steady decline in mortality, with a reduction of one sixth from 1950-51 to 1958-59. Among white males the age-adjusted death rate was quite stable, ranging between 11.0 and 11.6 per 100,000 annually over the entire decade. In contrast, the rates for nonwhite persons, both males and females, increased—22 per cent for males and 17 per cent for females between 1950-51 and 1958-59.

A closer insight into recent trends in diabetes mortality, taking into account age in addition to color and sex, may be obtained from the table on page 36. For young people in each color-sex category there were appreciable declines in death rates during the 1950's. This favorable trend reflects the considerable success in the treatment of severe diabetes among young patients during the early years of their disease. At ages twenty-five to forty-four, however, the rate increased considerably among white males, while white females experienced a slight decrease, much smaller than at other ages. Within this broad age group are many persons with juvenile diabetes of long duration who have had severe and irreversible complications. This is probably responsible for the unfavorable trends among white persons in early adult life. The pattern is different among nonwhites, but the variations in their diabetes mortality rates are influenced by chance fluctuations due to the relatively small number of deaths from the disease among them.

At ages forty-five and over, the death rates from diabetes among white males were relatively stable during the 1950's. However, white females showed a considerable improvement, the reductions between 1950-51 and 1958-59 amounting to about one fourth at ages forty-five to sixty-four, and to about one tenth at ages sixty-five and over. The difference between

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