Insulin Resistance

Response to Insulin from Various Animal Sources, Including Human

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SUMMARY

The comparative usefulness of crystalline human, porcine, dealaninated porcine and bovine insulin in the treatment of insulin resistance was studied.

A series of normals, insulin-sensitive diabetics, and insulin-resistant diabetics received various types of insulin in acute intravenous tolerance tests. The insulin-resistant patients were far less responsive to all forms of insulin in these tests than were the normals and insulin-sensitive diabetic patients. No positive correlation was found between responsiveness to a particular type of insulin in an acute tolerance test and responsiveness during a prolonged clinical trial.

Seven patients with insulin resistance received clinical trials with at least one insulin other than bovine. Three of these patients were found to be more sensitive to either human, porcine or dealaninated porcine than to bovine insulin. Insulin binding capacities of sera from the responsive patients were somewhat higher than the binding capacities of the nonresponders. The insulin binding capacities of sera from the responsive patients were just as high or higher for the insulin to which the patient responded as they were for bovine insulin. Serum from the responsive patients in general antagonized added porcine, dealaninated porcine and bovine insulin to the same degree in vitro. Adipose tissue from the responders was no more sensitive to human or porcine than to bovine insulin in vitro.

The increased sensitivity of these patients to insulin from a nonbovine source may be due to a difference in kinetics of the antigen-antibody reaction in vivo which is not demonstrable in vitro.

Patients with obesity and insulin resistance responded to a reducing diet. Three of four such patients placed on 600 to 900 calorie diets lost their need for insulin over a four- to six-week period.

Insulin resistance, defined as an insulin requirement of 200 U. or more per day in the absence of acidosis, is probably multifactorial in etiology.[1] Factors responsible for antagonism to insulin may reside in the serum, liver, renal cortex or perhaps in less specialized tissues of the body such as adipose tissue. Serum factors capable of insulin antagonism include antibodies, hormones, lipoproteins and perhaps others, but antibodies appear to be the most significant factor in this area. The possibility that insulin antibodies were species specific prompted Lowell to try human insulin in two patients with insulin resistance, and he met with success in one.[2,3] Subsequently Yalow and Berson have provided further evidence that there are differing degrees of affinity of insulin antibody for insulins from various animal sources, but there is no true species specificity.[4] The order of affinity of insulin antibody for insulin in vitro is as follows:

Bovine $\cong$ ovine $>>$ porcine $>$ equine $>$ human $\sim$ dealaninated porcine.*

This paper is a report on the results of clinical trials with insulin from various animal sources in eight insulin-resistant patients and on comparative results of tolerance tests with the various insulins in normal subjects and in insulin-sensitive and insulin-resistant diabetic patients.

MATERIALS AND METHODS

Intravenous insulin tolerance tests were performed in normal subjects and in insulin-sensitive and insulin-resistant diabetic patients. The normal and insulin-sensitive diabetic patients received 0.1 unit and the insulin-resistant patients 1.0 unit of crystalline insulin per kilogram of body weight. The various species of crystalline insulin preparations which were utilized in these tests and in the clinical trials were as follows: bovine, porcine, dealaninated porcine and crystalline human.† During clinical trials the insulin given was divided into three doses per day and was administered subcutaneously. Blood glu-

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*Porcine insulin from which the terminal amino acid, alanine, has been removed from the B chain.

†Furnished by Dr. W. R. Kirtley of The Eli Lilly Company, Indianapolis, Indiana.

cose levels were determined in duplicate by the Nelson modification of the Somogyi method.[5]

The eight patients with insulin resistance were admitted to the Clinical Research Unit at the University Hospital, Birmingham, Alabama. They were placed on rigidly controlled dietary regimens and the only variable during the study was the source and dosage of insulin employed.

Subcutaneous fat tissue was obtained by surgical biopsy from the patients with insulin resistance in order to test its responsiveness to insulin in vitro, utilizing an incubation technic similar to that of Martin and Renold.[6,7] The fat tissue was incubated in Krebs-bicarbonate buffer in the presence of 2 milliunits per milliliter of added crystalline insulin and the conversion of C_1-14-labeled glucose to glycogen was determined and recorded as counts per minute per gram of fat.

Serum antagonism to added bovine insulin (2 milliunits per milliliter) was measured by comparing the effect of each species of crystalline insulin (2 milliunits per milliliter) in buffer with the effect of the same type and quantity of insulin added to the patient's serum on the conversion of C_1-14-labeled glucose to C-14-O_2, in the rat epididymal fat assay system of Martin and Renold.[6,7]

The insulin binding capacity of the patients' sera was measured by the method of Yalow and Berson.[8]

RESULTS

The results of the insulin tolerance tests are presented in figures 1 through 5. The normals and insulin-sensitive diabetic patients responded to all species of insulin with an earlier and significantly greater percentage fall in blood glucose levels than did diabetic patients with insulin resistance. Patient W.J. (figure 4) is not included in table 1. He is presented only to demonstrate the lack of correlation between the insulin tolerance test and the subsequent clinical trial. He responded dramatically to porcine insulin during the tolerance test but required just as much porcine as bovine insulin for maintenance.

Table 1 is a summary of the clinical histories and experimental results of the eight patients with insulin resistance.

Clinical trials with insulin from various animal sources were conducted in seven of these patients and the results are depicted in figures 6 through 12.

Human insulin was available in very limited quantities and therefore was utilized in only two patients, K.S. (figure 6), and E.M.W. (figure 8). K.S. was more responsive to human, to porcine and to dealaninated porcine than to bovine insulin whereas no significant differences in response were apparent in E.M.W. Obesity and a comparatively low serum insulin binding capacity distinguish the nonresponder (E.M.W.) from the responder (K.S.).

Three patients, M.H. (figure 9), R.H. (figure 11)

INSULIN TOLERANCE TESTS IN NORMALS
(AVERAGE OF TWO NORMALS)

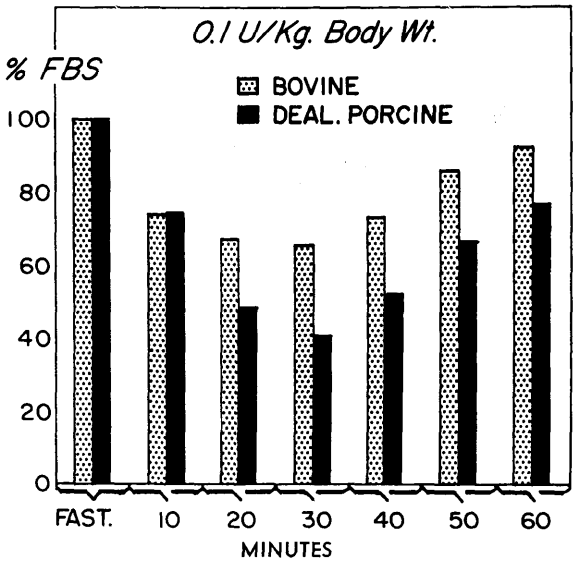

FIGURE 1

INSULIN TOLERANCE TESTS IN INSULIN-SENSITIVE
DIABETIC PATIENTS (AVERAGE OF THIRTEEN PATIENTS)

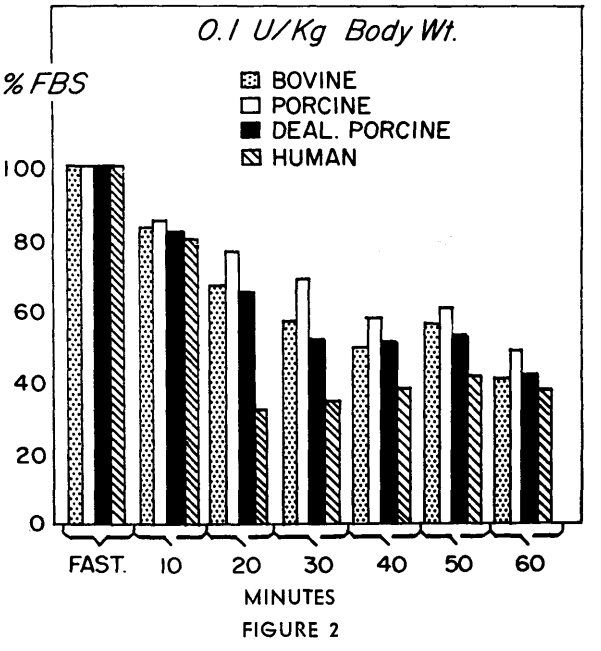

FIGURE 2

INSULIN TOLERANCE TESTS IN INSULIN-RESISTANT PATIENTS (AVERAGE OF FOUR PATIENTS)

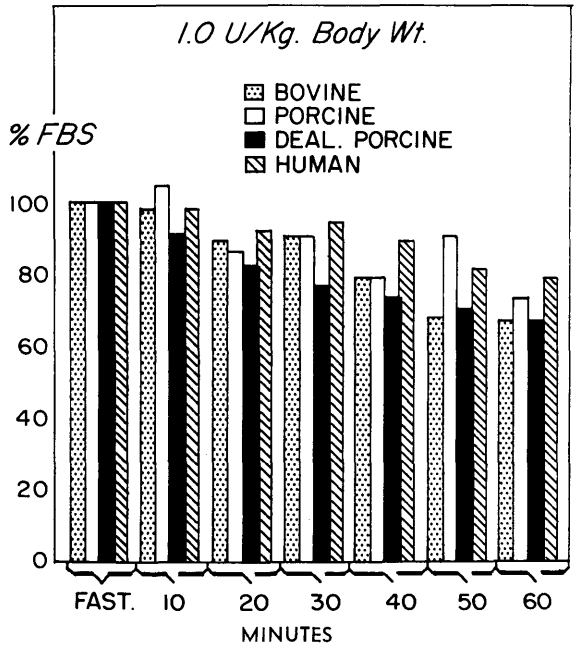

FIGURE 3

INSULIN TOLERANCE TESTS IN PATIENT W. J. (INSULIN RESISTANT)

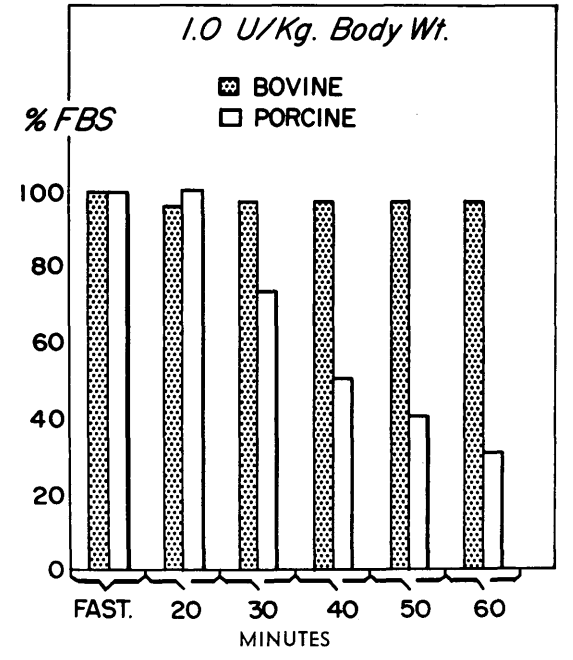

FIG. 4. A forty-four-year-old colored male with insulin resistance of fifteen years' duration who by insulin tolerance test appeared to be less resistant to porcine than to bovine insulin. A clinical trial failed to confirm this observation.

INSULIN TOLERANCE TESTS IN PATIENT R. H.

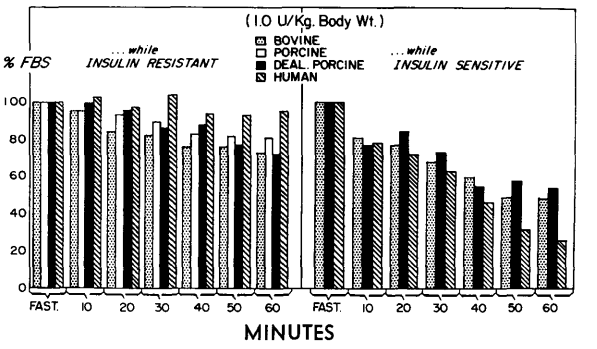

FIG. 5. R. H. is a sixty-three-year-old obese white woman with diabetes of ten years' duration. This figure is a graphic representation of the results of intravenous insulin tolerance tests performed before and after a forty-pound weight reduction. Crystalline insulin from various animal sources and in the amount of 1.0 unit per kilogram of body weight, as indicated in the figure, was used in each of these tests. The tests on the left of this figure were performed before and those on the right after the weight reduction. Although her sensitivity to insulin increased following the weight loss, it did not return to normal. See also figure 11.

and P.K. (figure 12) received clinical trials with bovine, porcine and dealaninated porcine insulin. Only M.H. responded significantly better to dealaninated porcine than to bovine insulin. She was essentially equally as responsive to porcine as to dealaninated porcine insulin. Again the serum insulin binding capacity was somewhat higher in the patient who responded better to porcine and to dealaninated porcine than to bovine insulin.

Patients J.L. (figure 7) and D.R. (figure 10) received clinical trials with only bovine and dealaninated porcine insulin. J.L. whose serum bound 270 units of bovine insulin per liter, developed profound hypoglycemia when he was changed from 330 units per day of bovine insulin to the same dose of dealaninated pork. D.R. was receiving only 120 units of bovine insulin per day when she was changed to the dealaninated preparation to which she was equally unresponsive. Her serum insulin binding capacity was less than 5 units per liter.

Patient M.G. was studied before the availability of human and dealaninated porcine insulin therefore received only bovine insulin. The results of her studies are not included in graph form but are summarized in table 1.

M.G., M.H. (figure 9), E.M.W. (figure 8) and R.H. (figure 11) all of whom were at least moderately obese were subsequently placed on diets of 600 to 900 calories per day and within a period of six weeks insulin was discontinued without loss of diabetic control, in all except E.M.W. Her insulin requirement decreased from 180 to 60 units per day. The increased sensitivity to in-

TABLE 1

Insulin-resistant patients

Patient	Age	Sex	Duration of diabetes mellitus (years)	Duration of insulin resistance (months)	Obese	Insulin binding capacity (beef) (U/L)	"Serum antagonists"* (per cent)	Activity of subcutaneous fat† (cpm./gm. fat)	Insulin requirement for regulation (units/day) Beef	Deal. pork	Pork	Human
K.S.	52	F	4	17	No	360	41	16,210	700	220	250	—
									150	60	—	30
J.L.	57	M	9	33	No	218	43	14,500	330	150	—	—
E.M.W.	45	F	20	12	Moderately	14	47	16,091	180	150	180	180
M.H.	64	F	5	36	Moderately	13	70	30,263	180	60	90	—
M.G.	58	F	15	27	Yes	<5	94.7	2,250	300	—	—	—
D.R.	64	F	12	24	Yes	<5	92	19,610	120	120	—	—
R.H.	63	F	10	1	Yes	31	—	5,035	180	180	180	—
P.K.‡	55	F	2	1	Moderately	40	77	8,999	200	170	170	—

*Expressed as activity of 2 mU of beef insulin in patients' serum as compared to 2mU of beef insulin in buffer. (Average of four normal sera = 102 per cent.)

†In actual conversion of C-14-glucose into glycogen. (Two-milliunits of beef insulin/ml.)

‡Acromegaly.

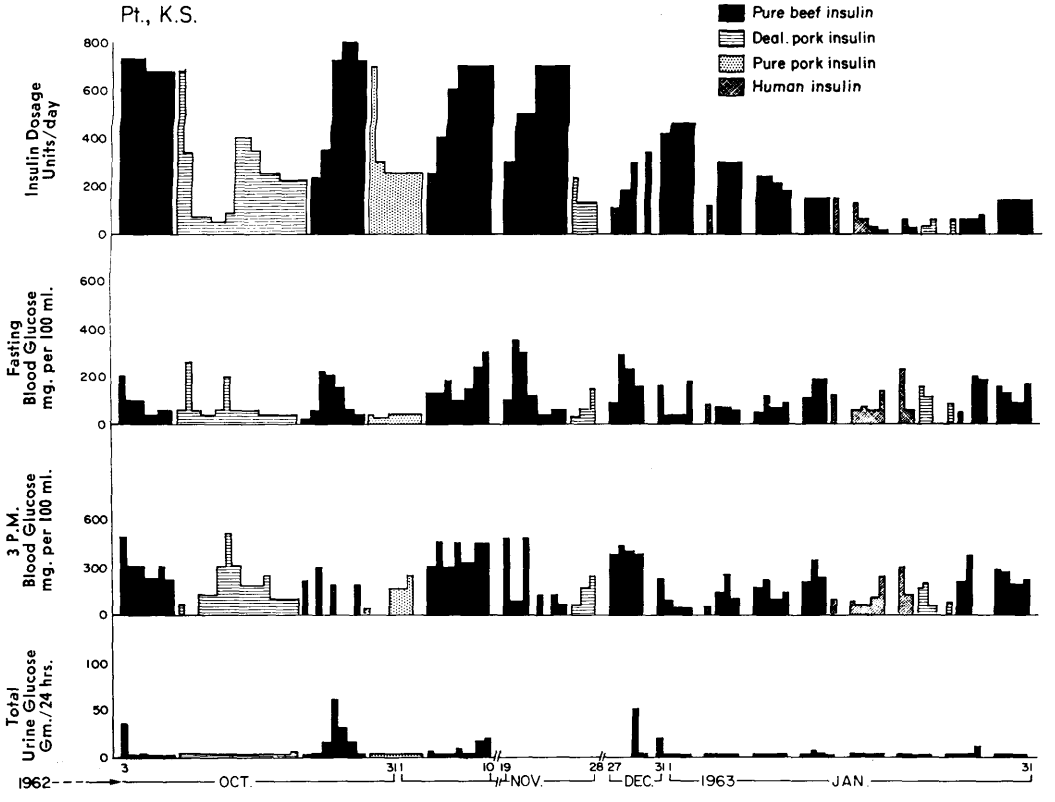

FIG. 6. K.S. is a fifty-two-year-old colored woman, ketoacidosis prone, who had been diabetic for four years and insulin resistant for seventeen months when these studies were performed. She was not obese and her serum insulin binding capacity for bovine insulin was 360 units per liter. The patient had previously required as much as 1,200 units of insulin per day during the year preceding this evaluation. She was regulated on 700 units of crystalline bovine insulin per day, and then the same dose of dealaninated porcine insulin was substituted. She quickly became hypoglycemic, and the dealaninated porcine was decreased to 70 units per day. Postprandial (3:00 p.m.) hyperglycemia occurred over a five-day period and the dose was increased to 400 units and subsequently reduced to 220 units per day. Bovine insulin was then reinstituted and the requirement increased again to 700 units per day. Porcine insulin then produced comparable results to the dealaninated porcine. Subsequent trials with dealaninated pork and human insulin consistently revealed them to be more effective than bovine insulin.

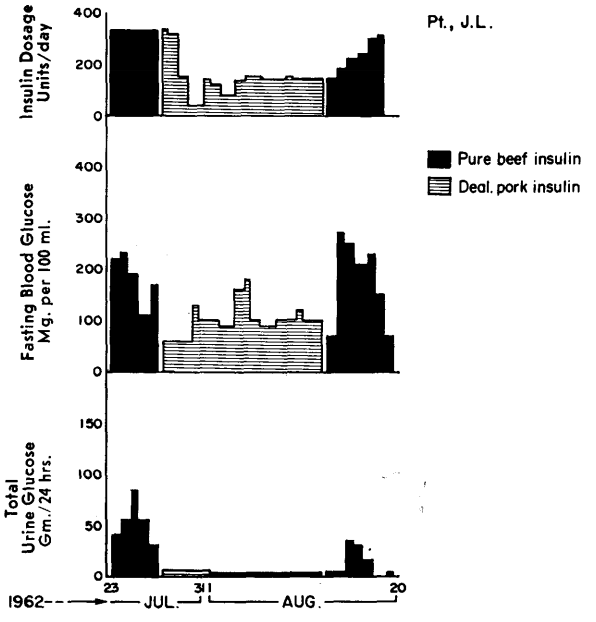

FIG. 7. J.L. is a fifty-seven-year-old white man with diabetes mellitus of nine years' duration who two and one-half years prior to admission developed an insulin requirement of greater than 200 units per day. He did not respond to bovine, porcine or dealaninated porcine insulin during intravenous insulin tolerance testing but responded rather dramatically to dealaninated porcine insulin during the clinical trial as shown in this figure. He was stabilized on 330 units of bovine insulin; however, when the same dose of dealaninated porcine was substituted profound hypoglycemia resulted, necessitating a 50 per cent reduction in the total insulin dosage.

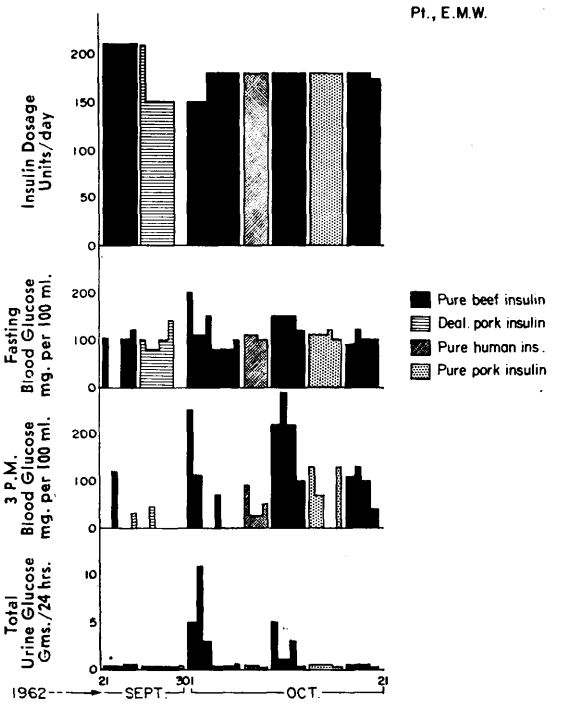

FIG. 8. E.M.W. is a forty-five-year-old moderately obese colored woman who has had diabetes mellitus for twenty years and insulin resistance for ten months prior to these studies. She was regulated with 210 units of crystalline bovine insulin per day and then given a trial of dealaninated porcine insulin. The latter was decreased to 150 units without causing a significant change in her diabetic regulation. She was subsequently changed to bovine insulin again and was well regulated on 180 units per day. Clinical trials with the same dose of human insulin and pork insulin, each preceded by a period of treatment with bovine insulin, revealed no increased sensitivity to either of these insulins. The decreased insulin requirement produced by the 900 calorie diet described in the text is not shown here.

sulin following the dietary restriction in patient R.H. is depicted in figure 5.

After noting the increased responsiveness of patients K.S., J.L., and M.H. to dealaninated porcine insulin, we then tested the binding capacity of their sera for dealaninated porcine and porcine insulin. The results are as follows:

1. K.S.—Bovine 360, Porcine 428, and dealaninated porcine 500 units per liter.
2. J.L.—Bovine 218, Porcine 330, and dealaninated porcine 315 units per liter.
3. M.H.—Bovine 55, Porcine 110, and dealaninated porcine 200 units per liter.

Thus it becomes apparent that from a quantitative standpoint, failure of the antibody to bind the dealaninated porcine and porcine insulin was not the explanation for the increased sensitivity to these preparations.

The possibility that serum from the three responders antagonized porcine or dealaninated porcine less than bovine was then explored, utilizing the same in vitro system as had been employed with the bovine insulin. The results are expressed as activity of two milliunits of the insulin in question, in the patients' serum, as compared to two milliunits of the same insulin in buffer, considering the latter to be 100 per cent activity. Results of this evaluation are as follows:

K.S.—Bovine 41 per cent, Porcine 39 per cent, and dealaninated porcine 42 per cent.

J.L.—Bovine 43 per cent, Porcine 41 per cent, and dealaninated porcine 15 per cent.

M.H.—Bovine 70 per cent, Porcine 77 per cent, and dealaninated porcine 149 per cent.

With the exception of a greater antagonism of the dealaninated porcine insulin by M.H. and a lesser antagonism of dealaninated porcine insulin by J.L., there is no apparent evidence of less circulating antagonism to dealaninated porcine or to porcine than to bovine insulin.

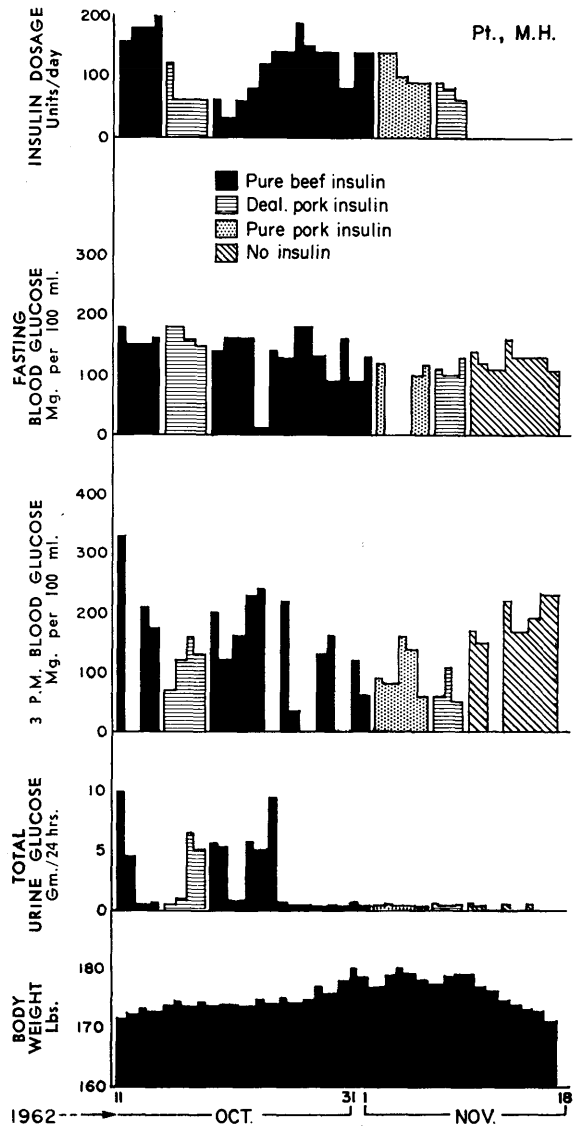

FIG. 9. M.H. is a sixty-four-year-old moderately obese colored woman who had diabetes mellitus for five years and insulin resistance for three years prior to this admission. She was stabilized with 180 units of crystalline bovine insulin per day. When dealaninated porcine insulin was substituted for this it was necessary to reduce the dose to 60 units per day in order to prevent hypoglycemia. A return to bovine insulin resulted in hyperglycemia, especially in the afternoon, and the dose was increased to 140 units per day. The same degree of regulation or perhaps even better was then obtained with 90 units of porcine or with 90 units of dealaninated porcine insulin. The dealaninated porcine insulin was then decreased and finally discontinued in mid-November. Her fasting blood glucose remained normal after all insulin was discontinued, however, postprandial hyperglycemia (3:00 p.m.) recurred.

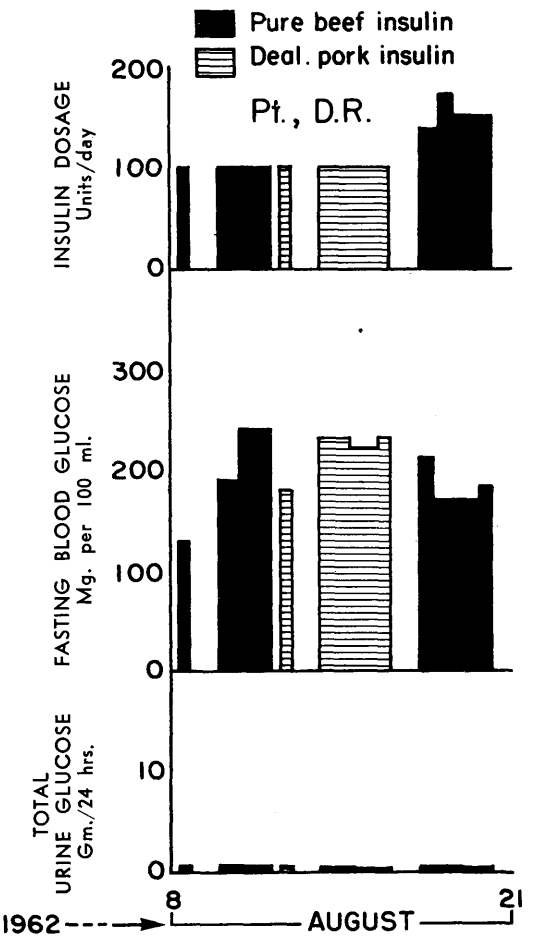

FIG. 10. D.R. is a sixty-four-year-old markedly obese white woman who developed diabetes mellitus twelve years, and insulin resistance two years prior to these studies. Although she had been taking 200 to 300 units of insulin per day for two years prior to hospital admission, the dose was decreased to 120 units of crystalline bovine insulin per day after she was stabilized on a 1500 calorie diet in the hospital. Substitution of a similar dose of dealaninated porcine insulin produced no change in the state of diabetic regulation. Subsequently returning to bovine and increasing the dose to 150 units per day produced a better degree of diabetic control.

Comparative effectiveness of bovine, porcine and human insulin on the conversion of labeled glucose to glycogen in these three patients' adipose tissue revealed the following:

K.S.—Bovine 16,210 CPM* per gram fat
Porcine 17,210 CPM per gram fat
Human 11,777 CPM per gram fat

J.L.—Bovine 14,500 CPM per gram fat
Porcine 12,000 CPM per gram fat
Human 6,900 CPM per gram fat

M.H.—Bovine 30,263 CPM per gram fat
Porcine 31,538 CPM per gram fat
Human 27,437 CPM per gram fat

*CPM = Counts per minute

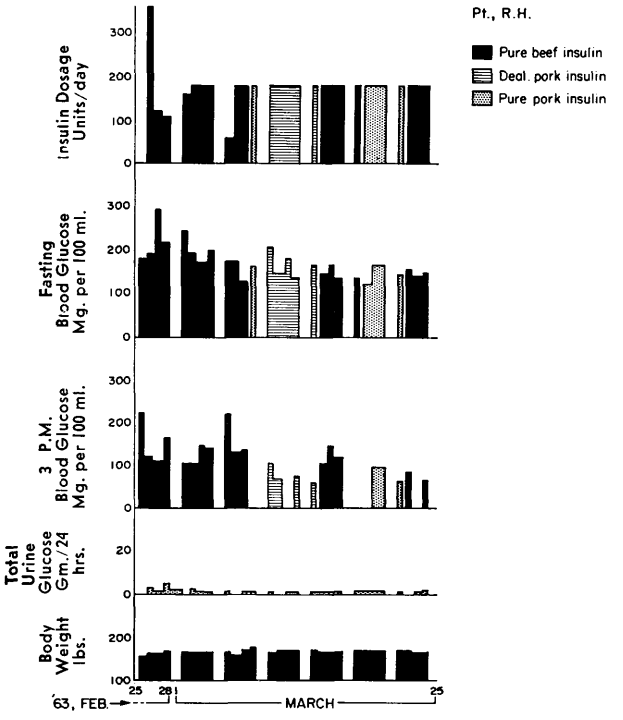

FIG. 11. R.H. is a sixty-three-year-old obese white woman who had been diabetic for ten years and insulin-resistant for one year prior to hospital admission. This patient was regulated with 180 units of crystalline bovine insulin. She was then given a short trial of porcine insulin followed by a longer trial of dealaninated porcine insulin and subsequently a second trial of porcine insulin without any significant change in her state of diabetic regulation other than a slight decrease in her 3:00 p.m. blood glucose.

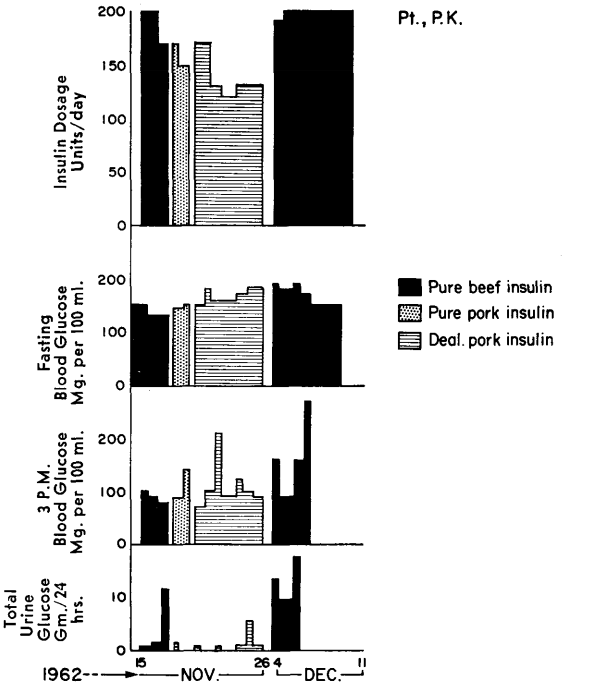

FIG. 12. P.K. is a fifty-five-year-old white woman with acromegaly and diabetes mellitus of two years' duration. The patient was regulated with 170 units of crystalline bovine insulin. The same dose of dealaninated porcine was then substituted and subsequently reduced to 150 units per day. Blood glucose levels increased when the dose was reduced. She was then placed on 170 units of dealaninated porcine insulin per day. An attempt to reduce the latter again resulted in hyperglycemia. Because of rapidly progressing visual loss the study was terminated, and a total hypophysectomy was performed. She was maintained on 200 units of crystalline bovine insulin per day during the operation and the postoperative period.

Human and porcine insulin were found to be no more effective than bovine insulin in isolated fat tissue from the patients who clinically responded better to insulin of a nonbovine origin.

DISCUSSION

The intravenous insulin tolerance tests did not prove helpful in predicting whether a patient would respond to a given species of insulin in longer clinical trials. A striking example is W.J., (figure 4), who exhibited a good response to porcine insulin during the tolerance tests but in prolonged clinical maintenance trials with this insulin, was comparatively unresponsive.

Although the normal subjects appear to be more responsive to dealaninated porcine insulin than to bovine insulin in the acute insulin tolerance tests, this difference is not very striking and the number of patient trials is too few to warrant conclusions (figure 1). This may have been due to a greater potency of the dealaninated insulin than was estimated by the supplier.

That patients with insulin resistance may respond better to human insulin than to bovine insulin was first demonstrated by Lowell.[2,3] The results of our clinical trials with human insulin confirm those of Lowell in that one of our patients was more sensitive to human than to bovine insulin whereas the second patient was equally unresponsive to insulin from all species tested. The responsive patient was also more sensitive to porcine and to dealaninated porcine than to bovine insulin.

The most consistent point of differential between the insulin-resistant patients who responded better to insulin from a nonbovine source than to bovine is the higher serum insulin binding capacities of the responders.

Dealaninated porcine insulin was not significantly more potent than unmodified porcine insulin in the five insulin-resistant patients who received clinical trials with both, nor in the diabetic patients who received intravenous tolerance tests with both.

Human insulin, dealaninated porcine and unmodified

porcine insulin produced comparable results in the insulin-resistant patient, K.S. (figure 6), who was more sensitive to these than to bovine insulin. Whether dealaninated porcine or human insulin will prove to be effective when porcine is not effective must await further study.

The similar total binding capacity of serum from patients K.S. and J.L. for all types of insulin studied suggests that binding capacity per se is not particularly helpful in predicting clinical responsiveness to insulin from a given animal source. Perhaps this seeming paradox is due to differences in the affinity of the antibody binding sites for the various types of insulin and subsequently to the kinetics of the reaction. Actually, these two patients responded much better to dealaninated porcine insulin than to bovine but had higher binding capacities for the dealaninated porcine than for the bovine variety. Yalow and Berson have shown that the higher the antibody titer the lower is the equilibrium constant for formation of the insulin-antibody complex, and they have suggested that perhaps the production of higher antibody titers is associated with the production of antibodies with a lower affinity for the antigen.[9] Insulin is actually univalent in its reaction with antibody but two different antibody sites are demonstrable in most antisera.[9] Furthermore, in vitro, there are two distinct types of antigen-antibody reactions from the standpoint of the rate of dissociation.[9] In one, the rate is 3 per cent per minute to 25 per cent per minute, and in the other it is 0.01 per cent per minute to 1 per cent per minute.[9] The insulin binding capacity was determined in these studies by an in vitro method involving a four-day equilibration period of antigen and antibody at 4° C. before quantitative measurement is made. It is not surprising that the values obtained do not necessarily correlate exactly with an in vivo reaction that occurs in minutes.

Antigenicity of insulin depends, at least partially, upon some distinction in the sequence of amino acids in positions 8-10 of the A chain and possibly on the terminal amino acid of the B chain.[8,10] These differences are not, however, the sole basis for immunologic individuality. Yalow and Berson[8] and Prout[10] have suggested that the structural integrity and arrangement (steric configuration) of the insulin molecule are also important factors.

Since the antagonist studies were conducted in the adipose tissue assay system it is not surprising that the antagonism of added crystalline insulin correlated well with the antibody binding capacities of the respective sera. Insulin antibodies are the only known antagonists found in serum that affect the response of adipose tissue to insulin. We have no ready explanation for the rather marked antagonism of added bovine insulin by the serum of E.M.W., whose insulin binding capacity was low. Many serum factors including the synalbumin of Vallance-Owen[11] antagonize the action of insulin on the diaphragm. Whether the serum of the patients in this study contained antagonists other than insulin antibodies was not determined. The low antibody titers found in the sera of the obese insulin-resistant patients are unexplained but suggest that factors other than specific insulin antibodies are responsible for insulin resistance in these patients.

The diminished responsiveness to insulin of adipose tissue from two of the three obese, insulin-resistant patients as compared to adipose tissue taken from thin, insulin resistant patients is not readily explained. It may be that this tissue is over-insulinized as suggested by Rabinowitz[12] and therefore cannot respond to the further addition of insulin. Perhaps the marked distortion of intracellular constituents by the large intracellular fat globules interferes with normal cell function and responsiveness to insulin. In any case, the increased responsiveness of the obese patient to insulin following weight loss suggests that the adipose tissue may play a part in this type of insulin resistance (figures 5 and 11).

The obese patients do not have adequate antibody titers to account for their large insulin requirement. In the patients with serum insulin binding capacities of 200 units per liter, as much as 350 units of insulin per day could possibly be destroyed by the sequestration of the antigen-antibody complex by the normal immunological disposal mechanisms inherent in the body.[8] This supposition was derived by Yalow[8] and based on the thesis that insulin antibody is distributed in a space equivalent to approximately seven liters of plasma and that soluble antigen-antibody complexes have an average half-life of two to three days. Thus, it would not be difficult to assume that the binding of insulin by antibody is a major causative factor of the resistance in patients with high insulin binding capacities. Since this cannot account, however, for some of the described patients with insulin resistance, other factors such as local tissue resistance must also be considered.

SUMMARIO IN INTERLINGUA

Resistentia Contra Insulina: Responsa a Insulinas de Varie Origines—Animal e Human

Esseva studiate le comparative utilitate, in le tractamento de resistentia contra insulina, de insulina crystallin human, porcin, dealaninate porcin, e bovin.

Un serie de subjectos normal, de diabeticos sensibile pro insulin, e de diabeticos resistente contra insulina recipeva varie typos de insulina in acute tests de tolerantia intravenose. Le patientes resistente contra insulina esseva multo minus responsive a omne le formas de insulina in iste tests que le normales e le penicillino-sensibiles. Esseva trovate nulle correlation positive inter le responsivitate a un typo particular de insulina in un acute test de tolerantia e le responsivitate durante un prolongate essayo clinic.

Septe patientes con resistentia contra insulina recipeva essayos clinic con al minus un insulina altere que insulina bovin. Esseva trovate que tres de iste patientes esseva plus sensibile pro insulinas human, porcin, o dealaninate porcin que pro insulina bovin. Le capacitates insulino-ligatori de seros ab le responsive patientes esseva plus alte que illos de seros ab non-responsivos. Le capacitates insulino-ligatori de seros ab responsivos esseva equal o superior pro le insulina al qual le patientes responseva in comparation illos pro insulina bovin. Sero ab le responsivos antagonisava generalmente addite insulina porcin, dealaninate porcin, e bovin al mesme grado in vitro. Tissu adipose ab le responsivos non esseva plus sensibile pro insulina human o porcin que pro insulina bovin in vitro.

Le augmentate sensibilitate de iste patientes pro insulina ab un fonte nonbovin es possibilemente le resultato de un differentia de cinetica in le reaction antigeno-anticorpore inter le ambiente in vivo e illo in vitro.

Patientes con obesitate e resistentia contra insulina respondeva a un dieta de reduction de peso. Tres de quatro tal patientes qui recipeva dietas diurne de 600 a 900 calorias perdeva lor requirimento pro insulina intra un periodo de inter quatro e sex septimanas.

ACKNOWLEDGMENT

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