The Effect of Insulin-binding Serum Globulin on Insulin Requirement

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An insulin-binding globulin has been demonstrated in the serum of most patients previously treated with insulin.[1] Although this insulin-binding globulin may alter the biologic activity of insulin,[2-5] it has not been possible to demonstrate a direct correlation between the insulin requirement of diabetic patients and the insulin-binding capacity of their sera.[2,4,5] Thus, the clinical implications of the insulin-binding globulin are not apparent.

Characteristics of the binding globulin other than its total binding capacity for insulin may account for the lack of correlation with insulin requirements previously reported. Firstly, the reversibility and degree of dissociation between the bound and unbound insulin may influence the clinical response to insulin. Secondly, the extent to which the binding globulin has been saturated may alter insulin availability. Thirdly, the rate at which the binding globulin is produced may change during the course of diabetes mellitus. Clinical observations that can be related to alterations in these characteristics of the insulin-binding globulin will be described.

METHODS

Insulin labeled with iodine 131* (insulin I^{131}) was given to patients by intravenous injection in dosages varying from 0.3 to 3.6 U of insulin and 75 to 150 microcuries of $iodine^{131}$. Venous blood samples were obtained following injection at the intervals shown in the experimental detail. Serum was partitioned by precipitation with tricholoroacetic acid (TCA) into TCA soluble and TCA insoluble portions on 0.1 ml. of each serum sample. The intact insulin contained in each serum was separated by electrophoretic-convection flow on paper.[1,6] All analyses were carried out in duplicate. Strips used in electrophoresis were stained with 5 per cent bromphenol blue to differentiate the protein fractions. All samples were counted in a shielded well-type scintillation counter connected through a linear pre-amplifier to a scaler. The monitoring apparatus had a sensitivity of 10,000 counts per minute per 0.01 millicuries of radioiodine over a background of 180 counts per minute. Counts were carried out to sufficient numbers as to reduce the error of counting to less than 3 per cent. The quantity of radioactivity injected into each patient was determined by the "serum phantom" technic previously described.[7]

Propylthiouracil, 100 mg., and potassium iodide, 0.5 gm., were given to each subject every eight hours for two days prior to the study and continued for five days after injection of the radioactive material to prevent thyroidal accumulation of $iodine^{131}$ and to promote excretion of the degradation products of the radioinsulin. Studies of excretion revealed that approximately 85 per cent of the injected radioactivity could be recovered in the urine in seventy-two hours.

In these studies the radioactivity in each of the partitions has been expressed as the percentile of the injected material contained in the total body plasma at each time interval. Plasma volume was assumed to be 5 per cent of the body weight. This method of calculation has been used in order to allow correlation in studies of different subjects. The iodine space, previously determined as being approximately 25 per cent of total body weight at equilibrium,[8] was used to calculate the free $iodine^{131}$ contained in the body at specific times. The sum of the $iodine^{131}$ excreted in the urine and the $iodine^{131}$ contained in the iodine space is a measure of the degradation of the injected radioinsulin for any given time interval.

RESULTS

The clinical characteristics of the patients selected for study are shown in table 1. Patients I, II, III, who had diabetes mellitus, had optimal insulin dosages of 190, 140 and 80 units per day respectively. At the time of the initial study they were on inadequate quantities of insulin and under poor diabetic control. The fourth patient, also a diabetic, had received insulin for twenty

* Obtained from Abbott Laboratories.

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TABLE 1

Characteristics of patients studied

No.	Patient	Age	Sex	Race	Duration of insulin therapy (yr.)	Insulin dose at test (U./day)	Optimal insulin (U./day)	Clinical status at test
I	W.S.	34	M	N	1.8	45	190	Poor control
II	E.S.	60	F	N	6.0	90	140	Poor control
III	B.B.	40	F	N	3.0	60	80	Poor control
IV	B.H.	39	M	N	20.0	32	32	Brittle, early onset
V	A.M.	74	M	N	0	0	10	Mild diabetes, no insulin
VI	M.V.	34	F	N	0	40	40	Mild diabetes, insulin, four days
VII	J.D.	50	M	W	0	0	0	No diabetes, functional hypoglycemia

years and could be described as insulin sensitive; he had wide variations in the level of blood glucose typical of the "brittle" diabetic. Two patients, V and VI, had mild diabetes of several years' duration; patient V had not received insulin, and patient VI had received insulin for only four days. Patient VII, without diabetes mellitus, had been hospitalized for hypoglycemia as a consequence of previous gastric surgery.

These patients were given radioinsulin by intravenous injection and the percentage of the injected insulin that is contained in the total body plasma of each patient at intervals is shown in figure 1. These patients retained from 79 to 3 per cent of the radioinsulin at 120 minutes following injection. The percentage of the injected insulin that is retained by the insulin treated patients (I-IV) is in the same order as their optimal insulin requirements. Patients not previously treated with insulin retained little radioinsulin in their serum after 120 minutes.

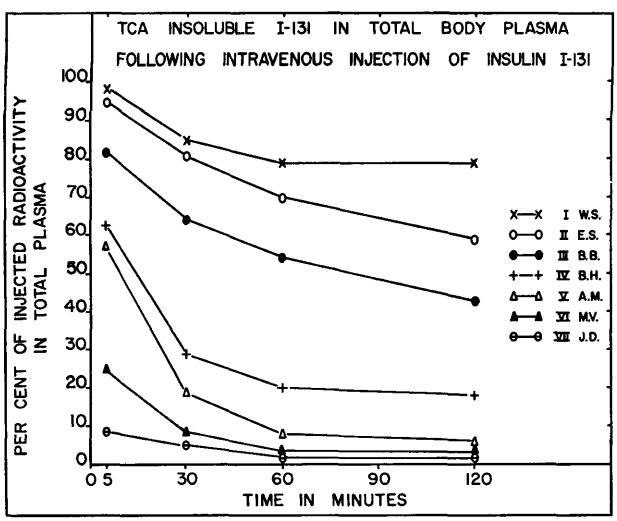

FIG. 1. The per cent of injected insulin I^{131} contained in the total body plasma as the TCA insoluble I^{131} has been determined in each patient. Compare with table 1 for the clinical characteristics of each patient.

Insulin bound to the insulin-binding protein is protected from degradation as shown in table 2. There is an inverse relationship between insulin retention and insulin degradation. The patients showing the highest retention of insulin in the total body plasma show the least degradation of insulin at sixty and 120 minutes following injection. The patients not previously treated with insulin degrade the injected insulin rapidly.

TABLE 2

Correlation between the radioactivity in the TCA precipitate and degradation of insulin

Patient		Time (in minutes) after intravenous insulin I^{131}			
		Per cent in TCA precipitate		Per cent of insulin degradation	
		60 min.	120 min.	60 min.	120 min.
I	W.S.	79	79	9	14
II	E.S.	70	59	20	27
III	B.B.	54	43	23	32
IV	B.H.	20	17	45	50
V	A.M.	9	8	56	—
VI	M.V.	4	3	58	62
VII	J.D.	4	3	67	—

It seemed likely that an increased proportion of injected insulin would remain unbound and become available for peripheral use if it were possible to sat-

urate the insulin-binding globulin. In figure 2 is shown the amount of radioactivity contained in the body plasma on three occasions in Patient I, W.S., while receiving 45, 90 or 190 units, respectively, of insulin by daily injection. Both the total insulin, as the TCA precipitate, and the free insulin, separated promptly by convection flow, are shown. At the time of the first test, the patient was receiving 45 units of insulin by daily injection and he retained 79 per cent of the injected radioinsulin at sixty and 120 minutes. At the time of the second test, eight days later, his insulin had been advanced by increments to 90 units per day and he retained 71 and 60 per cent of the radioinsulin at sixty and 120 minutes, respectively. The third test was completed ten days following the second, after the optimal dosage of insulin had been established at 190 units per day. On this occasion he retained only 48 and 43 per cent of the radioinsulin at sixty and 120 minutes. The amount of injected insulin remaining in the unbound form at five minutes after injection increased from 2.2 per cent in test I to 11.1 per cent in test III, as more of the binding protein became saturated with the insulin given by daily injection. An inverse relationship between the binding and degradation of insulin was also noted in these experiments. Degradation of the radioinsulin at 120 minutes following injection increased from 14 to 22 and 33 per cent, respectively, in the three experiments.

From the results shown in figure 2, it is clear that some insulin remains in the free form despite the presence of the insulin-binding globulin. Since free insulin is degraded quickly in vivo,[7] this suggests that some degree of dissociation between the bound and unbound insulin occurred in these patients, as has been shown previously in vitro.[9] The degree of dissociation can be expressed as the percentage of the total insulin that is found in the unbound form. Measurements of total insulin and free insulin were made on two occasions in Patient II, E.S., and in Patient III, B.B., without insulin loading. The results of these experiments and of the previous studies in Patient I, W.S., are shown in table 3. A relatively constant portion of the total insulin is in the free form throughout the period of study in patients E.S. and B.B. and in tests I and II of W.S. This is consistent with the hypothesis that there is a dissociation constant between the bound and unbound insulin by which insulin becomes available for peripheral use. The proportion of total insulin in the unbound form was greatly increased by the saturation of the binding globulin in Patient W.S. (test III).

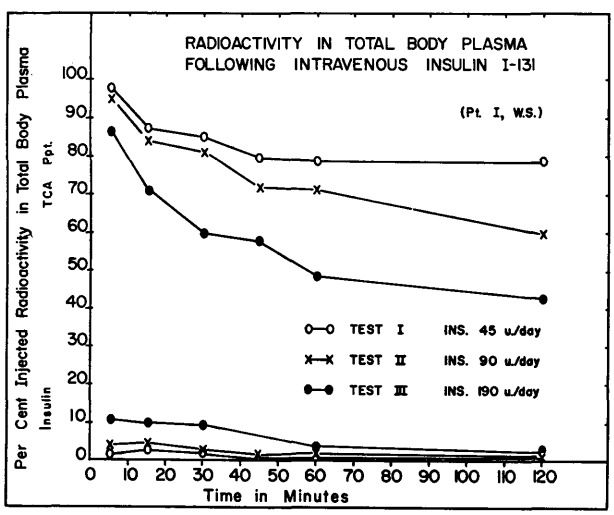

FIG. 2. The per cent of injected insulin I^{131} contained in total body plasma has been measured as the bound insulin (TCA precipitate) and unbound insulin (separated by electrophoresis). Results in one individual are shown at three dosages of insulin. Less insulin is bound and more insulin remains in the unbound form as the insulin-binding globulin becomes saturated by the increased insulin dosage.

TABLE 3

The per cent of total insulin found in unbound form in serum obtained at intervals after intravenous administration of insulin I^{131}

Patient	Test	Minutes after insulin I^{131}			
		5	30	60	120
II E.S.	I	3.3	2.7	2.3	2.5
	II	2.2	2.4	2.2	2.2
III B.B.	I	4.4	3.9	3.2	4.1
I W.S.	I	2.1	1.2	0.9	0.9
	II	3.3	2.2	1.7	1.3
	III	17.6	13.0	5.4	3.4

Although raising the dosage of insulin in patients whose serum contained large quantities of insulin-binding globulin allows a larger proportion of insulin to become available for peripheral use, increasing the dosage may also act as an added stimulus to further production of the insulin-binding globulin. The sera of patients previously treated with insulin were diluted serially with isotonic saline. Each serum dilution was placed with unlabeled insulin in combination with insulin I^{131} in a total concentration of 0.2 units of insulin per ml. The mixture of serum and insulin was allowed to equilibrate over a sixty-minute period. The unbound and bound insulin were separated in each dilution by means of the electrophoretic-convection technic and the radioactivity in each portion was determined as previously stated. The insulin-binding protein complex travels with the gamma globulin when separated by this

technic[1] although it may move more rapidly in other systems.[10] The results were expressed as the percentile of the total insulin I^{131} bound to the serum globulin. The serum of each patient was tested again for the insulin-binding capacity after one to three months on their optimal insulin dosages. The results of the first test are shown in figure 3. The serum showing the highest insulin-binding in all dilutions was obtained from the patient requiring 190 units of insulin per day (I, W.S.). The least insulin-binding was obtained in the serum of Patient IV, B.H. Patients II, E.S., and III, B.B., had binding capacities that were intermediate between these two parameters.

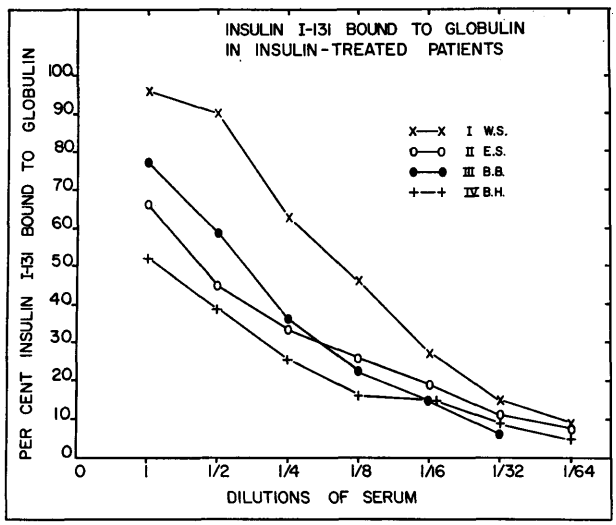

FIG. 3. The per cent of insulin I^{131} bound to globulin in serial dilutions of the sera of insulin treated patients. The insulin-binding properties shown here, in vitro, are of the same order as the insulin I^{131} retention in total body plasma shown in figure 1.

The insulin dosage of Patient B.H. remained unchanged and no change in the insulin-binding globulin was found after three months of continued insulin therapy. An increase in the amount of insulin-binding globulin was noted, however, in patients E.S. and B.B., after three months and one month, respectively, on the increased dosage of insulin (figure 4). The results of the tests on Patient I are shown in figure 5. There was no change in the quantity of insulin-binding globulin before and two months after the institution of the optimal insulin dosage. Methyl prednisolone (Medrol) therapy, 16 mg. per day, was instituted for one month prior to a third test. As shown in figure 5, there was a decrease in the insulin-binding globulin in all dilutions following institution of steroid therapy. The patient remained well controlled throughout the initial period of adrenal steroid therapy. Subsequently, while

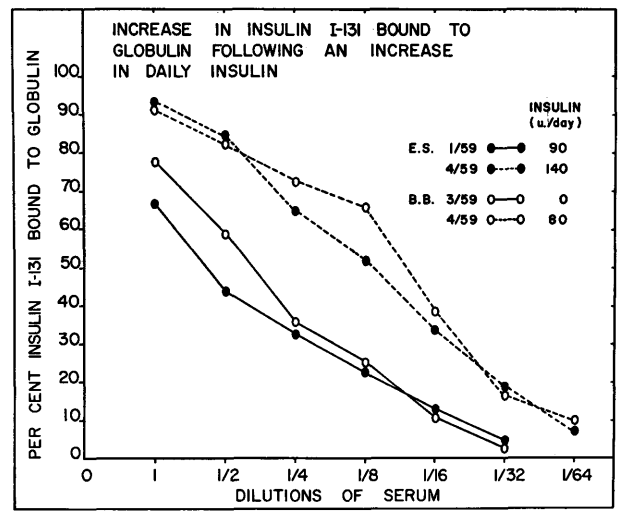

FIG. 4. The per cent of insulin I^{131} bound to globulin in serial dilution of the sera of patients II, E.S., and III, B.B., before, and after the increase in daily insulin dosage. There is an increase in the total binding capacity of both sera following the change in insulin therapy.

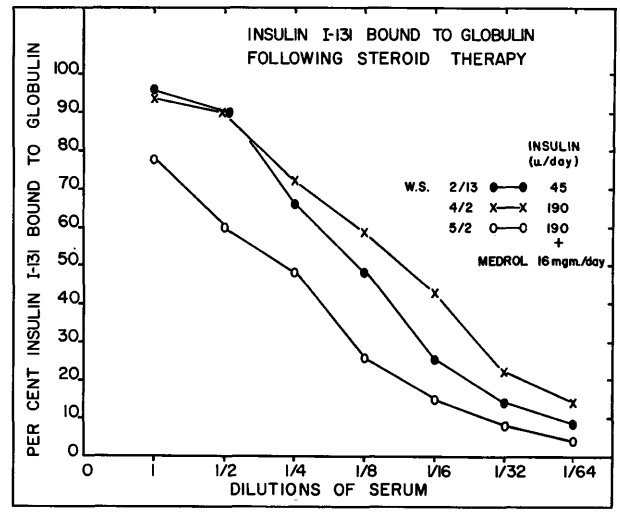

FIG. 5. The per cent of insulin I^{131} bound to globulin in serial dilutions of the sera of patient I, W.S. There was no change in the total binding capacity after approximately two months on the increased dosage of daily insulin. The binding capacity was decreased one month following the institution of methyl prednisolone (Medrol), 16 mg./day.

continuing on this therapy, his insulin dosage was reduced to 160 units per day following several mild episodes of sweating. These were interpreted as being due to hypoglycemia although blood sugar determinations were not obtained at the times of the symptoms.

DISCUSSION

Previous investigators have shown a good correlation, in general, between the insulin-binding and insulin-

nhibiting effects of sera of patients previously treated with insulin.[2,4,11] Kalant and his associates[5] reported hat patients previously treated with insulin for six nonths or longer, had a decrease in the effect of in-ulin during a glucose-insulin tolerance test. Failure to ind a good correlation between the daily insulin re-uirements of insulin-treated patients and the insulin-inding capacity of their sera has cast doubt, however, n the clinical significance of insulin-binding globu-in.[2,4,5] Many of the patients studied for this possible orrelation had complications of their illness that would e expected to raise insulin requirements without nec-ssarily affecting the binding capacity. This possibility annot be excluded from studies in which the clinical letails of the patients have been omitted. In addition, insulin antagonists of other types may possibly obscure he relationship.[11]

The studies on the effect of the saturation of insulin-inding globulin by increased dosages of daily insulin eported here, illustrate a further difficulty in demon-trating a *direct* correlation between insulin dosage and etention of radioinsulin. It was shown that as the in-ulin dosage is increased, insulin-binding globulin be-omes saturated and the percentage of insulin retained in the body plasma decreases. The degree of saturation of the binding globulin appears to be related to the atio between the insulin administered and the opti-mal dosage of insulin required. The ratio, in percentage, between the administered and optimal dosages of insulin in each patient studied has been tabulated in ascend-ing order and correlated with retention and degrada-tion of the radioinsulin at sixty and 120 minutes (table 4). When considered in this way, it can be seen that the percentage of radioinsulin degraded varies directly and the percentage of insulin retained varies inversely with the percentage of the optimal insulin dosage administered. Thus, it appears that the degree of saturation of the binding globulin rather than the insulin dosage is the crucial factor in the degree of retention or degradation of the radioinsulin.

Within the limitations of the procedure, it was possible to demonstrate that a relatively constant proportion of the injected insulin is present in the unbound form in patients with large quantities of binding globulin. The three studies in Patient I, W.S., show that more insulin is made available for peripheral use by the saturation of the binding globulin. Raising the daily dosage of the insulin in patients with insulin-binding globulin, increases the absolute quantity of available insulin, firstly, by a constant proportion of the increased dosage and secondly, as the binding globulin becomes saturated, by an increased proportion of the total insulin.

Patient IV, B.H., had early onset diabetes of the "brittle" type and had been treated with insulin for twenty years. This patient had less insulin-binding globulin than the other insulin-treated patients. There was no evidence that the total insulin-binding globulin of his serum changed over the period of several months. During this period, he continued to have wide fluctuations in the level of the blood glucose.

It is possible that the presence or absence of a large plasma insulin "depot" in the form of insulin reversibly bound to globulin may be the fundamental difference between "brittle" patients and patients less sensitive to insulin changes. The latter group of patients may be sustained between injections of exogenous insulin either by small amounts of endogenous insulin or by the dissociation of free insulin from the insulin-binding protein. Patients with "brittle" diabetes, on the other hand, are lacking in endogenous insulin stores,[12,13] and may lack the sustained supply of insulin afforded by dissociation from the insulin-binding globulin. This interesting possibility requires further study.

There is some question as to whether or not estimates of the total quantity of insulin-binding globulin in sera obtained at different times and under different circumstances are comparable. On the basis of the dissociation of the bound and unbound insulin in vitro,[9] it has been assumed that the radioinsulin serves as an accurate measure of the total binding capacity of the serum providing sufficient time is allowed to achieve a steady state after mixing of the insulin and serum. Berson and Yalow[9] have reported that a rapidly dissociating and a slowly dissociating insulin-binding com-

TABLE 4

Comparison of degree of saturation of insulin-binding globulin with degradation and retention of radioinsulin in vivo

Patient	Test	Per cent saturation*	Per cent degradation 60 min.	Per cent degradation 120 min.	Per cent retention 60 min.	Per cent retention 120 min.
I W.S.	I	24	9	14	79	79
I W.S.	II	47	14	20	72	63
II E.S.	—	64	20	27	70	59
III B.B.	—	75	23	32	54	43
I W.S.	III	100	26	31	50	43
IV B.H.	—	100	45	50	20	17

*Per cent saturation $= \frac{\text{Insulin dosage at test}}{\text{Optimal insulin dosage}} \times 100$

See text for discussion.

plex can be demonstrated in the sera of insulin treated patients. These insulin-binding globulins are possibly related to the administration of insulins from two different animal species. A number of dissociation curves between the two extremes are shown, however, and these curves would also be consistent with the progressive dissociation of an insulin-protein complex that becomes larger with time. Dilution studies of the sera would not conclusively differentiate between these two possibilities.[9] In either case, it is questionable whether a tightly bound single complex or a large aggregate would be labeled by the radioinsulin added to the serum. Studies of the serum of patient I, W.S., before and after insulin loading, show no change in the binding capacity of the serum. This is in agreement with the assumption that the total binding capacity of the serum, rather than the unsaturated binding sites, are measured by the technics used.

High levels of binding globulin may not be accurately measured by the use of a single ratio between the insulin and the binding globulin. Under these circumstances, it will not be possible to differentiate between any of the sera that bind most of the available insulin. By using serial dilutions of the sera, with a constant quantity of insulin labeled with I^{131}, however, measurement of high levels of the binding globulin is possible. This is illustrated by the studies on the sera of Patient W.S. There was little change in the quantity of insulin-binding globulin following the increase in the daily dosage of insulin. After the addition of corticoid therapy, there was a reduction in the total binding capacity from approximately 400 units of insulin to approximately 160 units of insulin per liter of body plasma. Subsequently, as the corticoid was continued, there was a modest reduction in the daily insulin requirement.

Field[4] has reported the effect of ACTH on the insulin requirement of a patient with chronic insulin resistance. The daily insulin dosage was 375 units before and 30 units after ACTH therapy. The ability of one milliliter of this patient's undiluted serum to bind 0.2 units of insulin labeled with I^{131} remained the same before and after the ACTH therapy. However, it is difficult to be certain that total binding capacity was not reduced under these circumstances without further dilution studies.

There was an increase in the total insulin-binding capacity in the sera of patients II, E.S., and III, B.B., after the daily insulin dosage was increased. No change in clinical response to the daily dosage of insulin was noted as a result of the increase in the total binding capacity of their sera. Studies of insulin I^{131} retention and degradation were not carried out at the time of this increase in the binding globulin.

The present studies indicate that there is some correlation between the level of the insulin-binding globulin and the insulin requirements of diabetic patients. Calculation of the total quantity of insulin that may be bound in vivo, on the basis of the total binding capacity in vitro and the use of this figure as a basis for the daily insulin dosage, may not be realistic, however. Thus, Patient W.S. required 190 units of insulin per day but bound approximately 400 units of insulin per liter of plasma. The degree of saturation as the result of previous insulin, the rate of production, or turnover of the binding globulin and the degree of dissociation between unbound and bound insulin appear to influence insulin requirements and the total binding capacity.

SUMMARY

An insulin-binding globulin in the sera of patients previously treated with insulin has been shown to have an effect on their insulin requirements. The amount of insulin that is available for peripheral use is inversely proportional to the quantity of unsaturated binding protein of the serum. Some injected insulin is found in the unbound form despite high levels of insulin-binding globulin, however, presumably by dissociation from the insulin bound to globulin. Increasing the daily dosage of insulin saturates the binding globulin and allows a greater proportion of the injected insulin to remain in the unbound or free form. The increased insulin dosage may stimulate further production of the insulin-binding globulin. Administration of methyl prednisolone decreased the total binding capacity of the serum in one patient tested. Thus, the degree of saturation of the binding globulin as a result of previous insulin therapy, the degree of dissociation between the bound and unbound insulin, the rate of production of insulin-binding globulin in addition to the total insulin-binding capacity of the serum, appear to influence insulin requirements in diabetic patients previously treated with insulin.

SUMMARIO IN INTERLINGUA

Le Effecto de Globulina Insulino-Ligante del Sero Super le Requirimento de Insulina

Esseva monstrate que un globulina insulino-ligante in le seros de patientes previemente tractate con insulina ha un effecto super lor requirimento de insulina. Le quantitate de insulina disponibile pro uso peripheric es inversemente proportional al quantitate de non saturate globulina insulino-ligante in le sero. Tamen, un

certe quantitate del injicite insulina es trovate in forma non-ligate in despecto del presentia de alte nivellos de globulina insulino-ligante, presumitemente in consequentia de dissociation ab le insulina ligate a globulina. Le augmentation del dosage diurne de insulina resulta in le saturation del globulina ligante e permitte a un plus grande proportion del injicite insulina remaner in le forma non-ligate o libere. Le augmentate dosage de insulina pote stimular additionalmente le production de globulina insulino-ligante. In un patiente il esseva trovate que le administration de un steroide adrenal reduceva le total capacitate ligatori del sero. Assi il pare que le requirimento de insulina in diabeticos previemente tractate con insulina depende—a parte le total capacitate insulino-ligante del sero—de tres factores, i.e. (1) le grado de saturation del globulina ligante que es un resultato del previe therapia a insulina, (2) le grado de dissociation inter le ligate e le non ligate insulina, e (3) le production de globulina insulino-ligante.

ACKNOWLEDGMENT

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In 1948, D. E. Green proposed that not only the electron-transfer mechanism but also the mechanisms for citric cycle oxidation, oxidative phosphorylation, and fatty acid oxidation were associated with one and the same particulate complex, which was called the "cyclophorase system." It was postulated that the large number of enzymes and all the auxiliary coenzymes which implemented each of these sequential processes were linked together in a very precise manner within the structural framework of a giant macromolecule. The complex was assumed to be the unit of enzymatic action in the same sense that the polyfunctional pyruvic dehydrogenase has been defined previously as a unit of enzyme action. It is unnecessary in this paper to summarize the various lines of evidence which led to the cyclophorase concept. To be sure, the evidence first presented was more circumstantial than conclusive. It was probably not so much the incomplete nature of the evidence that engendered widespread skepticism as it was the strangeness of the concept and the multiplicity of reactions encompassed by the cyclophorase system. But the groundwork had been laid for the more sympathetic reception which came when Potter and Schneider and, independently, Lehninger and Kennedy established that the mitochondrion did, in fact, carry out precisely the sequences postulated for the cyclophorase system.

From an article by David E. Green and Johan Jarnefelt in *Perspectives in Biology and Medicine,* Vol. 2, page 163, 1959.