

SHORT COMMUNICATIONS

Antibody Studies in Patients on Mixed Bovine/Porcine Insulins

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Summary. A study of two groups of diabetic sera has been carried out with reference to total insulin binding capacity and preferential binding of beef and pork insulin. Group I (25 patients) received insulin containing predominantly beef insulin with a beef/pork ratio of 3 : 1 approximately; Group II (13 patients) received insulin containing at least 97% pork insulin. In Group I the mean total insulin binding capacity was 15.38U/litre and in 21 beef insulin was preferentially bound with reference to pork insulin. Group II was divided into two subgroups: group IIa, 7 patients, the sera bound beef > pork, and the mean insulin binding capacity was 8 U/l. Group IIb, 6 patients, the sera bound pork > beef or showed no preference, and the mean insulin binding capacity was 3.1 U/l. The mean insulin dose in Group IIa was 34.8 and in Group IIb was 24. The mean insulin binding capacity and mean insulin dose were significantly different ($P < 0.05$) between Group IIa and Group IIb. The possible significance of the results is discussed.

Etudes d'anticorps chez des patients traités par un mélange d'insuline bovine et porcine

Résumé. Les auteurs ont effectué une étude de deux groupes de sérums de diabétiques, en ce qui concerne la capacité totale de liaison à l'insuline et la liaison préférentielle avec l'insuline de bœuf et de porc. Le groupe I (25 patients) a reçu principalement de l'insuline de bœuf, avec un rapport bœuf/porc d'environ 3 : 1; le groupe II (13 patients) a reçu de l'insuline contenant au moins 97% d'insuline de porc. Dans le groupe I la capacité moyenne de liaison avec l'insuline était de 15.38 U/litre, et dans 21 cas l'insuline de bœuf était liée de façon préférentielle par rapport à l'insuline de porc. Le groupe II a été divisé en deux sous-groupes: le groupe IIa (7 patients) dont les sérums liaient plus l'insuline de bœuf que celle de porc, la capacité moyenne de liaison avec l'insuline était de 8 U/l. Le groupe IIb (6 patients) dont les sérums liaient l'insuline de porc mieux que celle de bœuf, ou ne montraient pas de préférence, la capacité moyenne de liaison

avec l'insuline était de 3.1 U/l. La dose moyenne d'insuline dans le groupe IIa était de 34.8 et dans le groupe IIb, elle était de 24. La capacité moyenne de liaison avec l'insuline et la dose moyenne d'insuline étaient significativement différentes ($p < 0.05$) entre le groupe IIa et le groupe IIb. L'importance éventuelle de ces résultats est discutée.

Antikörper-Untersuchungen bei Diabetikern nach Behandlung mit Misch-(Rinder/Schweine)-Insulinpräparaten

Zusammenfassung. Bei den Seren von 2 Gruppen insulinbehandelter Diabetiker wurde die totale Bindungskapazität für Rinder- und Schweineinsulin bestimmt. Die Patienten aus Gruppe 1 (25 Personen) waren mit einem Mischinsulin behandelt worden, das Rinder- und Schweineinsulin im Verhältnis von 3 : 1 enthielt. Die Patienten aus Gruppe 2 (13 Personen) wurden mit nahezu reinem Schweineinsulin (97%) behandelt. Die Insulinbindungskapazität betrug in Gruppe 1 durchschnittlich 15.38 E/l. In 21 Fällen wurde Rinderinsulin stärker als Schweineinsulin gebunden. — Die Patienten aus Gruppe 2 wurden in 2 Untergruppen aufgeteilt: In Gruppe 2a (7 Patienten) zeigte das Serum durchschnittlich eine Insulinbindungskapazität von 8 E/l, Rinderinsulin wurde stärker gebunden als Schweineinsulin. Gruppe 2b bestand aus 6 Patienten. Die mittlere Insulinbindungskapazität betrug 3.1 E/l, die Seren zeigten entweder eine stärkere Bindungsfähigkeit für Schweine-Insulin, oder keine Bevorzugung einer Insulinart. Die mittlere Insulintagesdosis betrug in Gruppe 2a 34.8 und in Gruppe 2b 24 E. Ein Vergleich der Werte für die mittlere Insulinbindungskapazität und die mittlere Insulindosis der Gruppen 2a und 2b zeigte einen statistisch gesicherten Unterschied. Die Ergebnisse werden diskutiert.

Key-words: Insulin antibodies, dose, total binding capacity, preferential binding, beef/pork insulin antibodies.

Introduction

In a previous communication it was shown that in a study of diabetic patients treated with an insulin preparation containing from 80 to 90% beef insulin, 38 out of 42 patients developed antibody which bound beef > pork insulin by a factor of 1.8, mean value; range 1.1–5.0 (DEVLIN et al., 1967). It was also shown by relating the alteration in insulin requirement on changing from beef to pork insulin to the study *in vitro*

of preferential binding of species of insulin by antibody that insulin dose is directly related to antibody (DEVLIN et al., 1967).

In order to clarify further this relationship, two further groups of patients have been studied, (i) a group treated with Danish insulins which contained up to 30% porcine insulin (sera obtained through courtesy of Novo Institute, Copenhagen) and (ii) a group treated with Novo semi-lente insulin which is prepared from porcine pancreas. The results of this study are presented in this communication.

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Materials and Methods

Patients. The mean age, sex, duration of diabetes and insulin dose in the two groups of patients studied is set out in Table 1. Total binding capacity was estimated as described by BERSON and YALOW (1959), and preferential beef/pork insulin binding as previously described (DEVLIN and BRIEN, 1965). Beef insulin-¹²⁵I and porcine insulin-¹³¹I were obtained from the Radiochemical Centre, Amersham.

Table 1. Mean age, sex, duration of diabetes and insulin dose in patients

	Mean age, years	Mean duration diabetes (years)	Sex Male	Mean insulin Female dose (units)
Group I.	48.6	21.1	13	38.6
Range	12-76	1/4-44		16-100
Group II.	34.2	0.7	14	29.3
Range	11-70	1/12-21/12		12-60

Group I. Patients treated with insulin up to 30% Porcine.

Group II. Patients treated with insulin semi-lente Novo, 97% Porcine.

Results

Group I. Of the 25 sera in this group (species source, porcine: bovine, 30 : 70 approximately), in 21 there was detectable beef preference by a factor ranging from 1.1 to 2.5, mean 1.41. No particular preference was detected in 3, and in one pork preference was found. The total insulin binding capacity in this group ranged from 1.1 to 41 units/litre, mean 15.38.

Group II. This group is subdivided into two further groups depending on whether insulin therapy lasted for

more than 3 months (group IIa), or less than 3 months (group IIb). Complete data with reference to age, sex, duration of therapy, insulin requirements and total insulin binding capacity are set out in Table 2, for group IIa. It can be seen that the mean insulin dose, and total insulin binding capacity are higher in those patients whose antibodies bind beef > pork insulin. This difference was significant for both insulin dose and total binding capacity ($p < 0.05$). Similar data with reference to group IIb, i.e. on insulin for less than 3

months are set out in Table 3. Antibody titres were low, and absolute determination of preferential binding factors, therefore, for technical reasons was less reliable. The two interesting observations arising from Group IIb were the low antibody levels, and also the change in antibody levels and in species preferential binding in one patient changed to predominantly bovine insulin.

Table 3. Group IIb. Age, sex, insulin dose and total binding capacity in patients on semi-lente (Novo) for less than 3 months

Sera Exhibiting Pork Preference by Antibody					
Age	Sex	Dose	Factor	Total Binding Capacity U/litre	
		Initial	Final		
38	M	24	0	1.2	1.56
63 ^b	M	12	0	1.4 ^a	low
58	F	24	0	1.7 ^a	very low
48	M	20	0	1.2 ^a	low
54 ^b	M	32	0	nil	4.8
70	M	12	0	nil	low

Sera Exhibiting Beef Preference by Antibody					
Age	Sex	Dose	Factor	Total Binding Capacity U/litre	
		Initial	Final		
18	M	52	52	2.1	10.1
63 ^b	M	8	0	2.6	increased

^a Preferential binding factor less reliable with low antibody levels.

^b Changed over to British insulin for 1 month.

Discussion

Although no direct relationship has been shown to exist between antibody levels and insulin dose in a group of non-resistant diabetic patients (MOINAT, 1958; BERSON and YALOW, 1959), and were PALUMBO, MOLNAR and TAUZE (1964) unable to demonstrate any correlation between insulin binding proteins and the increased insulin requirements of menstruation and pregnancy, a definite relationship has been shown to

Table 2. Group IIa. Age, sex, duration of therapy, insulin dose and total insulin binding capacity in patients on semi-lente (Novo) for more than 3 months

Sera Exhibiting beef Preference by Antibody					
Duration (years)	Age	Sex	Dose	Total Binding Capacity U/litre	Factor.
2 1/2	17	M	44	8.65	1.1
5/12	12	M	24	5.8	1.6
9/12	11	F	48	9.9	1.5
1	15	F	32	11.0	1.3
1	12	F	40	4.4	2.8
1 1/2	35	M	32	2.6	1.9
2 1/2	19	F	24	14.0	1.3
Mean:	14/12	17.2	34.8	8.0	1.3

Sera Exhibiting Pork Preference or Nil Preference by Antibody					
Duration (years)	Age	Sex	Dose	Total Binding Capacity U/litre	Factor.
2 1/2	14	M	24	5.9	1.7
1 1/2	18	M	36	4.5	2.0
1 1/2	27	M	24	7.2	1.6
3 1/2		M	20	1.56	1.5
1 1/2	40	F	16	very low	nil
3/12	24	F	24	very low	nil
Mean:	1.2	24.6	24	3.1	2.2

exist in the individual patient from studies with bovine and porcine insulin (DEVLIN et al., 1966). A significant rise in antibody level with increase in insulin requirements was also shown by KÜHNAU et al. (1967). In a previous study it was postulated that the factors relating antibody to insulin dose were threefold (DEVLIN, 1967).

1. Total binding capacity.
2. Equilibrium constants of reaction in patient.

$$\frac{k}{k'} \cdot K = \frac{(\text{antigen : antibody})}{(\text{antigen}) (\text{antibody})}$$
3. Biological requirements.

The level of free hormone, i.e. antigen concentration, is determined by (1) and (2) and the required level is set by (3).

The present study introduces a further aspect of this relationship. Since the introduction of current techniques, using labelled insulin to study antibody related — insulin resistance as in the study of FELDMAN et al. (1963), no case of insulin resistance in which antibodies binding porcine > bovine insulin has been reported. The greater antigenicity of beef insulin compared with porcine insulin in the human subject, is again confirmed by the development of antibodies which bind beef > pork insulin despite the presence of at least 97% porcine insulin in the preparation used (semi-lente, Novo). The corollary of this observation, i.e. that the exhibition of porcine insulin alone should be accompanied by the development of a lower antibody titre and correspondingly lower insulin requirements, receives some confirmation from the two subgroups of Group IIa, i.e. those who develop antibodies binding bovine > porcine and those who develop the reverse or showed no specific preference. It would seem that when the immune mechanism fails to recognize the relatively minor quantity of bovine insulin (3%) in the preparation used, and responds to the porcine insulin only, a significantly lower antibody titre and insulin requirement is found. An alternative explanation for the observation made could be that the study is demonstrating differences in immunologic competence between groups within the same species. Further study with absolutely pure porcine insulin and comparison of the results so obtained with group IIa will answer this question.

It is interesting to note that where there is the development of antibodies specific for bovine insulin, neither the total binding capacity nor the insulin requirement is significantly different despite gross differences in the proportions of bovine/porcine insulin

(*vide* Group I and Group IIa). The differences which were found were not statistically significant. It is more than likely, however, that with a much larger group, statistically significant differences would emerge, in view of the well known relationship between antigen dose and antibody response (STEVENS, 1956). A further point which must be made with reference to this aspect of the study is that Group I were Danish diabetics and Group II were Irish, and cognisance of the well recognized differences of immunologic competence within groups of the same species must be made.

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References

- BERSON, S.A., and R.S. YALOW: Quantitative aspects of the reaction between insulin and insulin antibody. *J. clin. Invest.* **38**, 1996–2016 (1959).
- DEVLIN, J.G., F. BRIEN, and N. STEPHENSON: Relation between antibody and insulin dose. *B.M.J.* **1**, 542–544 (1967).
- — — Effect of alteration of species source of insulin on insulin antibody levels. *Lancet* **1966 II** 883–884.
- — — Relationship between differential antibody binding capacity and clinical requirements of beef and pork insulin. *Metabolism* **14**, 1034–1036 (1965).
- — — Hormones and antibodies. *Irish J. med. Sci.* **397** – 406 (1967).
- FELDMAN, R., G.M. GRODSKY, F.W. KUHOUT, and N.B. MCWILLIAMS: Immunologic studies in a diabetic subject resistant to bovine insulin but sensitive to porcine insulin. *Amer. J. Med.* **35**, 411–417 (1963).
- KÜHNAU Jr. J., u. H.W.I. MEYER: Über die Bedeutung insulinneutralisierender zirkulierender Antikörper für die Insulintherapie bei Diabetes mellitus. *Excerpta medica, International Congress Series No. 104*, p. 77. (1967).
- MOINAT, P.: A quantitative estimation of antibodies to exogenous insulin in diabetic subjects. *Diabetes* **7**, 462–467 (1958).
- PALUMBO, P.J., G.D. MOLNAR, and W.N. TAUXE: Serum protein binding of exogenous insulin in menstruating and pregnant diabetic patients. *Diabetes* **13**, 634–638 (1964).
- STEVENS, K.M.: Some considerations on the antigen dose — antibody response relationship. *J. Immunol.* **76**, 187–191 (1956).

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