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Glomerular Structural Changes in Rabbits on Treatment with Bovine and Porcine Insulin

A Morphometric Analysis

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Summary. Weak nonspecific immunological stimuli can irritate the glomerular mesangium as observed following administration of insulin preparations of varying degrees of purity. In the present study further substances were investigated with regard to this effect. We wished to examine which substances obtained during purification of insulin are mainly responsible for the antigenicity, and whether porcine and bovine MC insulin have the same antigenic properties. Rabbits were treated for up to 90 days with bovine MC insulin, bovine proinsulin, bovine a+b-component, porcine a-component and porcine b-component. The kidneys were analysed morphometrically and antibody titers to bovine insulin, a-component, porcine PP and proinsulin were determined in the various test groups. It was found that bovine MC insulin and porcine MC insulin possess the same immunological activity, i.e. no antibody formation to either of the two insulins was demonstrable. Similarly, there were no differences in the morphometric findings; slight transient mesangial changes were demonstrable after both insulins. However a-component and b-component showed a pronounced immunogenic potency with antibody formation. Marked and partly persisting mesangial alterations were demonstrable, with the antigenicity of the a-component being particularly marked.

The implication of the study is that a "pure" or optimally purified insulin should be used in the therapy of diabetes mellitus.

Key words: Glomerular structure – Morphometry – Monocomponent insulin – Antibody formation – Mesangium.

Introduction

Although there is now no doubt that the renal changes of the diabetic are a consequence of the metabolic disorder, additional slight nonspecific immuno-

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logical reactions can influence the course of diabetic glomerulosclerosis (Bloodworth 1968; Wehner et al. 1978). This is particularly important in diabetes mellitus since mesangial function appears to be disturbed and the uptake and elimination of immune complexes is delayed (Mauer et al. 1975; Wehner et al. 1979). It is evident from earlier investigations (Wehner et al. 1977) in which we have demonstrated that the severity of the glomerular lesions increases with the degree of the antigenicity of insulin preparations, that weak immunological stimuli can also irritate the nondiabetic mesangium. The purpose of the present study was to investigate this finding using further insulin preparations. At the same time we wished to examine whether the antigenicity of bovine and porcine MC insulin differ and which components of the various preparations are mainly responsible for the antigenicity.

Material and Methods

Experimental Animals

A total of 86 mature male (4–5 months old) rabbits (Belgian giants, piebalds) were allocated to the following test groups.

Test Groups

1. Bovine MC Insulin Group. Thirteen animals received 20 units of bovine MC insulin without Freund's adjuvant three times weekly, injected into the cervical region. Four animals were killed after 30 days, five animals after 60 days, and the other four animals after 90 days.

2. Bovine Proinsulin Group. Fifteen animals received 0.5 ml of a bovine proinsulin preparation (1.6 mg/ml) without Freund's adjuvant three times weekly, injected into the cervical region. Five animals were killed after 30, 60 and 90 days.

3. Bovine a+b-Component Group. Thirteen animals received 0.5 ml of a+b-component (1.6 mg/ml, pH 2.8–2.9) without Freund's adjuvant three times weekly, injected subcutaneously into the cervical region. Five animals were killed after 30 and 60 days and the other three animals after 90 days.

4. Porcine a-Component Group. Eighteen animals received 0.5 ml of a-component (1.6 mg/ml, pH 2.8–2.9) without Freund's adjuvant three times weekly, injected into the cervical region. Six animals were killed after 30, 60 and 90 days.

5. Porcine b-Component Group. Eighteen animals received 0.5 ml of b-component (1.6 mg/ml, pH 2.8–2.9) without Freund's adjuvant three times weekly, injected into the cervical region. Six animals were killed after 30, 60 and 90 days.

6. Control Group. Nine untreated animals served as the control group. All animals had free access to a 6% glucose solution (to avoid hypoglycaemic episodes) and to food (Altromin standard feed).

Insulin Preparations

1. Bovine MC Insulin. This is a monocomponent insulin from bovine pancreas in neutral solution. Its potency was 40 units per ml.

2. Proinsulin. This is a proinsulin preparation from bovine pancreas in acid solution. The concentration was 1.6 mg proinsulin per ml.

3. a-Component and b-Component. These are partial fractions which result from preparation of purified crystalline bovine insulin and porcine insulin by gel filtration on Sephadex G 50 and subsequent ion exchange chromatography. a-Component consists of numerous proteins with molecular weights of more than 15,000 (average 25,000) whose structure and origin have not been adequately elucidated. b-Component consists of proteins with a molecular weight of between 6,000 and 12,000 including proinsulin, intermediary and dimer. This solution showed an insulin potency in the experimental animal corresponding to 6–8 units per ml.

Histological Methods

The kidneys of the animals were removed immediately after killing, fixed for 24 h in buffered 4% formalin solution, fixed again for 2 h in buffered 2% osmic acid and embedded in plexiglass. Thin sections 0.1–1 μ in thickness prepared with an ultramicrotom were silver impregnated using the method of Movat (Wehner 1974).

Morphometric Methods

The morphometric analysis (Wehner 1974) was carried out as a blind study, i.e. the investigator was not aware to which group the preparations under evaluation belonged. They were not allocated to the various groups until after evaluation. Twenty different glomeruli were evaluated per animal using a projection microscope (Reichert Visopan). The following variables were determined: 1. Using the point counting technique (objective plane 63/0.8, 160/0.17) with a 6 μ distance between points, the cut glomerular surface (μ^2), the cut mesangial surface (μ^2) and the resulting percentage fraction of the mesangium of the glomerular surface were measured. The glomerular surface corresponds to the surface which is bounded by Bowman's capsule. 2. By direct nuclear count and differentiation (oil immersion 100/1.25) the total number of glomerular cells and that of the various types of glomerular cells (endothelial, epithelial and mesangial cells) and the resulting percentage distribution were estimated. To avoid double counts cells already counted and classified were marked on the glass slide with washable colour signs. 3. The glomerular cell concentration as total cell count/1,000 μ^2 of the glomerular surface. 4. The mesangial cell concentration as mesangial cell count/100 μ^2 of the mesangial surface.

Antibody Determinations

The following antibodies were determined in the serum of the animals which were exsanguinated immediately after killing:

1. Antibodies to bovine insulin.
2. Antibodies to a-component.
3. Antibodies to porcine PP (pancreatic polypeptide).
4. Antibodies to bovine proinsulin.
5. Antibodies to porcine proinsulin.

The mentioned antibody titers were determined by the method of Heding et al. The results must be corrected for the nonspecific fraction of antibody binding (for bovine insulin = 0.05 mμ/ml, for porcine PP = 1.0 ng/ml, for a-component = 25.0 ng/ml).

Statistics

The statistical analysis of the results was carried out with Student's *t*-test. The limit for the error probability chosen was 5% or $2p = 0.05$. Statistical significances are shown in each case in the text.

Results

1. Morphometric Findings

Normal Controls. The following mean values were obtained for the glomeruli of the untreated control animals. With a mean glomerular cut surface of 7972.7 $\mu^2$and a mean total cell count of 48.22 the following distribution resulted: 12.6 (26.2%) epithelial cells, 20.3 (42.01%) endothelial cells and 15.3 (31.7%) mesangial cells. The mesangial surface was 417 μ^2 and the percentage mesangial fraction was 5.23%. The glomerular cell concentration was 6.05/1,000 μ^2 and the mesangial cell concentration was 3.67/100 μ^2 (Table 1).

Bovine MC Insulin Group. No statistically significant differences were found after 30 days' treatment when compared with the control group. The first statistically significant changes were not apparent until after 60 days, namely an increase in the percentage mesangial fraction to 5.5% and a resulting reduction in the mesangial cell concentration to 3.54/100 μ^2. The total cell count was reduced after 90 days to 41.7, the percentage fraction of the epithelial cells had increased to 30.4% and the mesangial cell concentration was further reduced to 3.28/100 μ^2 (Table 2, Fig. 1).

Bovine Proinsulin Group. Significant changes were already demonstrable after 30 days' treatment. These consisted of an increase in the glomerular cell concentration to 7.9 cells/1,000 μ^2 and an increase in the mesangial surface to 5.45%. After 60 days the epithelial cell content was still increased to 29.9%, whereas after 90 days the changes consisted only of a decrease in the mesangial cell concentration to 3.5/100 μ^2 (Table 2).

Bovine a+b-Component Group. After 30 days the mesangial area was significantly increased to 5.23% but it returned to normal again during the further course of the study. After 60 days glomerular surface had increased to 9,993 μ^2 and the mesangial surface to 436 μ^2; correspondingly the mesangial cell concentration was reduced to 3.3 cells/100 μ^2. After 90 days all determined values were within the normal range again (Table 3).

Porcine a-Component Group. The main significant morphometric finding after 90 days was an enlargement of the glomeruli with an increase in the glomerular surface to 9,948 μ^2. The mesangial surface was also significantly enlarged after 60 and 90 days with values of 655 μ^2 and 646 μ^2 respectively. The glomerular cell concentration showed a corresponding decrease when compared with the normal values to 4.32 cells/1,000 μ^2, likewise the mesangial cell concentration to 2.13/100 μ^2 (Table4, Fig. 2).

Porcine b-Component Group. Significant changes were demonstrable already after 60 days' treatment with b-component which corresponded to those observed in the a-component group. The glomerular surface was enlarged to 9,326 μ^2 and the mesangial surface to 659 μ^2. Corresponding to these marked changes

Table 1. Morphometric findings in normal rabbits (mean ± SD)

	Normal ($n=9$)
Total glomerular area (μ^2)	7,972.7 ± 479.53
Total glomerular cells	48.22 ± 2.61
No. of epithelial cells	16.62 ± 0.82
Epithelial cells (%)	26.22 ± 0.91
No. of mesangial cells	15.33 ± 1.3
Mesangial cells (%)	31.75 ± 1.9
No. of endothelial cells	20.32 ± 1.1
Endothelial cells (%)	42.01 ± 1.05
Total mesangial area (μ^2)	417.4 ± 21.05
Mesangial area in % of total glomerular area	5.23 ± 0.23
Total glomerular cells/ 1,000 μ^2 glomerular area	6.05 ± 0.44
Mesangial cells/100 μ^2 mesangial area	3.67 ± 0.41

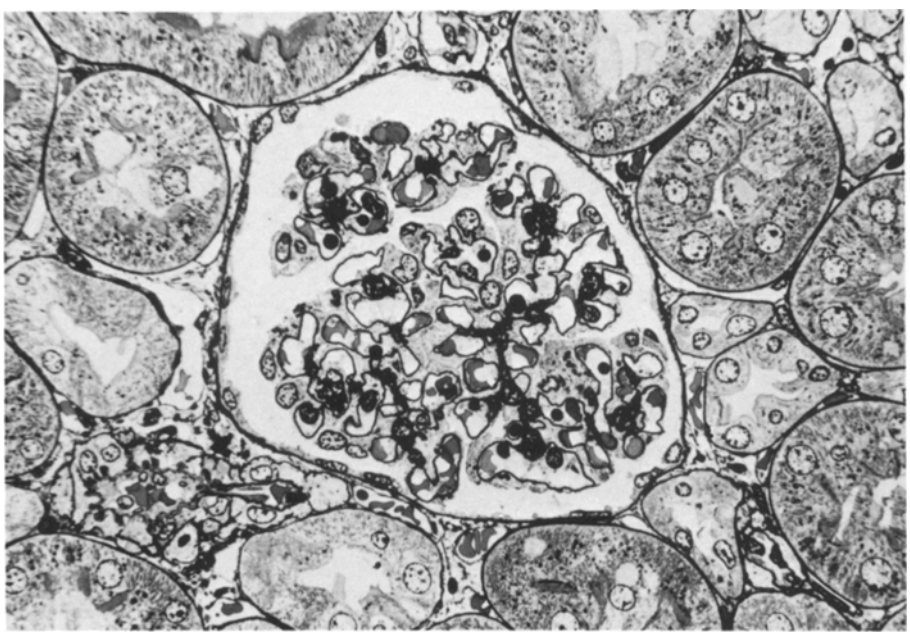

Fig. 1. Glomerulus of a rabbit after 90 days' treatment with bovine MC insulin. (Silver impregnation after Movat, 528:1)

Table 2. Morphometric findings in rabbits treated with bovine MC insulin and bovine proinsulin (mean ± SD; normal values see Table 1)

	MC-insulin ($n=13$)			Proinsulin ($n=15$)		
	30 days	60 days	90 days	30 days	60 days	90 days
Total glomerular area (μ^2)	7,398 ± 364	7,445.5 ± 353	7,388 ± 482	7,189 ± 436	7,371 ± 442	7,935 ± 649
Total glomerular cells	47.9 ± 2.76	48.2 ± 1.8	41.7 ± 1.5	56.5 ± 4.3	45.8 ± 1.2	48.4 ± 2.9
No. of epithelial cells	13.99 ± 1.0	13.39 ± 0.5	12.6 ± 0.3	15.3 ± 0.6	13.6 ± 0.5	12.6 ± 0.4
Epithelial cells (%)	29.4 ± 2.0	27.8 ± 0.9	30.4 ± 0.9	27.6 ± 2.2	29.9 ± 1.6	26.1 ± 1.0
No. of mesangial cells	13.4 ± 1.5	14.3 ± 0.7	11.2 ± 0.7	14.7 ± 1.9	12.6 ± 0.7	13.8 ± 1.0
Mesangial cells (%)	27.8 ± 2.0	29.6 ± 0.5	26.3 ± 0.7	25.7 ± 1.2	27.6 ± 1.3	28.4 ± 0.6
No. of endothelial cells	20.5 ± 1.1	20.5 ± 0.9	17.8 ± 0.6	26.4 ± 2.3	19.5 ± 1.2	22.1 ± 1.6
Endothelial cells (%)	42.8 ± 0.5	42.6 ± 0.6	42.8 ± 0.5	46.7 ± 1.4	42.5 ± 1.5	45.5 ± 0.9
Total mesangial area (μ^2)	381 ± 31	407 ± 29	344 ± 23	393 ± 39	348 ± 25	394 ± 26
Mesangial area in % of total glomerular area	5.2 ± 0.4	5.5 ± 0.3	4.65 ± 0.1	5.45 ± 0.3	4.74 ± 0.2	5.0 ± 0.2
Total glomerular cells/ 1,000 μ^2 glomerular area	6.5 ± 0.3	6.5 ± 0.2	5.7 ± 0.3	7.9 ± 0.3	6.3 ± 0.3	6.2 ± 0.2
Mesangial cells/ 100 μ^2 mesangial area	3.49 ± 0.1	3.54 ± 0.1	3.28 ± 0.1	3.74 ± 0.3	3.64 ± 0.1	3.5 ± 0.2

Table 3. Morphometric findings in rabbits treated with bovine a+b-component (mean ± SD; normal values see Table 1)

	a+b-component ($n=13$)		
	30 days	60 days	90 days
Total glomerular area (μ^2)	7,873 ±189	9,993 ±103	8,946 ±623
Total glomerular cells	52.7± 3.3	47.7 ± 2.6	47.6 ± 3.5
No. of epithelial cells	13.1± 0.5	12.1 ± 0.9	10.9 ± 0.6
Epithelial cells (%)	25.3± 2.1	25.3 ± 1.2	23.2 ± 2.3
No. of mesangial cells	16.0± 1.0	14.5 ± 0.4	14.3 ± 1.4
Mesangial cells (%)	30.4± 0.7	30.7 ± 1.1	29.9 ± 1.1
No. of endothelial cells	23.6± 2.5	21.0 ± 1.6	22.3 ± 2.6
Endothelial cells (%)	44.3± 2.2	44.0 ± 1.1	46.8 ± 2.4
Total mesangial area (μ^2)	419 ± 24	436 ± 19	354 ± 25.4
Mesangial area in % of total glomerular area	5.3± 0.2	4.4 ± 0.2	3.96± 0.2
Total glomerular cells/ 1,000 μ^2 glomerular area	6.7± 0.3	4.98± 0.3	5.4 ± 0.6
Mesangial cells/ 100 μ^2 mesangial area	3.8± 0.2	3.3 ± 0.2	4.1 ± 0.5

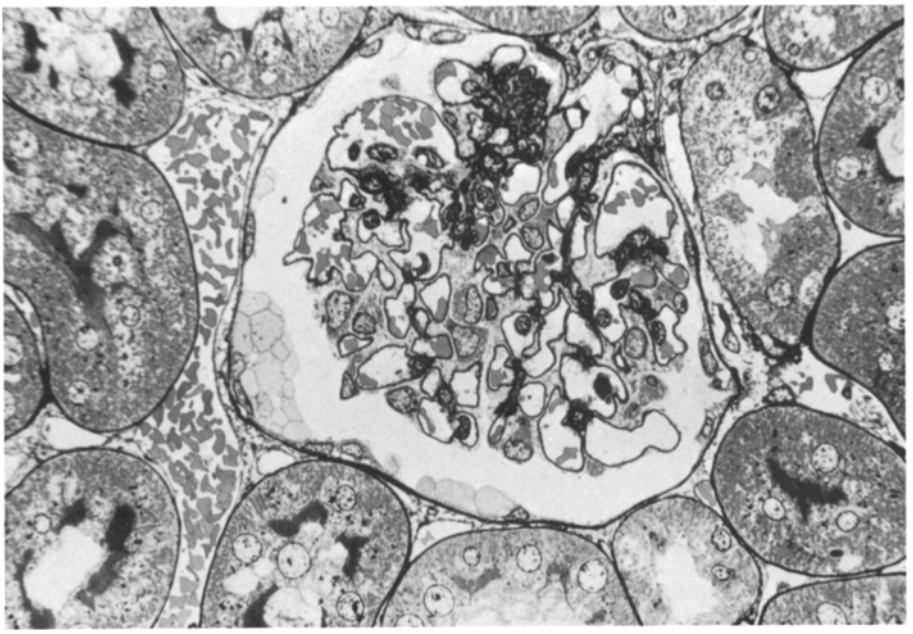

Fig. 2. Glomerulus of a rabbit after 90 days' treatment with porcine a-component. (Silver impregnation after Movat, 528:1)

Table 4. Morphometric findings in rabbits treated with porcine a-component and porcine b-component (mean ± SD; normal values see Table 1)

	a-component ($n=18$)			b-component ($n=18$)		
	30 days	60 days	90 days	30 days	60 days	90 days
Total glomerular area (μ^2)	9,051 ± 552	8,836 ± 1,449	9,948 ± 807	7,988 ± 937	9,326 ± 864	8,365 ± 665
Total glomerular cells	45.9 ± 3.2	43.6 ± 4.6	42.7 ± 5.2	40.2 ± 6.3	41.0 ± 4.5	40.7 ± 4.7
No. of epithelial cells	12.1 ± 1.1	12.1 ± 1.8	10.7 ± 1.4	12.5 ± 0.8	11.6 ± 1.5	11.4 ± 0.7
Epithelial cells (%)	26.5 ± 2.1	27.5 ± 2.6	25.2 ± 3.6	31.6 ± 4.2	28.2 ± 2.4	28.0 ± 3.2
No. of mesangial cells	14.3 ± 1.9	13.4 ± 1.8	13.7 ± 2.5	11.2 ± 3.3	12.7 ± 1.9	12.5 ± 2.3
Mesangial cells (%)	31.3 ± 3.2	30.7 ± 3.4	32.0 ± 4.2	27.4 ± 3.9	30.8 ± 2.1	30.6 ± 3.9
No. of endothelial cells	19.4 ± 2.3	18.2 ± 2.6	18.4 ± 3.1	16.5 ± 3.1	16.8 ± 1.8	16.9 ± 1.9
Endothelial cells (%)	42.2 ± 2.9	41.7 ± 3.3	42.8 ± 2.9	41.0 ± 2.4	41.0 ± 1.1	41.5 ± 0.7
Total mesangial area (μ^2)	562 ± 64	655 ± 159	646 ± 57	534 ± 97	659 ± 107	585 ± 107
Mesangial area in % of total glomerular area	6.2 ± 0.9	7.4 ± 0.9	6.5 ± 0.8	6.7 ± 0.9	7.1 ± 0.9	6.9 ± 1.0
Total glomerular cells/ 1,000 μ^2 glomerular area	5.1 ± 0.4	5.0 ± 0.7	4.3 ± 0.6	5.0 ± 0.4	4.4 ± 0.3	4.9 ± 0.4
Mesangial cells/100 μ^2 Mesangial area	2.56 ± 0.2	2.1 ± 0.3	2.1 ± 0.3	2.1 ± 0.3	1.9 ± 0.2	2.1 ± 0.3

Table 5. Antibody titers to bovine MC insulin, porcine PP and a-component in the various test groups (mean ± SD)

Experimental groups (days)	MC-bovine-insulin (μU/ml)	Porcine-PP (ng/ml)	a-component (ng/ml)
MC-bovine	(Detection-Limit) 0.05	(Detection-Limit) 1.0	(Detection-Limit) 25.0
30 ($n=4$)	0.0	0.08 ± 0.05	6.0 ± 3.6
60 ($n=5$)	0.01	0.52 ± 0.13	4.4 ± 2.2
90 ($n=4$)	0.0	0.18 ± 0.14	0.0
Proinsulin-bovine			
30 ($n=5$)	0.08 ± 0.04	0.56 ± 0.07	34.0 ± 19.4
60 ($n=5$)	0.21 ± 0.12	0.70 ± 0.14	4.8 ± 1.8
90 ($n=5$)	0.04 ± 0.02	0.68 ± 0.14	9.2 ± 4.1
a+b-component (bovine)			
30 ($n=5$)	3.3 ± 1.0	2.96 ± 2.23	87.2 ± 22.8
60 ($n=5$)	1.27 ± 0.4	14.7 ± 7.62	189.3 ± 21.6
90 ($n=3$)	2.05 ± 1.7	23.67 ± 22.3	218.7 ± 42.1
a-component (porcine)			
30 ($n=6$)	3.0 ± 2.5	13.1 ± 26.4	203 ± 162
60 ($n=6$)	1.7 ± 0.9	28.0 ± 42	402 ± 23
90 ($n=6$)	2.8 ± 2.1	13.8 ± 14	455 ± 46
b-component (porcine)			
30 ($n=6$)	0.5 ± 0.6	12.1 ± 28	255 ± 99
60 ($n=6$)	0.05 ± 0.07	1.4 ± 0.7	93 ± 75
90 ($n=6$)	0.29 ± 0.3	1.9 ± 2.9	194 ± 56

there was a reduction in the glomerular cell concentration to 4.4 cells/1,000 μ^2 and in the mesangial cell concentration to 1.93 cells/100 μ^2. After 90 days the values showed a tendency to return to normal again (Table 4).

2. *Immunological Findings*

It should first be mentioned that determinations of the antibody titers to bovine and porcine proinsulin did not show any demonstrable antibody titers in 90% of the tested sera after on repeated determinations. The antibody titer was below the limit of detection in the other sera.

Bovine MC Insulin Group. In this group no value for the three antibody titers determined exceeded the limit of detection (Table 5).

Proinsulin Group. The mean antibody titers to MC insulin had increased after 30 days to 0.08 mU/ml, after 60 days to 0.21 mU/ml and after 90 days they

had fallen to 0.04 mU/ml. The antibody titers to porcine PP were below the limit of detection. After 30 days the antibody titers to a-component of 34.0 ng/ml were just above the limit of detection but after 60 and 90 days they were far below this limit (Table 5).

Bovine a+b-Component Group. All determined antibody titers in this group were significantly above the limit of detection and greater than in the other test groups. The mean antibody titer to MC insulin was 3.33 mU/ml after 30 days, 1.27 mU/ml after 60 days and 2.05 mU/ml after 90 days. The mean antibody titer to porcine PP increased from 2.96 ng/ml after 30 days to 14.7 ng/ml after 60 days and to 23.67 ng/ml after 90 days. The highest values were found for the antibody titers to a-component. After 30 days the mean titer was 87.2 ng/ml, after 60 days it was 189.3 ng/ml and after 90 days there was a further increase to 218.7 ng/ml (Table 5).

Porcine a-Component group. The mean antibody titer to MC insulin was 3.01 mU/ml after 30 days, 1.7 mU/ml after 60 days and 2.81 mU/ml after 90 days. The peak antibody titer to porcine PP of 28.00 ng/ml was present after 60 days. After 30 and 90 days the values were lower, namely 13.1 and 13.8 ng/ml respectively. There was a significant increase in the antibody titer to a-component to 203.33 ng/ml after 30 days, the value was 402.00 ng/ml after 60 days and 455 ng/ml after 90 days (Table 5).

Porcine b-Component Group. The antibody titer to MC insulin was 0.51 mU/ml after 30 days and it fell to 0.05 mU/ml after 60 days (limit of detection). A slight increase to 0.29 mU/ml was found after 90 days. The antibody titer to porcine PP did not show any significant differences. The mean value after 30 days was 12.1 ng/ml, it was 1.4 ng/ml after 60 days and 1.93 ng/ml after 90 days. The antibody titer to a-component was 255 ng/ml after 30 days, it was 93 ng/ml after 60 days and 194 ng/ml after 90 days (Table 5).

Discussion

It is apparent that immunization of rabbits over 90 days with bovine MC insulin, proinsulin and a+b-component leads to morphologically demonstrable changes consisting of transitory thickening of the mesangium and a transient increase in the glomerular cell concentration. The most marked changes were observed in the group of animals treated with a+b-component. Similar changes were also observed in the animals treated with porcine a-component and b-component. The most pronounced changes here were found in the group treated with a-component. Antibody formation also showed a similar course. Whereas antibodies to MC insulin, porcine PP and a-component were not demonstrable in the group treated with bovine MC insulin, and were practically no longer demonstrable for proinsulin, the investigations showed a marked increase in the titer in the test groups which had received a+b-component, a-component

and b-component. The highest titers to a-component were found in the groups treated with a + b-component and a-component.

This shows that a-component elicits the greatest antibody formation, i.e. it has the most potent immunogenic effect. It also causes the most pronounced morphological changes. This is consistent with its average molecular weight of 25,000; the antigen-antibody complexes are particularly large for a-component (greater than 160,000). These constitute a particular immunological stimulus for the mesangium of the renal glomeruli which in our investigation were found to be particularly irritable structures, which reacted, at least transiently, to even mild immunological stimuli.

These findings confirm our investigations performed with porcine MC insulin and bovine a + b-component. In these investigations only slight transient changes were observed, without demonstrable antibodies in the MC insulin group, marked mesangial changes and antibody formation were found in the group treated with a + b-component (Wehner et al. 1977).

It is evident from these findings that bovine MC insulin and porcine MC insulin possess similar immunological activities, i.e. antibody formation to both insulins was not demonstrable in rabbits after treatment over 90 days, nor were there any marked morphological glomerular alterations. In contrast, a + b-components showed pronounced antigenicity with antibody formation and partly persistant morphological mesangial changes. a-Component – if it is not removed from insulin preparations – appears to possess the most potent antigenic effect and consequently induces the most pronounced morphological changes, by non-specific immunological reaction. Although diabetic microangiopathy is caused by the metabolic disorder it would not appear to be important that an additional immunological reaction may occur.

It is clear from these investigations that one should use insulin which has been highly purified in the therapy of diabetes mellitus, since contaminants induce slight glomerular changes which further potentiate the metabolism-induced mesangial lesions including disturbed phagocytic function (Wehner et al. 1979; Mauer et al. 1975; Bretzel et al. 1979). This is also suggested by investigations in diabetic KK mice which on treatment with MC insulin showed marked regression of the glomerulosclerotic changes (Wehner et al. 1978). On treatment with nonpurified insulins this effect was lacking (Wehner et al. 1978; Oppermann et al. 1979).

Various clinical and experimental studies are available which deal with the effect of insulin contaminants and the immunogenicity of "pure" insulin. These are fundamentally in agreement that pure insulin does not induce any, or at most an insignificant antibody formation and thus has proved to be of advantage in the treatment of diabetes (Freytag et al. 1973; Jansen and Freytag 1973; Schlichtkrull 1978; Andreani et al. 1972; Bruni et al. 1978; Frerichs et al. 1975; Korp and Levett 1973; Wehner et al. 1978).

Older work has also shown that after immunization with heterologous (non-purified) insulin morphological changes in the glomeruli can be demonstrated (Grieble 1960; Mohos et al. 1963; Ditscherlein et al. 1967; Fuchs et al. 1973; Wehner et al. 1970). There is now no doubt that the changes described in these papers were caused by the impurities contained in the insulin preparations used at that time.

Since the uptake of immune complexes by the mesangium in diabetes mellitus is delayed and since elimination is also retarded (Wehner et al. 1979) it is quite conceivable that circulating immune complexes in the diabetic are treated differently in the mesangium. Consequently they may play an *additional* role in the development of diabetic microangiopathy of the kidney. Irvine et al. (1978), Andersen (1976) and Bloodworth (1968) have drawn attention to this possibility.

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