INSULIN-INDUCED GLOMERULOSCLEROSIS IN THE RABBIT*

BY STEVEN C. MOHOS, M.D., GORDON R. HENNIGAR, M.D., AND JOHN A. FOGELMAN§

(From the Department of Pathology, State University of New York Downstate Medical Center, and the Institute of Pathology, Kings County Hospital Center, Brooklyn, New York)

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Following the reports of Kimmelstiel and Wilson (1) and Murakami (2) on the pathognomonic association of nodular glomerular lesions with human diabetes, a number of experiments have been performed on laboratory animals with the intent of producing similar lesions. Two approaches were pursued:

(a) In one, experiments were designed to induce the lesions in animals by virtue of their being diabetic. Alloxan-induced diabetes has generally failed to bring about these lesions. One of us (3) studied the effects of alloxan diabetes, alone and with other agents, in New Zealand albino rabbits. In these experiments 20 per cent of the animals made diabetic by alloxan, and maintained for 2 years with blood sugar levels of 400 to 750 mg per cent, showed an occasional glomerulus with foci of collagen or hylalinization. Similarly, alloxan diabetic rabbits when fed cholesterol singly or in combination with either desoxycorticosterone acetate or cortisone remained without apparent alteration of the glomeruli. The induction of the hypertensive state in the alloxan diabetic animal by bilateral renal artery constriction failed to produce significant glomerular lesions. Also, normal rabbits given cortisone alone did not produce glomerulosclerosis. These findings do not contradict the work of Rich et al. (4) who reported the “formation of large, often globoid masses recalling in some instances the Kimmelstiel-Wilson glomemlar lesions in diabetics” in rabbits given both horse serum and cortisone. However, our findings are in disagreement with the reports of Bloodworth and Hamwi (5) who found nodular lesions in most of the glomeruli in one of their alloxan diabetic rabbits kept on desoxycorticosterone acetate. Similarly we are in disagreement with the findings of Wilens and Stumpf (6) who observed nodular glomerular lesions in cortison-injected rabbits. Lawe (7) has shown that untreated Chinese hamsters with hereditary diabetes failed to develop these kidney lesions, although he observed the presenile appearance of glomerular changes that are usually associated with advanced age in that species.

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† Present address: Department of Pathology, New York Medical College, New York.

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(b) The other approach was based on the idea that perhaps not diabetes per se but the injected foreign insulin is responsible for these lesions on the basis of an immunological mechanism and diabetes has, if at all, only a modifying effect. Impetus was given to this line of thought by the recent observation of Berns et al. (8). Using fluorescein-labeling techniques they demonstrated the localization of insulin-binding antibodies in the nodular glomerular lesions of human diabetics. Griepke (9) has reported that injection of crystalline and depot insulin intravenously into rabbits was followed by minimal proliferation of glomerular epithelial cells, exudation of protein into Bowman's space, hyaline casts, and periglomerular fibrosis. He found similar lesions in 100 per cent of his control animals treated with normal human serum and interpreted the findings in his insulin injected animals as foreign protein-induced nephritis.

Our failure to induce glomerulosclerosis in guinea pigs with insulin alone or with insulin plus incomplete Freund's adjuvant (bayol F and falba without mycobacterium) led us to believe that perhaps the renal lesion is related to a state of delayed hypersensitivity. In order to test this hypothesis, delayed hypersensitivity was induced in rabbits by immunizing them with insulin incorporated into complete Freund's adjuvant (containing mycobacterium) and then eliciting the delayed hypersensitivity reaction by challenging with insulin alone. The challenge insulin was delivered repeatedly to the sensitized renal tissue in the form of subcutaneous injections.

The observations derived from these experiments support the hypothesis that the glomerulosclerosis may result from a delayed hypersensitivity reaction to insulin.

**Material and Methods**

**Insulin.**—Commercially available crystalline zinc beef insulin was incorporated into Freund's adjuvant for immunization and was used also for the challenge injections. Fifty to 400 units per animal were given per injection as indicated below.

**Immunization.**—For immunization, insulin was incorporated in the complete Freund's adjuvant consisting of heat-killed *Mycobacterium butyricum* 8 mg, bayol F 1.5 ml, and falba 1.5 ml (10). This preparation was injected intramuscularly at alternating sites. To prevent insulin shock the rabbits received 50 ml of 5 per cent glucose in aqueous solution subcutaneously in the loose dorsal neck skin prior to insulin injection and were given the same solution as drinking water *ad libitum* for 24 hours before and after each injection.

**Urine.**—Urine was collected daily using toluene as the preservative. The protein of the acidified urine was precipitated with 3 per cent sulfosalicylic acid and the turbidity read at 415 mu on a Zeiss M4QII spectrophotometer. The protein content was expressed in total milligrams per daily output.

**Determination of Antibody Titers.**—Blood was taken from the experimental animals before during, and after immunization and the precipitating insulin antibodies were demonstrated by agar plate precipitation. Total antibody titer determinations using Boyden's technique

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1. Esso Standard Oil Company, Linden, New Jersey.
4. Eli Lilly & Company, Indianapolis, through the courtesy of Dr. W. R. Kirtley.
(11) of tannic acid-treated, insulin-coated sheep red blood cells, as modified for insulin by Borduas and Grabar (12) were also used. All necessary controls including inhibition with insulin were included with each test serum.

Delayed Hypersensitivity.—To test for delayed hypersensitivity 40 units of insulin were injected intradermally into rabbits 3 months following their first immunization with insulin incorporated into complete Freund's adjuvant. A positive reaction was characterized by a red wheal which appeared approximately 48 hours after the intradermal challenge. As controls, 0.1 per cent phenol in saline was injected intradermally at a nearby site, on the same animal, and non-immunized rabbits were skin-tested for the same insulin in like manner.

Tagging of Insulin.—The method of Mayersbach (13) using the fluorescent dye dimethyl-1-naphthylamine sulfonic acid (DIS) was adapted for tagging insulin. One-hundred ml of beef insulin containing 80 units per ml (approximately 320 mg insulin in toto) were evaporated in a dialyzing bag by air currents to about 25 ml. The pH of the concentrated insulin was adjusted to 8.2 with 0.5 M NaHCO₃. At 0°C, 3 ml of dioxane was added, dropwise, while stirring constantly. Fifteen mg DIS was dissolved in 1.5 ml acetone and added dropwise during a period of approximately 30 minutes. The solution was maintained at 0° and occasionally mixed until it cleared which occurs in approximately 3 to 4 hours. After clearing it was placed in Visking tubing and dialyzed against distilled water which had been adjusted to pH 8 with 0.1 M NaOH. Dialysis was continued until no detectable fluorescence was observed in the dialysate. It was kept refrigerated and used within 2 days.

The electrophoretic mobility of DIS-insulin was found to be essentially the same as that of untagged insulin, separating into two fractions as seen in Text-fig. 1. The electrophoresis was performed on cellulose acetate strips (microphore) using equal amounts of Laurell's barbital buffer (14) and Aronsson's tris-EDTA-boric acid buffer (15). The strips were fixed with 3 per cent sulfosalicylic acid and stained with ponceau S. The fluorescence in ultraviolet light of unstained strips coincided with the stained pattern. The electropositive portion of the separation was slightly heavier in the DIS-tagged insulin (Text-fig. 1) presumably because of a decrement in the net positive charge of the insulin molecules when DIS reacts with the NH₂ groups.

Histological Procedures.—Kidneys, adrenals, liver, spleen, pancreas, and lungs were fixed in 10 per cent solution of neutral-buffered formalin USP and embedded in paraffin. Hematoxylin and eosin, periodic acid-Schiff, Congo red, Alcian blue, Masson's trichrome, Verhoeff's van Gieson, Giemsa, fast green-fuchsin, Gram, and Ziehl-Neelsen's stains were used as needed on all specimens.

In the fluorescent studies, 4 parts of absolute alcohol and 1 part of glacial acetic acid were used as a fixative for 2 to 4 hours. Alcohol and methylbenzoate were used for dehydration and paraffin for embedding. Four micron sections were mounted on ultraviolet transparent slides, deparaffinized in xylol and alcohol, and covered with glycerol gelatin which had been buffered to pH 8 with McIlvaine's buffer. The slides were examined under ultraviolet light using ultraviolet transmitting filter UG11 and a darkfield condenser in a Reichert Zetopan ultraviolet microscope. Studies of the localization of DIS-insulin on paraffin-embedded sections were performed according to the technique of Berns and Blumenthal (8).

Experimental.—Thirty-four hybrid rabbits divided into 4 groups were used for the experiment. They were about 3 to 4 months of age at the onset of the experiment.

Group I: Twenty-one rabbits were scheduled to receive, at monthly intervals, 5 intramuscular injections of increasing doses (50, 70, 90, 100, 120 units) of insulin incorporated into Freund's adjuvant. Following immunization they were to receive 7 insulin challenge injections ranging from 50 to 400 units subcutaneously at 10 day intervals. Six of the rabbits died during immunization before blood could be drawn for antibody titer determination. Seven died

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Gelman Instrument Company, Chelsea, Michigan.
INSULIN-INDUCED GLOMERULONEPHROSIS during the insulin challenges in spite of the measures taken to avoid "insulin shock." The 6 rabbits which died during immunization were excluded from the study as were 2 of the 7 which died during the insulin challenging. The latter 2 were excluded because they showed severe

**TABLE I**

*Kidney Lesions in Relation to Insulin Antibody Titer and Proteinuria*

<table>
<thead>
<tr>
<th>Group I rabbit No.</th>
<th>No. of F.A.* + insulin injections</th>
<th>No. of insulin challenge</th>
<th>Proteinuria</th>
<th>Insulin antibody titer</th>
<th>Kidney lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>11</td>
<td>–</td>
<td>8, 0</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>7</td>
<td>++</td>
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<td>5</td>
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<td>64</td>
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<tr>
<td>9</td>
<td>5</td>
<td>7</td>
<td>+</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>1-0</td>
<td>5</td>
<td>7</td>
<td>+</td>
<td>256</td>
<td>+</td>
</tr>
<tr>
<td>1-1</td>
<td>9</td>
<td>11</td>
<td>+</td>
<td>32, 32</td>
<td>+</td>
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<tr>
<td>1-2</td>
<td>5</td>
<td>1</td>
<td>+</td>
<td>16</td>
<td>+</td>
</tr>
<tr>
<td>1-3</td>
<td>5</td>
<td>7</td>
<td>+</td>
<td>16</td>
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<td>5</td>
<td>1</td>
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<td>1064</td>
<td>+</td>
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<tr>
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<td>5</td>
<td>1</td>
<td>–</td>
<td>256</td>
<td>–</td>
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<tr>
<td>2-0</td>
<td>5</td>
<td>5</td>
<td>+</td>
<td>4</td>
<td>+</td>
</tr>
</tbody>
</table>

*F.A., Freund's adjuvant.

The remaining 13 animals in this group (1, 3, 5-7, 9-13, 15, 19, and 20) and the number of injections they received are listed on Table I. The animals were sacrificed with intravenous nembutal from 1 to 5 days following the 7th injection except for those which died earlier as a result of the insulin challenge, and 2 animals (1 and 11) which received 4 additional immunizing and 4 additional challenge injections; i.e., 9 immunizations and 11 challenges. The latter 2 were kept for fluorescent studies and were sacrificed in connection with those studies as described below.
Group II: Six rabbits (a, b, c, d, e, f) received 5 injections of complete Freund's adjuvant intramuscularly at monthly intervals.

Group III: Five rabbits (A, B, C, D, E) received 7 insulin injections each (35, 50, 80, 100, 100, 100 units) at 10 day intervals subcutaneously.

Insulin antibody titers, daily urine, and urinary protein output were determined on groups I, II, and III and their organs examined on the gross and by histological techniques.

Group IV: For fluorescent insulin studies to determine whether insulin would localize in the glomeruli of the insulin-Freund's adjuvant immunized animals, 2 rabbits, Nos. 1 and 11, were selected from group I. No. 11 exhibited proteinuria after insulin challenges whereas No. 1 did not. Three other animals were used: rabbit (A) from Group III which had been subjected earlier to insulin injections only; and 2 normal untreated animals. Rabbits 1, 11, (A), and 1 normal rabbit received DIS-tagged insulin intravenously; No. 11 received a total of 4 ml containing approximately 50 mg of the DIS-tagged insulin and each of the others received 7 ml containing approximately 90 mg. The other normal rabbit did not receive DIS-tagged insulin. To prevent possible anaphylaxis they were first desensitized with 0.1 ml DIS-insulin given intravenously which was followed 30 minutes later by an additional 1 ml of DIS-insulin. They were also given 50 ml of 5 per cent glucose in water subcutaneously and aqueous glucose to drink ad libitum. Initially they tolerated the injections well but in about 5 hours they began to show signs of "insulin shock." They were anesthetized with intravenous Nembutal and perfused with cold Ringer's solution until the returning fluid was in the gross free of blood. Their organs were taken and examined for fluorescence using the fluorescent techniques described above.

RESULTS

Group I.—Reciprocals of antiinsulin titers at the end of immunization, as determined by Boyden's hemagglutination technique, are shown in Table I. Titors are also given on sera taken just before the fluorescent studies were undertaken on rabbits 1 and 11. In the case of No. 11 the titer was also the same; i.e., 32 at the end of experimentation. In No. 1 the titer declined from 8 to zero. It is interesting to note this decline in antibody titer in No. 1 and to note also that this animal became very obese in the course of the experiment.

Semiquantitative agar plate estimates for precipitating antibodies did not parallel the total antibody titer as determined by hemagglutination (Table I). The presence of precipitating insulin antibodies is shown with sera 6 and 7 taken 30 days after the 3rd immunization (Text-fig. 2). Two lines can be clearly discerned (16).

Skin tests for delayed hypersensitivity revealed that all rabbits with proteinuria and kidney lesions showed a strong skin reaction which persisted for about 1 week, became indurated, and developed a small necrotic center (Fig. 2). Rabbits 5, 15, and 19 also showed a positive skin reaction but which lasted only 2 or 3 days while No. 1 failed to react. There was no positive reaction to phenol or in non-immunized controls. It was also observed that rabbits with prominent kidney lesions revealed a marked granulomatous reaction at the site of immunization which decreased in size after many weeks. In contrast non-reactive rabbits walled off the injected material apparently preventing its dissemination and forming a sterile abscess.

Urine examination revealed that 9 of the 13 rabbits had proteinuria during
the repeated immunizations and also after the insulin challenges. Usually there was a 2 day lag between the challenge and the ensuing proteinuria. At first the proteinuria was slight and returned to normal within a few days. The proteinuria became more severe with repeated challenges and finally failed to return to normal values. The characteristic urinary response is illustrated in Text-fig. 3 which shows the urine volumes and total urinary protein in 2 non-responsive rabbits (Nos. 1 and 5), in a responsive rabbit (No. 11), and in a non-immune group III control animal (A) which received only insulin. In animals 1, 5, and 11 the response is shown both after injection of the insulin incorporated in Freund's adjuvant and after insulin alone. The response of rabbit (A) after 2 different doses of insulin is also recorded. The effects (marked proteinuria) on No. 11 are obvious as are the absence of such effects in the other three.

Examinations of the urines for sugar and acetone using clinistest® and acetest® were uniformly negative.

In the gross, the involved kidneys showed minute cortical scars scattered throughout (Fig. 1).

The histologic findings in the kidneys of the 13 rabbits are listed in Table 1. The essential changes included protein casts, the number of which paralleled the degree of proteinuria. The glomerular damage varied from minimal localized or diffuse thickening of the basement membrane to very severe, PAS-positive, Congo red-negative irregular thickening of the loops with occasional nodule-
Text-Fig. 3. Daily urinary volume and urinary protein in a responsive immunized animal (11), non-responsive immune animals (1 and 5), and in a control (A) following injection of Freund’s adjuvant with insulin (F.A. INS.) and insulin (INS.) alone. Note that animal 11 exhibits a delayed and significant proteinuria.
like formations reminiscent of those seen in human diabetics (Figs. 4 and 5) (17). The cellular components of the glomeruli in the earlier phase showed an increase, but later in the course of the disease they decreased in number until complete obliteration of the glomerulus supervened.

The histologic changes often involved closely associated nephrons and in such cases they readily explained the gross cortical scars. In other instances, however, these glomerular changes occurred in individual glomeruli with no apparent fibrosis around them. In none of the cases did we observe the presence of emboli or thrombus formation. Vascular sclerosis caused by endothelial proliferation, as described by Blumenthal et al. (18), in human diabetics was frequently seen. Cellular infiltrates with or without fibrous components were often found in the interstitium, frequently in association with damaged glomeruli. The cells were varied in appearance. There were lymphocytes, plasma cells and occasionally a few histiocytes. In animals which died soon after an insulin challenge the cellular infiltrates were mainly neutrophils which in the rabbit are called pseudoeosinophils because of their eosinophilic cytoplasmic granules (19). In these animals the pseudoeosinophils frequently showed disintegration and were often closely attached to the glomerular wall, often adhering to glomerular endothelial cells containing refractile material, presumably altered mycobacterium (Fig. 3). That DIS-tagged mycobacteria are taken up by glomerular cells could be demonstrated in association with other studies using Freund's adjuvant (20). Such pseudoeosinophils were found consistently in all reacting animals, particularly in the spleen, less frequently in the lungs and liver. They were often phagocytized by histiocytes.

Other organs often involved were the lungs, the liver, and the spleen. In the lungs of rabbits 5, 6, 11, 15, and 19 there were many scattered small foci of large cells with pale cytoplasm having the appearance of histiocytes or epithelioid cells. Interspersed with these cells were many lymphocytes, plasma cells, and polymorphonuclear leukocytes. Acid-fast-negative granules were often found in the histiocytes. These foci were in the interstitium often obliterating the alveolar lumen. In the liver small accumulations of lymphocytes, plasma cells and occasionally polymorphonuclear leukocytes were noted, mainly in the perportal areas as in rabbits 5, 6, 9 to 11. Only occasionally was a nodule of epithelioid cells present. In a few instances the infiltrate was mizonal and included large reticuloendothelial cells and regenerating liver cells.

The spleen deserves particular attention. In almost all animals the sinuses were distended and filled with large histiocytes often containing granules, presumably ingested or altered mycobacteria, but which were not acid-fast. Steiner (21) was also unable to demonstrate the injected mycobacterium with the acid-fast stain. The histiocytes showed signs of disintegration. These disintegrating cells frequently engulfed polymorphonuclear leukocytes or were surrounded by large number of polymorphonuclear leukocytes and plasma...
cells (22) as in animals 5, 10, and 20. The malphigian follicles were large and cellular and occasionally contained lakes of proteinaceous material. There were no cell infiltrates in the pancreas. The islets of Langerhans were large. The alpha cells were prominent.

The zona fasciculata of the adrenal glands were wide and pale but granulomas and cell infiltrates were conspicuously absent.

Group III.—The rabbits of this group which received only insulin did not show any alterations. The urine output of one of these rabbits (A) is recorded in Text-fig. 3 after receiving 50 and 100 units of insulin. There was no significant increase in the total protein excreted. Precipitating insulin antibodies could not be detected in the sera of these animals, and gross and histological examination disclosed normal kidneys.

Group IV.—Fluorescent studies were performed to observe the localization of DIS-insulin given intravenously to insulin-immunized and non-immunized animals. No obvious fluorescence occurred in the already established glomerular lesions in animal 11 of group I. However, fresh glomerular lesions which did show fluorescence occurred in the same animal apparently as a result of the intravenous challenge with the DIS-insulin (Fig. 7). The protein casts (Fig. 8) also fluoresced as did the brushborders of the convoluted tubules. In contrast to the established lesions these fresh glomerular lesions elicited by the intravenous administration of DIS-insulin were Congo red-positive (Fig. 6) and gave only a faint periodic acid-Schiff reaction. Glomerular fluorescence was absent in the other DIS-insulin injected rabbits.

It was also observed that the capillary basement membranes showed a strong fluorescence in all the DIS-insulin–injected animals whether or not they were immunized. No fluorescence was observed in the normal control which did not receive DIS-insulin.

In vitro application of DIS-insulin to deparaffinized kidney sections from each of the groups (8) failed to localize in the glomerular lesions.

DISCUSSION

Our findings can be condensed into the following points:

1. Insulin alone in large quantities does not produce glomerulosclerosis and this is essentially in agreement with the findings of Grieble (9).

2. Immunization with insulin incorporated in complete Freund’s adjuvant and followed by subcutaneous challenges with insulin alone results in glomerulosclerosis of progressive severity and is associated with increasing proteinuria.
Generally, the delayed hypersensitivity to insulin correlates with the severity of kidney involvement and proteinuria. No such correlation exists between the circulating insulin antibody titers and the kidney lesions.

3. Immunization with complete Freund's adjuvant alone results also in glomerulosclerosis. However, fewer glomeruli are involved.

4. The proteinaceous glomerular deposits are PAS-positive and Congo red-negative as are the human diabetic glomerular lesions. In the early phase of these lesions disintegrating pseudoeosinophils often are found to be attached to glomerular endothelial cells containing acid-fast-negative granules, which are presumably altered mycobacteria.

5. It becomes apparent that there is a difference between the glomerular lesions induced by subcutaneous or intravenous insulin challenge. DIS-tagged insulin given intravenously localizes in fresh glomerular lesions which are Congo red-positive. Thus they are different from the Congo red-negative glomerulosclerotic lesions induced by subcutaneous insulin challenges in animals with delayed hypersensitivity to insulin. These subcutaneous insulin-induced glomerular lesions did not show obvious fluorescence either with intravenously injected DIS-insulin or when DIS-insulin was applied \textit{in vitro} on deparaffinized sections.

We can offer three explanations to interpret the development of these glomerular lesions and will analyze our findings relative to them:

(a) They are a type of foreign protein nephritis, in which case, circulating antibodies are of primary importance (23). The absence of lesions in 4 immunized rabbits (Nos. 1, 5, 15, and 19) even in the presence of high antibody titer (Nos. 15, 19) and the lack of correlation in the other 9 rabbits between the antibody titers and the severity of glomerular lesions indicates that circulating antibodies are not of primary importance in the development of these kidney lesions in the rabbit. However, in the case of intravenous insulin challenge, circulating antibodies may play a primary role. These lesions are probably established as a result of the interaction of intravenously given insulin with circulating antibodies. It is known that complexes formed between circulating antibodies and antigens are capable of producing such glomerular lesions? (23).

(b) They are due to a cellular, delayed type of hypersensitivity, in which case, during immunization with the insulin incorporated in Freund's adjuvant, the antigen (insulin) together with the adjuvant is deposited in the glomeruli. The deposits are in histiocytes and perhaps in activated glomerular endothelial cells. When insulin is injected again it reacts with the cells containing these antigenic deposits, or with their transformed products, the so-called transfer factor. This reaction leads to endothelial damage and glomerular lesions. This

\footnote{While this report was in preparation we learned that Hirata, Berns, and Blumenthal presented similar results at the Annual Meeting of the American Diabetes Association in July 1962, and reported it as an abstract in the program.}
would be in accord with Pappenheimer's hypersensitivity theory (24). The constant presence of strong, delayed type of skin hypersensitivity to insulin in animals with pronounced kidney lesions and the delayed onset of proteinuria after challenges are in favor of the pathognomonic association of these kidney lesions with a delayed hypersensitivity. Furthermore, the granulomatous reaction at the site of immunizing injection in the reactive animals in contrast to the sterile abscess formation in the non-reactive animals suggests that dissemination of antigenic material in general, and perhaps to the glomerular endothelial cells in particular, is of paramount importance. This is also suggested by the apparent presence of mycobacterium in glomerular cells in reactive rabbits.

This is in agreement with the findings of Steiner et al. (21) who have investigated the local and systemic effects of Freund's adjuvant and its fractions. They came to the conclusion that the changes found in different organs are caused by the "lymphohematogenous metastatic dissemination" of the injected material by phagocytes.

If either of these two immunological reactions is responsible for the lesions the poorly antigenic insulin may not be unique in bringing about these lesions and any other antigen such as albumin or globulin may act similarly.

(c) Insulin may have a chemical affinity for the basement membrane and this specific chemical preference in localization together with either of the above immunological mechanisms is responsible for the lesions. In this case other antigens not having such affinity to basement membrane would not be as effective as insulin in bringing about these lesions.

The localization of fluorescent insulin in the basement membrane of non-immunized rabbits is suggestive of a chemical affinity of insulin to this structure.

That such immunologically non-specific affinity may exist is indicated by the binding of insulin to different globulins of normal individuals as Mitchell (25), Steigerwald et al. (26) and Arquilla (27) have shown.

It is well known that the mycobacterium in the complete Freund's adjuvant is capable of initiating a delayed type of hypersensitivity against antigens which do not have this capacity, if the adjuvant is used together with these antigens. It is also known that once this delayed type of hypersensitivity is established for a "conventional" antigen, repeated challenges with "conventional" antigen alone will enhance the delayed hypersensitivity (28).

Therefore, once the animals develop a delayed hypersensitivity to insulin by the use of Freund's adjuvant further injection of insulin alone will suffice to enhance this hypersensitivity.

The fact that Freund's adjuvant alone is capable of inducing the glomerular lesions does not invalidate the possible role of insulin in the production of these lesions. Freund's adjuvant alone contains a protein antigen, tuberculoprotein,
which like insulin does not by itself produce delayed hypersensitivity unless in association with the waxy portion of the mycobacterium (29). Tuberculoprotein in Freund’s adjuvant may serve as an antigenic stimulus in the same manner as insulin incorporated in Freund’s adjuvant. Perhaps repeated infections such as with streptococcus (30) at the injection sites in diabetic patients or the frequent use of depot insulin have an adjuvant effect. If this is so, once the hypersensitivity is established to insulin, further injections with insulin alone will suffice to bring about a progressive glomerulosclerosis and proteinuria. Indeed, there is no other antigen that is clinically injected so consistently over such extensive periods of time as is insulin.

The modifying effect of diabetes on these lesions also must be investigated and in particular the antinflammatory effect of cortisone on delayed hypersensitivity to insulin.

The predominance of such vascular lesions in the kidneys may be explained by the extremely high blood flow through this organ and therefore an excessive exposure to antigenic stimulus. In an organ which is only 0.5 per cent of the total body weight, yet receives 20 per cent of the cardiac output, this represents a blood flow forty times greater than through the rest of the body.

**SUMMARY**

An attempt has been made to induce intercapillary glomerulosclerosis in rabbits by immunization with insulin incorporated in Freund’s adjuvant and followed by repeated challenges with subcutaneously given insulin. It was observed that lesions resembling human diabetic glomerulosclerosis with occasional nodule-like formation could be produced and that the challenge insulin injections produced proteinuria. The presence of a delayed type of hypersensitivity seemed necessary for the lesions to occur as did the dissemination of the immunizing material to the kidneys. The experiment also disclosed that intravenously given DIS-tagged insulin localizes in a subtly different kind of glomerular lesion with different staining properties. The significance of these findings and the possible role of insulin treatment in the pathogenesis of human diabetic glomerulosclerosis is discussed.

**BIBLIOGRAPHY**


3. Hennigar, G. R., unpublished observations.


**EXPLANATION OF PLATES**

**PLATE 73**

**FIG. 1.** Normal kidney (left) and kidney from animal with glomerular lesions (right). Note stippled appearance because of minute cortical scars. X 3.

**FIG. 2.** Positive delayed type of intradermal test to insulin (center) and negative control with phenol (arrow). Actual size.
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Plate 74

Fig. 3. Pseudoeosinophils (p) in glomerulus of an animal which died soon after insulin challenge. Note refractile body in endothelial cell (arrow). Giemsa. $\times$ 960.
(Mohos et al.: Insulin-induced glomerulosclerosis)
PLATE 75

Fig. 4. Low power view of kidney from rabbit 1-2. Note three glomeruli with significant morphological alterations resembling intercapillary glomerulosclerosis. Observe also protein casts. Hematoxylin eosin. × 60.

Fig. 5. High power view of a PAS-positive, Congo red-negative, non-fluorescent “established” glomerular lesion. PAS. × 340.
(Mohos et al.: Insulin-induced glomerulosclerosis)
**PLATE 76**

**Fig. 6.** High power view of Congo red- and fluorescence-positive “fresh” glomerular lesion. Congo red. $\times 600$.

**Fig. 7.** Ultraviolet microphotograph of a fluorescent “fresh” glomerular lesion, following intravenous injection of DIS-insulin. $\times 300$.

**Fig. 8.** Fluorescent cast in the same kidney as in Fig. 7. $\times 600$. 
(Mohos et al.: Insulin-induced glomerulosclerosis)