Persistent Grossly Elevated Plasma Immunoglobulin A Levels in Untreated Streptozocin-Induced Diabetic Rats

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Experimental diabetes mellitus was induced in adult male and female rats by injecting streptozocin (STZ; 60 mg/kg i.p.) in preparation for a screening survey of changes in the pattern of undenatured plasma proteins, as revealed by two-dimensional (2-D) gel electrophoresis followed by silver staining. As early as 8–12 days later, the 2-D gels revealed three high-molecular-weight plasma protein spots, which persisted for 150 days in the blood of untreated diabetic rats. Such spots were not seen in plasma of normal control rats. Evidence is presented for the presumptive characterization of these proteins as oligomers of immunoglobulin A (IgA). Specific measurement of total IgA content of diabetic plasma samples by single-radial immunodiffusion, after reduction with dithiothreitol and alkylation with iodoacetamide, reveals that IgA content increases linearly from control values of 11.1 ± 4.6 to 358 ± 249 mg/dl (means ± SE) 21 days after STZ and persists at these high levels for as long as 150 days. Diabetic rats injected daily with insulin showed IgA levels only two to four times higher than normal. Neither experiments designed to quantitate the rates of clearance (catabolism plus excretion) of ^{125}I-labeled secretory IgA from the circulation of normal and diabetic rats nor measurement of total IgA in the bile from diabetic and normal bile fistula rats supports the view that slowed clearance from the circulation or impaired biliary excretion in the diabetic rat causes observed gross hyperimmunoglobulinemia A. *Diabetes* 37:177–84, 1988

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In the course of a screening survey with two-dimensional (2-D) gel electrophoresis to detect qualitative and/or gross quantitative changes in the undenatured plasma proteins of untreated streptozocin-induced diabetic (STZ-D) rats, we observed the novel appearance of three major high-molecular-weight protein spots. These spots have not been encountered in numerous control gels of undenatured normal rat plasma or serum samples subjected to electrophoresis and silver staining at the same time as plasma samples from diabetic rats.

We describe the evidence for the presumptive characterization of these protein spots as immunoglobulin A (IgA) oligomers, the time course of development of increased IgA levels in the untreated STZ-D rat, the suppression of increased IgA levels by insulin treatment, and experiments done to determine whether increased IgA levels result from increased synthesis and/or decreased excretion or catabolism. Manifold increases in plasma IgA levels in rats as a consequence of total bile duct obstruction resulting from experimental ligation have been reported (1).

While our work was in progress, Triolo and co-workers (2,3) reported the occurrence in diabetic humans of modest increases in serum IgA levels and of circulating immune complexes containing IgA; the latter have been correlated with clinical evidence of microangiopathy.

MATERIALS AND METHODS

Induction of experimental diabetes mellitus. Adult Sprague-Dawley male rats (Holtzman, Madison, WI) weighing 300–450 g and females weighing 200–225 g were maintained on Purina rodent chow and water ad libitum in a 22 ± 2°C room with a 12-h light-dark cycle. Generally, groups of 16–18 rats were started at one time; diabetes was induced in 12, and the others served as controls. Obser-

vations described herein were made on plasma or serum samples taken from among >120 diabetic and 50 control rats. To induce diabetes, 32 mg/ml STZ (Sigma, St. Louis, MO) was freshly prepared in ice-cold isotonic citrate buffer (pH 4.5) and given (55–60 mg/kg body wt i.p.) to the rats, which were lightly anesthetized with ether after an 18-h fast; control rats received only citrate buffer. Diabetes was also induced in 4 of 6 male rats by giving 60 mg/kg body wt i.p. alloxan (Eastman-Kodak, Rochester, NY), followed 24 h later by an additional 100 mg/kg body wt alloxan in 50 mg/ml Ringer solution. Typically, the diabetic rats manifested gross polyuria, glycosuria (Tes-Tape 3–4+), and ketonuria (Acetest 0–2+) within 24–48 h. Blood glucose of diabetic rats ranged between 300 and 800 mg/dl as estimated by Chemstrip-bG (Boehringer Mannheim, Indianapolis, IN). When diabetic rats were treated with insulin, they received 1–2 U of protamine zinc insulin subcutaneously, usually between 1600 and 1700 h daily. When the rats were killed, under ether anesthesia, blood was obtained by cardiac puncture and mixed with heparin:sodium EDTA to yield plasma or after clotting to yield serum. In experiments in which repeated samples of blood were needed, rats were held in restraining cages, and blood was drawn from a nick at the end of the tail into heparinized glass capillary tubes (Clay-Adams, New York). With the alloxan-induced diabetic rats, plasma obtained 23 days after alloxan was given was examined by 2-D gel electrophoresis. Plasma and serum samples were preserved at −20 to −30°C.

Two-dimensional gel electrophoresis. Isoelectric focusing was carried out in glass capillary tubes, 1.5 mm internal diameter, essentially according to the method of O'Farrell (4). The 4% acrylamide, 0.11% bisacrylamide gel contained 2% ampholine (pH 3–10; Biolyte, Bio-Rad, Los Angeles, CA), but we avoided using urea, Nonidet P-40, and mercaptoethanol. Electrophoresis in the second dimension with a 4–20% linear acrylamide gel gradient (instead of 10–20%) permitted the electrophoretic migration of high-molecular-weight proteins.

Silver staining of the 2-D gels was carried out with essentially the procedure of Oakley et al. (5). Our procedure differed from that of Oakley in that the glutaraldehyde solution was buffered to pH 6.2 with a concentration of 0.05 M sodium citrate. In addition, three consecutive 5-min washes with double-distilled water were done after the gels were treated with the silver solution and before development with formaldehyde. Finally, to minimize the progressive background darkening occurring during storage, the gels were covered with a solution containing 0.02% methylamine and 0.05% citric acid (6). Gels were photographed after placing them in a Pyrex glass tray over an illuminated X-ray viewing box. Because electrophoresis of the specimens from normal and diabetic rats was carried out in an apparatus permitting simultaneous electrophoresis of two gels under identical conditions, and because the subsequent fixation, washing, and silver-staining reactions were carried out in the same vessels with the same reagents, differences in details of the respective patterns were regarded as primarily reflecting differences in the protein composition of the specimens and not as a result of artifactual differences resulting from the electrophoretic separation and staining.

Gel filtration of plasma or serum. Gel filtration was carried out on a column (76 × 2.5 cm) of Sepharose 6BCL conditioned with 0.1 M Tris-HCl buffer (pH 7.4) containing 0.3 M NaCl. The column had a void volume with respect to blue dextran (Pharmacia, Piscataway, NJ) of 145–155 ml. In a typical fractionation, 2.0 ml of plasma or serum was diluted with an equal volume of the Tris buffer, pumped onto the column, and followed by column buffer at a constant rate of 0.6–0.8 ml/min. After 130 ml of buffer had passed through the column, automatic fraction collection was started and

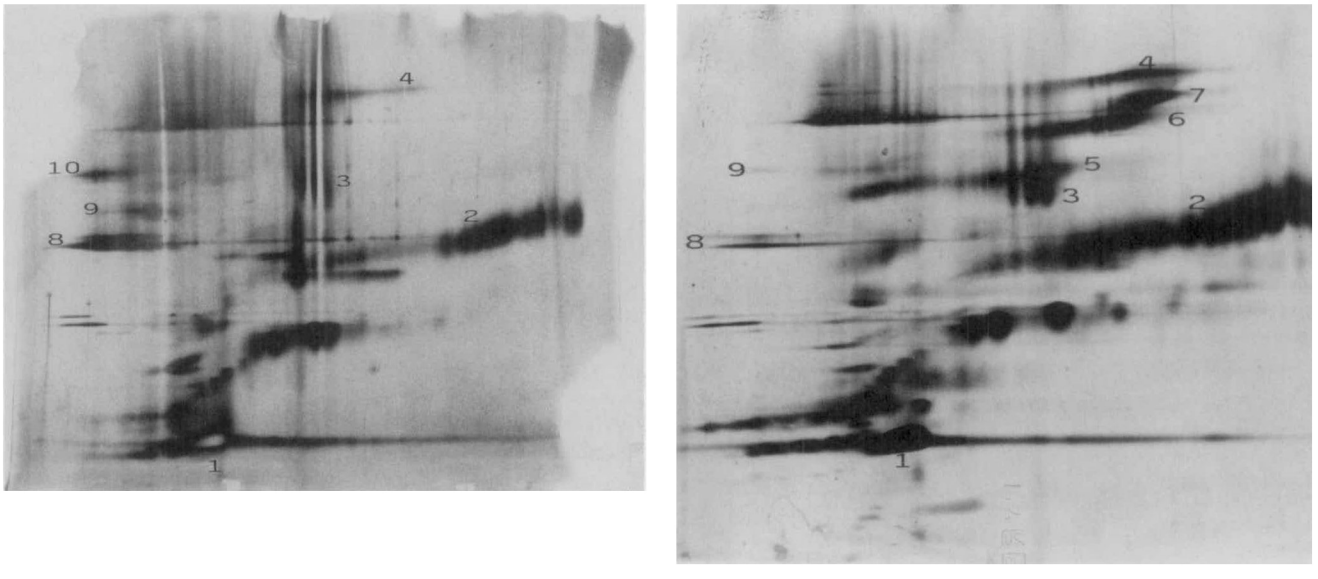

FIG. 1. Typical 2-dimensional gel patterns of undenatured rat plasma proteins after isoelectric focusing (anode at left) of 0.10 μl of plasma in 4% polyacrylamide gel; second dimension was run in linear 4–20% gradient polyacrylamide gel with anode at bottom. *Left*: from normal rat. *Right*: from untreated streptozocin-diabetic rat (11 wk after streptozocin). Presumptive identification of spots was based on comparison with spots of purified single proteins run under identical conditions: *1*, albumin; *2*, IgG; *3*, fibrinogen; *4*, IgM; *5*, IgA dimer; *6*, IgA trimer; *7*, IgA tetramer; *8*, *9*, and *10*, unidentified proteins of very low isoelectric point. Note that unidentified proteins *8*, *9*, and *10* of diabetic rats (*right*) are far fewer than those of normal rats (*left*).

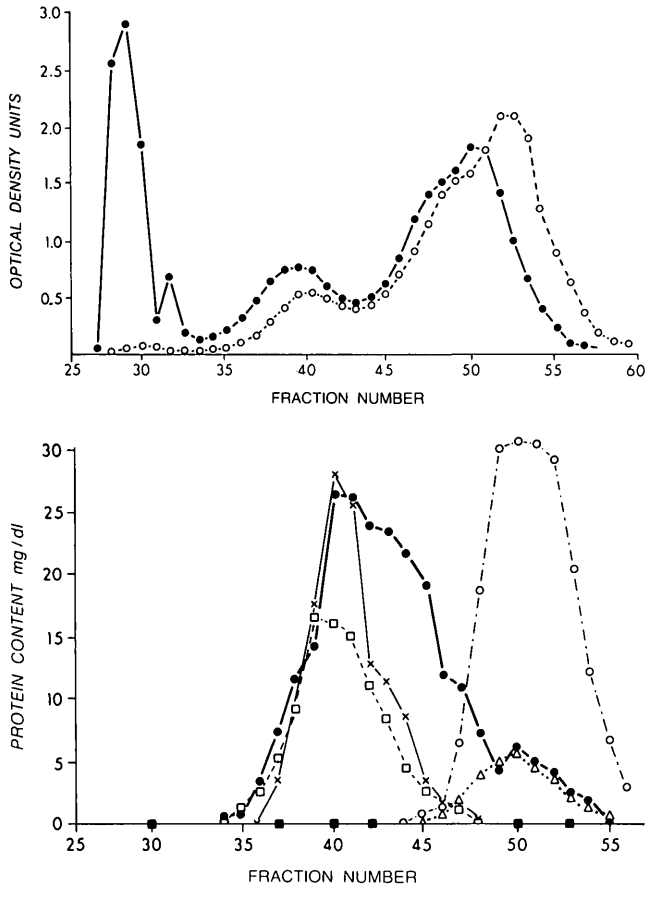

FIG. 2. *Top*: Sepharose 6BCL gel filtration patterns of plasma proteins in 2.0 ml plasma from normal control (○) and streptozocin-induced diabetic (●) rats (11 wk after streptozocin). Column was 76 × 2.5 cm; void volume (Blue Dextran 2000) was 145–155 ml (fractions 28–30); sample volume was 4.0 ml (2.0 ml plasma plus 2.0 ml Tris buffer, 0.1 M, pH 7.4). Note that area under high-molecular-weight peak (fractions 35–45) of diabetic pattern is grossly larger than that of normal. *Bottom*: fractions from gel filtrations (*top*) were analyzed for content of high-molecular-weight plasma proteins by single-radial immunodiffusion. X, α_1-macroglobulin; □, fibrinogen; △, C3 complement; ○, immunoglobulin G for diabetic plasma (normal control data similar; not shown); ■, IgA in normal plasma; ●, IgA in diabetic plasma.

continued until it totaled 400 ml. The optical density at 278 nM was measured for each fraction, and aliquots of each fraction were subjected to qualitative double immunodiffusion (Ouchterlony) analysis for specific plasma proteins; they were then subjected to quantitative single-radial immunodiffusion analysis by the method of Mancini et al. (7) as modified by Fahey and McKelvey (8).

Because Heremans (9) has emphasized the importance of routinely reducing and alkylating all IgA specimens of varying multimer content for a valid quantitative analysis of IgA content by single-radial immunodiffusion, both IgA standards and unknowns were first treated as follows: one volume of sample was mixed with an equal volume of 0.044 M dithiothreitol in 0.6 M degassed Tris-HCl buffer (pH 8.6) in a tightly capped conical microcentrifuge tube and allowed to stand at room temperature for 2 h; one volume of 0.048 M iodoacetamide in 0.6 M degassed Tris-HCl buffer (pH 8.6) was added, and the tightly capped mixture was allowed to stand overnight at room temperature. The samples were then subjected to single-radial immunodiffusion analysis.

Antisera. Antisera to rat IgA (α-chain specific), rat IgG (γ-chain specific), and rat IgM (μ-chain specific) were obtained from Cooper Biomedical (Malvern, PA) and PEL-Freez (Rogers, AR). Antisera to rat IgA were also prepared in our laboratory after first isolating secretory IgA (sIgA) antigen from the high-molecular-weight fractions of Sepharose 6BCL gel filtration of bile from normal bile fistula rats (10); only fractions found to react with the commercial α-chain–specific antiserum and that were free of significant protein contamination, as revealed by 2-D gel electrophoresis, were mixed with Freund's complete adjuvant and used to raise antisera in New Zealand white rabbits. The resulting antisera were freed of the nonspecific anti-rat Ig light-chain antibodies by repeated passage through an affinity column containing rat IgG bound to Sepharose 4BCL (11). Passages through an affinity column containing rat α_1-macroglobulin bound to Sepharose 4BCL and also through an affinity column containing whole–newborn-rat serum proteins bound to Sepharose 4BCL were also used to remove minor antibody impurities from some lots of anti-rat IgA antiserum as revealed by immunoelectrophoresis.

After absorption, the rabbit anti-rat IgA antisera (α-chain specific) were found to give reactions of identity with the two commercial goat anti-rat IgA (α-chain–specific) antisera when both were simultaneously compared by double immunodiffusion (Ouchterlony) against rat bile, diabetic rat serum, or an authentic specimen of rat oligomeric myeloma IgA (rat IR-22 myeloma IgA generously supplied by M. Cole, National Institutes of Health). Further absorption of some batches of anti-rat IgA antiserum by passage through an affinity column containing rat secretory component bound to divinyl sulfone–Sepharose 4BCL yielded antisera that did not react with purified rat secretory component.

Rabbit anti-rat IgA Sepharose 4BCL affinity column. A rabbit anti-rat IgA Sepharose 4BCL affinity column was prepared by first isolating the IgG fraction from 30 ml of absorbed rabbit anti-rat sIgA antiserum with a protein A–Sepharose column (Pharmacia) as described by Miller and Stone (12). After elution of the IgG, it was neutralized with excess 0.5 M potassium phosphate buffer (pH 7.4), con-

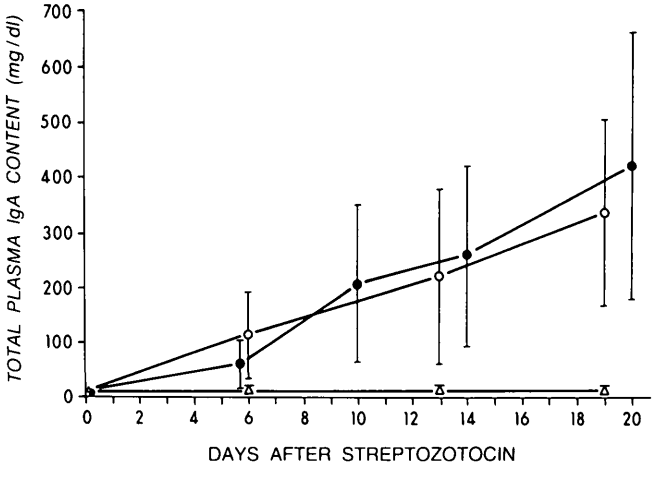

FIG. 3. Time course of increasing IgA content of plasma from 11 male (●) and 10 female (○) diabetic rats in first 20 days after induction of diabetes with streptozocin. △, Female control rats (n = 4). For IgA levels in diabetic rats at later times, see Table 1.

centrated by negative-pressure ultrafiltration, taken up in a small volume of 0.25 M $NaHCO_3$ (pH 9.2), and reacted with divinyl sulfone–Sepharose 4BCL as described by Sairam and Porath (11). The resulting rabbit anti-rat sIgA divinyl sulfone–Sepharose 4BCL was packed into a 0.9 × 20-cm Pharmacia column and used repeatedly, after appropriate elution and washing (11), to isolate IgA from normal rat serum and sIgA from the bile of a fistula rat. These eluted protein preparations were immediately neutralized with 0.5 M potassium phosphate buffer (pH 7.4) and concentrated by negative-pressure ultrafiltration with 0.2 M borate buffer (pH 8.0) in 0.16 M sodium chloride solution. After the protein content was measured, aliquots were taken to provide 50 μg of IgA or sIgA for iodination with 2.5 mCi of carrier-free ^{125}I (New England Nuclear, Wilmington, DE) by the oxidative ICl method of Helmkamp et al. (13); this procedure yielded a preparation of labeled protein with 1–2 atoms of iodine per molecule of IgA (reckoned as monomer) and a specific activity of 30–40 μCi/μg. The labeled protein was diluted to contain 0.8 g/dl of rat serum albumin as protective protein and then stored at −30°C.

^{125}I-sIgA clearance studies. To examine the possible role of impaired clearance (catabolism plus excretion) in the genesis of hyperimmunoglobulinemia, tracer doses of ^{125}I-IgA or ^{125}I-sIgA were given intravenously to normal control and untreated diabetic rats, and the time course of disappearance of ^{125}I activity from the circulation followed. Rats were lightly anesthetized with ether, and an incision was made in the skin of the inguinal region to reveal the femoral vein, into which 1.0 ml of solution containing 10 μCi of ^{125}I-labeled protein was injected in 5–10 s. The wound was then sutured, and a series of blood samples (0.5–0.6 ml each) was drawn from the tail vein tip at 10 min and 1, 6, 24, 48, and 72 h. The ^{125}I activity was measured in 100-μl aliquots of plasma, and the result was expressed as a percentage of the 10-min activity, which was assigned a value of 100%.

To control for the clearance of ^{125}I-sIgA from the circulation, particularly because isolation and iodination of the IgA may have altered the biological behavior of IgA (14), 2.0 ml of plasma from an untreated diabetic rat (with plasma IgA of 904 mg/dl) was injected into each of two normal rats (plasma IgA 9 and 20 mg/dl). The sampling procedure and timing were the same, and the time course of clearance was expressed in terms of the percentage of injected IgA remaining in the circulation based on the measured level of IgA at 10 min as 100%.

Rat secretory component. The secretory component was isolated from bile of fistula rats by the method of Underdown and Socken (15) and was used with Freund's complete adjuvant in rabbits to prepare an anti-rat secretory component antiserum. The resultant antiserum was absorbed by passage through an affinity column containing rat plasma proteins bound to divinyl sulfone–Sepharose 4BCL and reacted specifically with the original antigen on immunoelectrophoresis; however, the antiserum showed no reaction with rat (IR-22) myeloma IgA or with normal rat serum, and with rat bile it gave two arcs, corresponding to free secretory component and to sIgA as described by Altamirano et al. (10). Secretory component was also used to prepare an affinity column with secretory component covalently bound to Sepharose 4BCL through divinyl sulfone (4).

Rat α_1-macroglobulin affinity column. Using the method of Sairam and Porath (11), we prepared an affinity column for rat α_1-macroglobulin after isolating protein as described by Gauthier et al. (16). The rat α_1-macroglobulin was also used to raise an antiserum in New Zealand white rabbits, and the resulting antigen, after being found to react monospecifically against whole-rat serum and against the original antigen, was used for quantitative single-radial immunodiffusion analysis. Monospecific rabbit anti-rat fibrinogen antiserum was prepared as described by John and Miller (17).

Rat bile. We obtained bile from routine cannulation of the bile duct while carrying out isolated perfusion of livers (18,19) from normal and diabetic rats for collection periods of 12 or 24 h. Specimens of bile that had been stored at −30°C were available for analysis of secretory component and sIgA. Bile was also obtained from three normal and three diabetic rats, not treated with insulin, in which cannulation of the bile duct was carried out under ether anesthesia; PE10 polyethylene tubing cannula was attached to silastic tubing (Dow Corning 602-105) that was exteriorized to permit continuous collection of the total bile secreted. The rats studied in this procedure were maintained in a restraining holder, similar to that described by Bollman (20), and were allowed continuous access to food and water, which was mixed with an equal volume of Ringer solution.

RESULTS

Application of 2-D gels. When we applied 2-D gels (isoelectric focusing followed by polyacrylamide gradient gel electrophoresis) to a series of samples of plasma or serum from normal control and STZ-D rats not treated with insulin, under nondenaturing conditions, significant gross qualitative and quantitative differences were revealed in the silver-stained patterns of the proteins. Figure 1 shows typical protein patterns obtained from normal and diabetic rat plasma specimens. Characteristic changes associated with untreated diabetes have not been observed before 8–12 days of diabetes.

We found two major qualitative differences between the normal (Fig. 1, *left*) and diabetic (Fig. 1, *right*) protein patterns of our plasma specimens. Proteins 5, 6, and 7 are

TABLE 1
Persistent increase of plasma IgA levels in untreated streptozocin-induced diabetic rats is minimized by daily insulin treatment

Duration of diabetes (wk)	Insulin treatment (U/day)	*n*	Plasma IgA level (mg/dl)
6–10	0	15	551 ± 217
15–23	0	10	532 ± 336
6–9	1.5	4	42 ± 39
32–35	1.5	7	40 ± 26
51–53	1.5	5	15 ± 3

Samples of plasma were obtained by bleeding from the inferior vena cava incidental to the operation for the isolation and perfusion of livers from diabetic rats for other studies (20). Assays for total IgA were carried out by single-radial immunodiffusion, after reduction and alkylation, by comparison with similarly reduced and alkylated standards prepared from a pool of plasma from several diabetic rats; the IgA content of the latter was measured in comparison with a purified sample of rat sIgA isolated from a normal bile fistula rat. Plasma IgA levels are means ± SD.

TABLE 2
Comparison of total secretory component and secretory IgA in pooled bile secreted during 24-h perfusion of livers from normal and streptozocin-induced diabetic rats

	n	Total secretory component (mg)		Total sIgA (mg)	
Diabetic					
6–10 wk after streptozocin	7	1.45 ± 0.48	$P < .001$	3.63 ± 1.76	$P < .07$
After 8 wk with daily insulin treatment	5	3.76 ± 0.57	$P < .001$	2.25 ± 0.80	$P < .001$
			$P < .07$		$P < .005$
Normal	10	2.87 ± 1.14		1.19 ± 0.44	

All perfusions were carried out with standard perfusion medium of a suspension of bovine red cells in Krebs-Ringer bicarbonate buffer containing 3% bovine serum albumin, with a continuous infusion of glucose and a balanced mixture of amino acids (18). Total secretory component and total IgA content of total pooled bile collected during 24-h perfusion of livers isolated from normal and diabetic liver donors (20) are shown; values are means ± SD. Samples were stored at −30°C, then thawed and centrifuged to remove small amounts of precipitate; aliquots were analyzed by single-radial immunodiffusion after reduction and alkylation. Significant differences are based on Student's *t* test.

absent from the normal specimen, and the spot corresponding to protein 4 appears more intense in the diabetic than in the normal rat. The appearance of proteins 5, 6, and 7 occurred without exception in gels of plasma or serum specimens taken from more than 30 STZ-D rats 8–120 days after the onset of diabetes, untreated with insulin. Similar 2-D gel patterns with proteins 5, 6, and 7 were obtained with plasma specimens taken from 3 of the alloxan-induced diabetic rats after 23 days of diabetes. But these proteins did not appear in specimens taken from 16 diabetic rats treated with daily insulin injections, nor did they appear in gels of specimens taken from more than 20 normal control rats.

Figure 1 also reveals the disappearance of proteins 9 and 10, and the substantial decrease in the double protein band 8 in going from the normal to the diabetic state. Proteins 5, 6, and 7 have relatively high isoelectric ranges, their molecular weights are presumptively equal to or greater than that of fibrinogen (protein 3). The possible identity of proteins 5, 6, and 7 was suggested by the report of Orlans et al. (21), describing a procedure in which the triad of IgA dimer, trimer, and tetramer was portrayed in a 2-D gel of rat bile (with a 2.5–27% acrylamide gradient).

Gel filtration of diabetic and normal rat plasma on Sepharose 6BCL. Figure 2 (*top*) presents the results of a typical fractionation of samples of normal and STZ-D rat plasma on Sepharose 6BCL. The marked lipemia of the diabetic rat plasma specimen is reflected in the substantial optical density of the grossly turbid fractions 28–33. When subjected to Ouchterlony double-diffusion analysis, fractions 37–44 for both the normal and diabetic plasma reacted positively to specific antisera against rat fibrinogen, rat α_1-macroglobulin, and rat IgM (μ-chain specific). Fractions 36–44 of the diabetic rat plasma reacted very strongly to antiserum to rat IgA (α-chain specific), but the corresponding fractions from normal rat plasma were negative. Accordingly, quantitative single-radial immunodiffusion analysis of these gel-filtration fractions was carried out for the several proteins in the high-molecular-weight peak, and the results are presented in Fig. 2 (*bottom*). The broad size (mass) distribution of proteins reacting with specific anti-α-chain reagent is consistent with the occurrence of IgA oligomers, although the occurrence of IgA-reactive material in fractions 48–55 of the diabetic plasma support the presumption that there is also a significant increase in monomeric IgA with a gel-filtration behavior analogous to monomeric IgG (Fig. 2, *bottom*).

Time course of plasma IgA levels. For male and female rats the increase of plasma IgA levels for the first 20 days after STZ administration is presented in Fig. 3. The IgA levels of both male and female rats rose in linear fashion to mean values 30–40 times greater than those of the pre-STZ values and those of the normal female controls; the latter were not significantly different from plasma IgA levels of samples taken from 15 male rats of similar age with a mean ± SD of 9.5 ± 2.6 mg/dl. After 3 wk the plasma IgA levels of untreated STZ-D rats leveled off at mean values of ~500 mg/dl as indicated by analysis of samples taken from untreated STZ-D rats 6–23 wk after the onset of diabetes, at the time of death for isolated liver perfusion (Table 1). Daily treatment with insulin in comparable groups of male STZ-D

TABLE 3
Appearance of sIgA and secretory component during isolated perfusion of normal rat liver

Hour of bile collection	Bile volume (ml)	Total secretory component (mg)	Total IgA (mg)
1	1.0	0.20	1.11
2	1.1	0.27	0.12
3	0.80	0.34	0.02
4	0.65	0.35	0.01
5	0.57	0.34	0.01
6	0.55	0.38	0.00
7	0.47	0.34	0.00
8	0.46	0.29	0.00
9	0.38	0.24	0.00
10	0.35	0.20	0.00

Perfusion was carried out with standard perfusion medium of a suspension of bovine red cells in Krebs-Ringer bicarbonate buffer containing 3% bovine serum albumin, with a continuous infusion of glucose and a balanced mixture of amino acids (18). Fractionation of bile during isolated perfusion of normal rat liver reveals IgA present only in bile secreted during first 2 h; secretion of secretory component persisted throughout the 10-h course of perfusion. Measurement of the content of secretory component was made on samples of bile (without reduction and alkylation) compared with standards of purified rat secretory component by single-radial immunodiffusion.

TABLE 4
Total biliary excretion of IgA by untreated diabetic and normal bile fistula rats

	Total IgA in 24-h collection (mg)	
Day	Normal	Diabetic
2	47.5	72.5
3	42.7	82.8
4	63.4	83.4

Diabetic rat was administered streptozocin 13 mo before being killed for this experiment; at death, blood glucose was 320 mg/dl.

rats resulted in mean plasma IgA levels of 15–42 mg/dl (Table 1).

IgA content of bile secreted by rat liver and total bile fistula. Because the liver in normal rats plays a major role in excreting IgA as sIgA (22,23), we compared the IgA content in the total bile secreted during 24-h perfusion of livers isolated from normal rats and from STZ-D rats treated with insulin and untreated. Table 2 shows that the bile from perfusions of insulin-treated or untreated diabetic livers contained significantly more IgA than the bile from perfused normal rat livers ($P < .005$ and $P < .001$, respectively); the IgA content of bile from livers of diabetic rats was not significantly different from that of the bile secreted by perfused livers from insulin-treated diabetic rats ($P < .07$). Livers from both normal and insulin-treated diabetic rats secreted more secretory component in bile than the livers from untreated diabetic rats ($P < .001$).

Table 3 presents the results of a perfusion of a liver isolated from a normal rat in which the bile was collected in separate hourly fractions for 10 h. Analysis for the content of total IgA and of total secretory component in the separate fractions revealed that after the 1st or 2nd h of isolated perfusion, the bile fractions contain virtually no detectable IgA. However, the content of free secretory component increases and persists after the 1st h. These observations are compatible with the accepted view that the liver apparently does not synthesize IgA but is capable of secreting it as sIgA (23,24); when IgA is not available for conjugation and excretion with

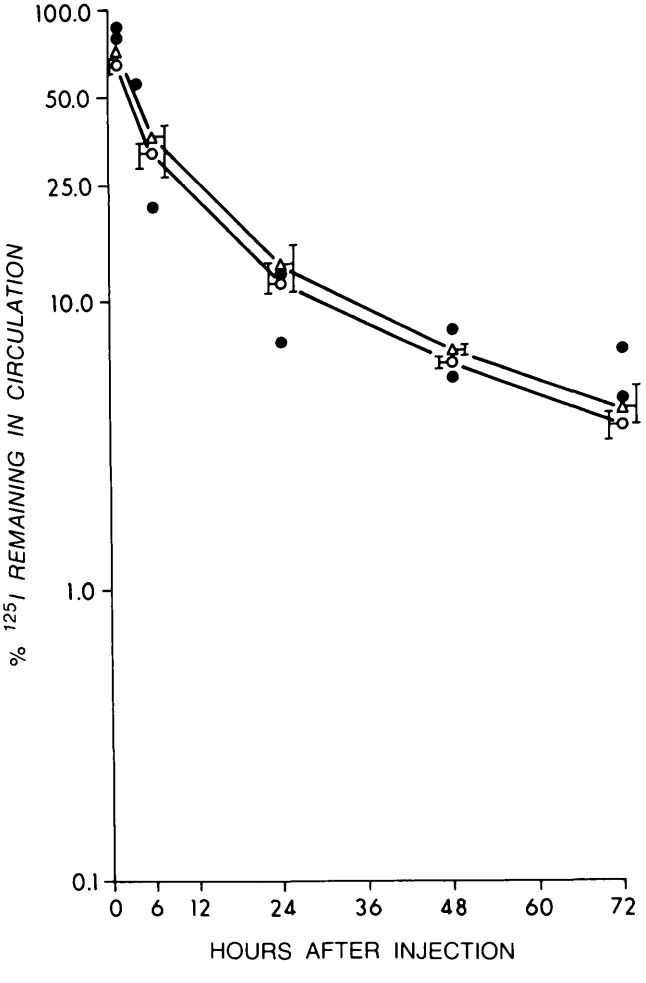

FIG. 4. Time course of disappearance of intravenously injected ^{125}I-labeled IgA from circulation of 4 normal (○) and 4 streptozocin-induced diabetic (△) rats (see RESULTS). Mean ± SD total initial plasma IgA content of 4 diabetic and 4 normal rats was 722 ± 289 and 10.0 ± 2.6 mg/dl, respectively. Calculation of percent ^{125}IgA remaining in circulation is based on ^{125}I activity of 100% at 10 min. *Points* are means, and *bars* show extremes. ●, Time course of disappearance of IgA from circulation of 2 normal control rats (plasma IgA content 9.0 and 20.0 mg/dl) after each was injected with 2.0 ml i.v. of plasma from untreated diabetic rat with IgA content of 920 mg/dl.

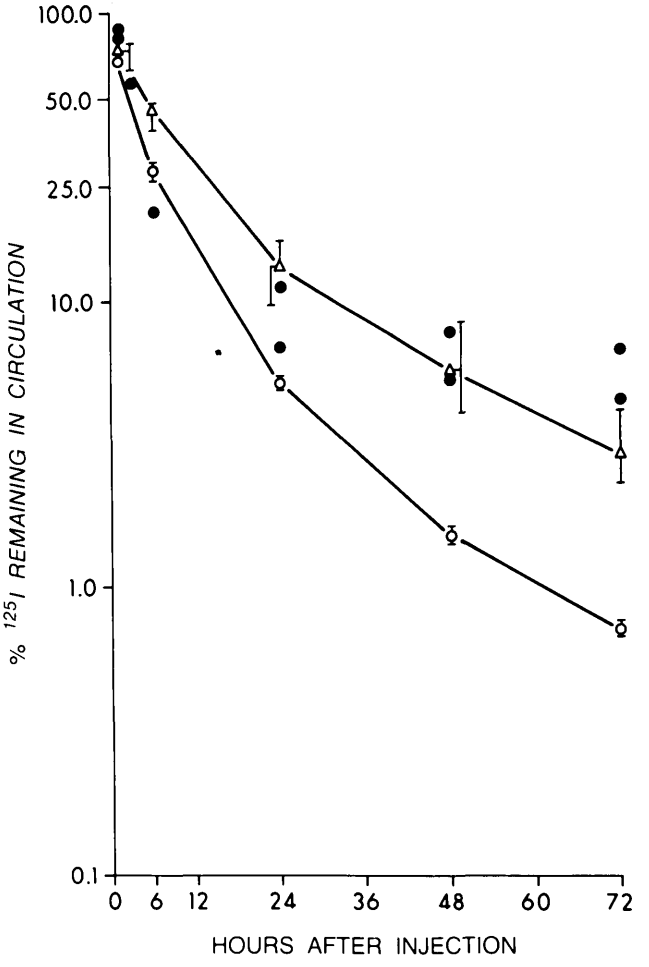

FIG. 5. Time course of disappearance of intravenously injected ^{125}I-labeled secretory (sIgA) (see RESULTS). Mean ± SD of total initial plasma IgA content of 4 diabetic (△) and 4 normal (○) rats was 628 ± 260 and 11.2 ± 8.2 mg/dl, respectively. Calculation of percent ^{125}I-sIgA remaining in circulating plasma is based on ^{125}I-sIgA of 100% at 10 min. *Points* are means, and *bars* show extremes. Disappearance of unlabeled IgA from 2 ml of diabetic plasma intravenously injected into each of 2 normal control rats (●) is included here for comparison with Fig. 4.

secretory component, free secretory component continues to be synthesized and excreted up through the 10th h of perfusion.

A comparison of the total biliary excretion of IgA by surviving normal and diabetic bile fistula rats is presented in Table 4. The data clearly indicate that the 24-h biliary excretion of IgA by the diabetic rat exceeded that of the normal rat and is consistent with the results of the isolated liver perfusions (Table 2) and with the view that the liver of the untreated diabetic rat is capable of excreting at least as much or more IgA in the form of sIgA than the liver of the normal rat. Further, impairment of the diabetic rat livers' capacity to excrete IgA cannot by itself be the cause of the hyperimmunoglobulinemia.

Clearance of ^{125}I-sIgA and unlabeled IgA. The clearance of intravenously injected ^{125}I-IgA was not significantly different in four diabetic rats compared with four normal controls (Fig. 4). The identity of the general course of disappearance of the ^{125}I-labeled and unlabeled IgA is compatible with the notion that the labeled proteins were not substantially altered by the preparation of the ^{125}I-IgA.

Figure 5 depicts the comparison of the clearance of ^{125}I-sIgA after intravenous administration to four normal and four untreated diabetic rats. The ^{125}I-sIgA used in these experiments was prepared from sIgA isolated from normal rat bile, which is mostly polymeric and bound to secretory component (22–24). The normal rat seems to have cleared the labeled sIgA from the circulation at a rate that, during the first 24 h, was ~10% faster than the rate for the diabetic rat.

DISCUSSION

The early occurrence and persistence of grossly increased levels of IgA in the plasma of untreated STZ-D rats is an interesting phenomenon for several reasons. *1*) The occurrence of hyperimmunoglobulinemia in several untreated alloxan-induced diabetic rats (and to a lesser but still significant degree in several BB/W diabetic rats treated with insulin; unpublished observations) suggests that rat experimental diabetes mellitus in general is associated with disordered IgA metabolism. *2*) Direct involvement of insulin in the regulation of IgA metabolism is suggested because the hyperimmunoglobulinemia A in insulin-treated diabetic rats is markedly attenuated, albeit to levels still two to three times higher than normal. The report by Triolo et al. (2,3) describing increased levels of IgA and IgA immune complexes in blood of both insulin-dependent and non-insulin-dependent human diabetes and their clinical observations indicating a correlation of increased plasma IgA levels and increased IgA immune complexes with renal and retinal complications of diabetes suggest a role for IgA in the pathogenesis of human renal and retinal microangiopathy.

Lemaitre-Coelho et al. (1) and others have demonstrated that total bile duct obstruction in the rat can cause increased levels of plasma IgA consisting largely of multimeric IgA; this finding raised the question of whether there was impaired excretion of IgA in the bile of the untreated diabetic rat. We measured the total excretion of sIgA in bile collected over several days from the total bile fistula secretion of normal and untreated STZ-D rats and found that a much greater amount of sIgA was secreted by the diabetic rat than by the normal bile fistula rat. Moreover, the greater-than-normal secretion of sIgA in the total bile from isolated perfused diabetic livers affirms the view that there is no demonstrable element of biliary obstruction to the secretion of sIgA by the diabetic liver.

There remained the possibility that pathways of metabolism or excretion other than the hepatobiliary may be impaired in the untreated diabetic rat. However, the intravenous clearance studies carried out with ^{125}I-labeled IgA and sIgA revealed that the clearance rates of the former were unchanged in the diabetic rat, and the clearance rates of the latter were ~10% slower in the diabetic rat; this result was not sufficient to account for the gross increase in IgA levels in the plasma. This slower clearance of ^{125}I-sIgA from the plasma of untreated diabetic rats must be interpreted in terms of the relatively larger pool of polymeric IgA in the untreated diabetic rat. Quantitative measurements of plasma IgA levels at the time of injection of the ^{125}I-IgA afford a basis for the measurement of the pool size, which indicates that the actual mass turnover rate of sIgA in the untreated diabetic rat must be substantially greater than it is in the normal rat; the implication is that the actual mass of IgA synthesized by the untreated diabetic rat to maintain the observed high plasma levels must be correspondingly much larger. For example, assuming an equal 10-ml plasma volume of distribution in both normal and diabetic rats and a normal plasma level of total IgA equal to 15 mg/dl and sIgA of <0.15 mg/dl (25), and with the fractional turnover rate of 4%/h for the first 24 h, then the calculated mass turnover rate for IgA equals ~0.06 mg/h in the normal rat. In the diabetic rat with the fractional turnover rate of sIgA of 3.6%/h and with plasma total IgA content of 500 mg/dl, the mass turnover rate equals 1.8 mg/h, or >30 times normal.

The observed rates of disappearance of intravenously injected IgA (labeled and unlabeled) are similar to what may be estimated from the observations of Vaerman et al. (26) on the rate of mesenteric lymph flow and its IgA content; they concluded that the number of times per day the circulating IgA pool is renewed due to the sole contribution of the gut wall was 6.5–32.5.

The interpretation of the data on the clearance of ^{125}I-sIgA (Fig. 5) from the plasma of normal and diabetic rats may be limited by a lack of quantitative measurement of the actual proportion of sIgA in the total plasma IgA, particularly of the untreated diabetic rat. We have recently developed a microradioimmunoassay for rat plasma sIgA with purified rat secretory component as a standard, which is analogous to the radioimmunoassay method of Delacroix and Vaerman (25) for measuring sIgA in human plasma. Our measurements indicate that the maximum content of sIgA in the plasma of normal and untreated diabetic rats is ~0.1% and 0.6%, respectively, of the total plasma IgA (unpublished data); this finding assumes that only one molecule of secretory component (70,000 M_r) is covalently bound to one molecule of multimeric IgA (average M_r 540,000).

The possibility of increased synthesis of IgA associated with either an increase in the number of IgA-producing cells, a grossly increased rate of IgA synthesis per cell, or both remains to be considered. Similar possibilities have been explored in attempts to define the pathogenesis of human idiopathic IgA nephropathy, and both increased numbers of IgA-producing cells and increased synthesis of IgA by them

in vitro have been demonstrated (27,28). In this connection, we have confirmed observations (29) that the untreated STZ-D rat manifests a grossly enlarged upper gastrointestinal tract, and in particular, hyperplasia of Peyer's patches and thickened hyperplastic-appearing duodenal and jejunal mucosa. Experiments seeking to define the proportion of gut-associated plasma cells producing IgA and quantitative measurement of IgA produced in cell culture by gut-associated lymphoid tissues should help define the potential contribution of these factors to the genesis of hyperimmunoglobulinemia in untreated experimental diabetes mellitus.

The demonstrated occurrence of increased levels of circulating IgA in rats after the oral or intraperitoneal administration of antigen (30) and the occurrence of increased levels of IgA and of circulating immune complexes containing IgA in human idiopathic IgA nephropathy (31,32) suggested that the hyperimmunoglobulinemia of the untreated diabetic rat may at least in part be the result of excessive antigenic stimulation and that increased levels of circulating immune complexes containing IgA could result. Furthermore, because of the known accumulation of immune complexes by the renal glomerular mesangium (33), it seemed reasonable to examine the untreated STZ-D rat for circulating immune complexes containing IgA and for renal involvement. (See companion article, this issue, p. 185.)

The literature on the regulation of the numbers and the productivity of IgA-producing cells has been reviewed by Elson (34). This regulation appears to involve a complex interaction of lymphoid B-lymphocyte precursors, so-called switch, helper, and suppressor T-lymphocytes, and the possible role of interleukins. Our work suggests the obvious speculation that insulin may be necessary for the normal action of one or more of these regulatory cell types in the control of IgA production.

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