Degradation of Circulating DNA by Extracorporeal Circulation over Nuclease Immobilized on Nylon Microcapsules

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ABSTRACT Studies were undertaken to determine whether deoxyribonuclease I, (DNase I) once immobilized on activated nylon microspheres, would be capable of degrading circulating DNA in vitro and in vivo in an extracorporeal circulation system in dogs. Nylon microspheres were prepared and after gentle hydrolysis and glutaraldehyde treatment, demonstrated a retention of up to 4.73 mg of Dnase I. In vitro studies showed that DNase I immobilized on microspheres degraded a significant percentage of ^{125}I-native DNA (nDNA) within 15 min. Mongrel dogs were injected with ^{125}I-nDNA and a variation in initial $t_{\frac{1}{2}}$ in individual animals was observed. Therefore, for experimental studies, ^{125}I-nDNA was injected and decay was recorded during a control period in which untreated microcapsules were utilized in the extracorporeal system. DNase I microspheres were then introduced into the extracorporeal circuit which resulted in an acceleration of degradation of acid precipitable ^{125}I-nDNA. When 200 μg of unlabeled DNA with ^{125}I-nDNA was injected, a similar augmentation of DNA degradation was noted after extracorporeal circulation over DNase I microcapsules. This effect could not be attributed to release of DNase I from the microspheres since no ^{131}I-DNase was detected in the serum or organs of the dogs at the conclusion of the experiments.

^{125}I-nDNA : anti-DNA complexes were passively injected into dogs and after a similar control period of circulation over untreated microcapsules. DNase I microspheres were introduced. Results showed a rapid acceleration in the degradation rate of ^{125}I-nDNA : anti-DNA complexes precipitable with $(NH_4)_2SO_4$. Extracorporeal circulation over nylon microspheres resulted in no significant alteration of the host's hematocrit or platelet count, and little residual cellular debris on the microcapsules. These data suggest that DNAase immobilized on nylon microspheres may have a potential role in the specific therapy of systemic lupus erythematosus, when it is desirable to hydrolyze DNA circulating free or in combination with antibody.

INTRODUCTION

The role of DNA : anti-DNA complexes in the pathogenesis of some cases of systemic lupus erythematosus (SLE)[1] has been suggested and there is now considerable evidence to support this possibility. DNA antibodies and DNA : anti-DNA complexes occur frequently in association with acute exacerbations of the disease and DNA has been shown occasionally in the sera of patients with acute lupus nephritis after disappearance of detectable anti-DNA (1–4). Further, DNA has been demonstrated along the glomerular basement membrane by fluorescent antibody technique and anti-DNA antibodies have been eluted from postmortem kidneys of patients with SLE (2, 3).

Therapy for SLE has largely been reliant upon immunosuppressive drugs that nonspecifically depress host immunity leaving the patient vulnerable to a wide variety of infectious agents. Another therapeutic approach would appear to be the specific removal from the circulation of the immune substances that are pathogenic in the disease. Preliminary studies utilizing an immuno-

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[1] *Abbreviations used in this paper:* DNase I, or DNase, deoxyribonuclease I; nDNA, native DNA; SLE, systemic lupus erythematosus; TCA, trichloroacetic acid.

adsorbent composed of DNA-cellulose entrapped in agarose showed the feasibility of specifically withdrawing DNA antibody from the circulation of actively immunized rabbits (5, 6). The degradation of circulating DNA or of DNA:anti-DNA complexes would be another therapeutic approach. To accomplish this, we prepared nylon microspheres approximately 1.1 mm in diameter, to which deoxyribonuclease I (DNase I) was conjugated. Similar micospheres, previously employed to encapsulate enzymes for the extracorporeal treatment of chronic renal failure (7, 8), have several properties which make them suitable for use in extracorporeal systems. These include (*a*) a large surface area to allow covalent conjugation of considerable quantities of protein, (*b*) minimal toxicity to the host due to chemical inertness, (*c*) structural stability which reduces the possibility of embolization, and (*d*) minimal thrombogenicity which avoids reduction in circulating platelets.

In previous studies, antigen or antibody has been conjugated to activated nylon in vitro and binding to radiolabeled antibody has been demonstrated, both in vitro and in vivo (9). In the present study, we subjected nylon microcapsules to gentle hydrolysis and treatment with glutaraldehyde, thereby creating a surface capabe of linking the enzyme DNase I.

DNase I is a heat stable exonuclease of mol wt 40,000 which is predominantly intracellular and dependent on divalent cations and an optimal pH for maximum activity (10–13). It is capable of cleaving DNA into low molecular weight fragments by hydrolyzing phosphodiester linkages. In the present study, we covalently conjugated DNase I to activated nylon microcapsules and have shown that this immobilized enzyme, when placed in an extracorporeal circuit, was capable of degrading significant quantities of DNA in vitro and in vivo. Degradation of DNA, both circulating free or complexed with anti-DNA, was observed. The immobilized deoxyribonuclease (DNase) was efficient, there was no detectable release of the enzyme into the host circulation or vital organs, and minimal alterations of host hematocrit and platelet counts.

METHODS

Animals. Mongrel dogs, weighing from 7.7 to 16.5 kg were employed in these studies.

Enzyme and antigen. DNase I (Worthington Biochemical Corp., Freehold, N. J.) contained 2,700 U/mg. Native calf thymus DNA (Worthington Biochemical Corp.) was deproteinized as previously described (4).

Labeling of enzyme and antigen. Deproteinized DNA was labeled with ^{125}I by the method of Comerford (14) and DNase was labeled with ^{131}I by the method of McConahey and Dixon (15).

Preparation of microcapsules. Nylon microcapsules were prepared as follows: a solution containing 9.2 g of 1,6-hexanediamine, 1.6 g of NaH_2CO_3, and 6.6 g of Na_2CO_3 in 100 ml of distilled water was prepared. A second solution consisting of 50 ml of chloroform, 200 ml of cyclohexane, and 2 ml of sebacolyl chloride was prepared and placed in a 500-ml beaker. A Pasteur pipette was tapered with heat to an internal diameter between 0.1 and 0.05 mm. The diamine solution was rapidly added dropwise into the second solution and solid nylon capsules 1.0–1.1 mm in diameter were formed at the bottom of the beaker. The capsules in the solution were swirled to separate them and then poured through a 20 mesh screen to dry them and remove debris. The capsules were then enclosed between a 42-mesh standard testing sieve and a 170 mesh sieve and washed with 2,000 ml of distilled water. Capsules were collected and stored in distilled water at 4°C.

Conjugation of DNase to microcapsules. Microcapsule chambers consisted of a glass cylinder 8 cm in length with an internal diameter of 2.5 cm with 40 mesh stainless steel screens at the ends (R. H. Allen Co., Boulder, Colorado). This chamber was filled with approximately 300 microcapsules 1.0–1.1 mm in diameter and then attached to a roller pump by polyethylene tubing. The capsules were washed with 2,000 ml of distilled water at 30°C and then gently hydrolyzed with 500 ml of 3 M HCl which was circulated at 30°C for 60 min at a flow rate of 30 ml/min. The capsules were washed with distilled water at 0°C. Glutaraldehyde, 4% wt/vol (Eastman Organic Chemicals Div., Rochester, N. Y.) in 0.1 M borate buffer pH 8.3 was circulated for 15 min at 0°C and the capsules then washed with 0.1 M borate buffer pH 8.3 at 0°C. The pump tubing was changed. DNase (15 mg) plus ^{131}I DNase (1 μg) as a marker was circulated in 50 ml of 0.1 M phosphate buffer pH 7.05 containing 100 mg of $MgCl_2$ at 0°C for 1 h. Capsules were then washed with 500 ml of 0.1 M phosphate buffer pH 7.05 containing 1,000 mg of $MgCl_2$ until the wash contained no ^{131}I counts per minute above background. The capsules were warmed to room temperature, and transferred in solution to a siliconized chamber for in vivo use.

In vivo extracorporeal system. Dogs were anesthetized with sodium pentabarbital and the femoral artery and vein cannulated with wide bore polyethylene tubing. Sodium heparin, 3 mg/kg, was injected intravenously and the femoral artery and venous catheters were connected to a Travenol hemodialysis pump (Travenol Laboratories, Deerfield, Ill.) and then to the microcapsule chamber. The latter had been coated with silicone (Clay Adams, Parsippany, N. J.) just before use. A three-way stop clock assembly made it possible to reverse the flow of blood through the chamber at 7–12-min intervals to prevent the microcapsules from impeding flow by clogging the screens. The chamber was connected to a bubble trap and then to the femoral vein. Heparinized blood was circulated through the extracorporeal system for 20 min before injection of DNA. Flow rate was sustained at 200 ml/min during the entire procedure. Samples of blood were withdrawn from the venous line and sampled for ^{125}I-nDNA and degradation products at various intervals. Schematic representation of this system is shown in Fig. 1.

Measurement of acid precipitable DNA. This was performed by adding 0.2 ml of dog serum to 1 ml of cold 0.1 M phosphate buffer pH 7.05. To this solution, 10% trichloroacetic acid (TCA) (1 ml) at 4°C was added. After centrifugation at 2,000 rpm for 15 min at 4°C, the supernate was removed with a Pasteur pipette. The precipitates were counted for 30 min in a Picker gamma scintillation counter. Values in graphs represent counts per minute of ^{125}I-nDNA precipitated by TCA.

Preparation of DNA:anti-DNA complexes. Various quantities of native DNA (nDNA) were added to 0.1 ml-

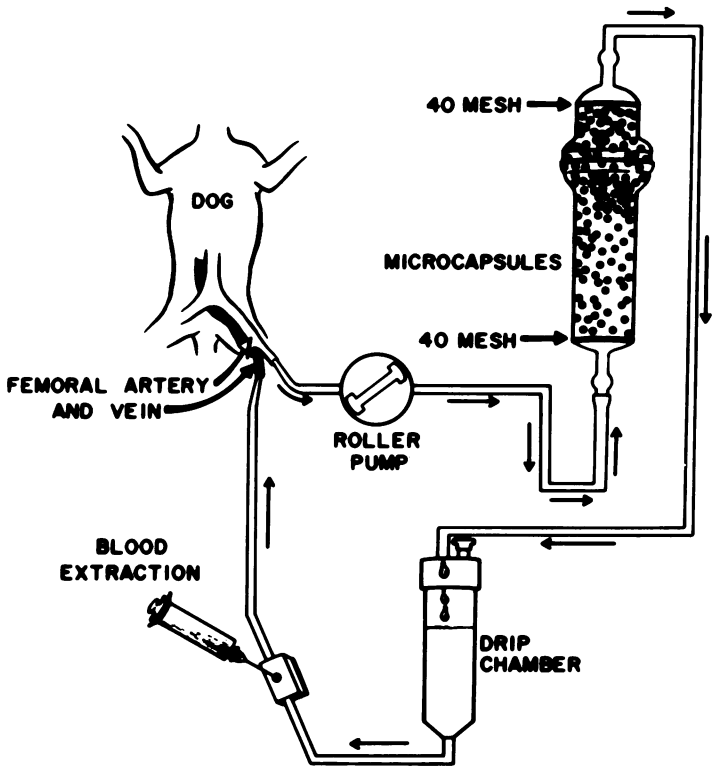


FIGURE 1 Schematic representation of the in vivo extracorporeal system is depicted.

portions of antisera to nDNA obtained from a patient with SLE. The mixture was incubated at 4°C for 72 h and then centrifuged at 2,000 rpm for 30 min at 4°C. The precipitates were washed three times in cold phosphate buffered saline pH 7.0 and redissolved in 0.05 ml of 0.1 N NaOH. The protein content was measured using the Lowry et al. method (16), with the OD read at 720-mu in a Beckman 25 spectrophotometer. The quantitative precipitin curve was plotted and the point in antigen excess at which no spontaneous precipitation occurred was determined. For the serum used, this point was at 62 μg of DNA/ml serum. Soluble DNA: anti-DNA complexes were prepared by incubating 62 μg of ^{125}I-nDNA with 1 ml of the same antiserum for 72 h at 4°C. The mixture was centrifuged at 2,000 rpm at 4°C for 30 min, the supernate was decanted, and an equal volume of saturated $(NH_4)_2SO_4$ was added to it. The mixture was incubated and centrifuged for 30 min at 4°C. The supernate was discarded, the precipitate redissolved in phosphate buffered saline pH 7.0 and the process repeated until the precipitate contained more than 95% of the ^{125}I-DNA present. The precipitate was redissolved in phosphate buffered saline pH 7.0 and dialyzed against phosphate buffered saline for 48 h. The final solution contained all the gamma globulin and also 10 μg of ^{125}I-nDNA in the DNA: anti-DNA complexes.

Measurement of DNA: anti-DNA complexes in vivo. To 0.5 ml of serum was added 0.5 ml of saturated $(NH_4)_2SO_4$ plus 5 ml of 50% saturated $(NH_4)_2SO_4$. The mixture was incubated for 30 min at 4°C and then centrifuged for 15 min at 4°C. The supernate was decanted and the precipitate was counted in a gamma scintillation counter (Picker Corp., Cleveland, Ohio).

Hematologic studies. Leukocyte counts on dog blood were performed in the Coulter counter (Coulter Electronics Inc., Hialeah, Fla.). Hematocrits were determined by centrifugation of whole blood in capillary tubes. Platelet counts were performed in a Neubauer counting chamber (Neubauer Mfg. Co., Minneapolis, Minn.).

RESULTS

Retention of DNase on microcapsules. DNase (15 mg) was circulated through a chamber containing approximately 300 microcapsules and conjugated to the nylon as described in Methods. After use of the capsules for in vitro or in vivo experiments, they were removed from the chamber, washed with 300 ml of saline and counted in a Picker gamma scintillation counter. The quantity of DNase I retained was calculated by determining the percentage of the total ^{131}I-DNase added which was bound to the capsules at the conclusion of the experiment. The results shown in Table I demonstrate that under the conditions described between 26.8 and 31.5% (4.02–4.73 mg) of DNase was retained on the microcapsules (Table I).

Degradation of labeled nDNA by immobilized DNase in vitro. Fresh normal dog blood (100 ml) was drawn from the femoral artery to which 300 U of sodium heparin was added. ^{125}I-nDNA, 3.0×10^5 cpm (2 μg) plus $MgCl_2$ (100 mg) was added to the blood. The

TABLE I

DNase I Retention on Microcapsules

Dog	Quantity of DNase added	^{131}I DNase added	^{131}I DNase recovered on microcapsules	DNase retained on microcapsules	Quantity of DNase retained on microcapsules
no.	*mg*	*cpm*	*cpm**	%	*mg*
4a	15	80,300	22,031	27.4	4.11
4b	15	64,550	17,310	26.8	4.02
4c	15	76,300	21,504	28.2	4.23
In vitro	15	126,580	39,913	31.5	4.73

* Microcapsules were washed with 500 ml saline and counted at the conclusion of in vitro or in vivo experiments.

mixture was incubated at 27°C for 15 min and then drawn into polyethylene tubing and circulated by a roller pump through a chamber containing DNase microcapsules at a flow rate of 200 ml/min. Results shown in Fig. 2 demonstrate an abrupt increase in the percentage of TCA soluble ^{125}I-nDNA shortly after circulation over Dnase microcapsules. ^{131}I-DNase, 4.73 mg, was recovered on the microcapsules at the conclusion of the experiment (Table I). There was no ^{131}I demonstrable above background counts per minute in a 2-ml sample of dog serum tested at the conclusion of the experiment.

Degradation rates for nDNA and nDNA: anti-DNA immune complexes in vivo. The $t_{\frac{1}{2}}$ of initial decay for DNA and nDNA: anti-DNA complexes after intravenous injection in control animals and for control periods (untreated microcapsule circulation) in experimental animals is shown in Fig. 3. In Group I, dogs weighing from 12.7 to 16.3 kg received 17 μg of ^{125}I-nDNA (3.0×10^6 cpm). In Group II, dogs weighing from 14.0 to 16.0 kg received 17 μg of ^{125}I-nDNA (3.0×10^6 cpm) and 200 μg of unlabeled nDNA. In Group III, dogs weighing from 7.7 to 13.7 kg received ^{125}I-nDNA: anti-DNA complexes containing 2 μg of ^{125}I-nDNA (10^6 cpm).

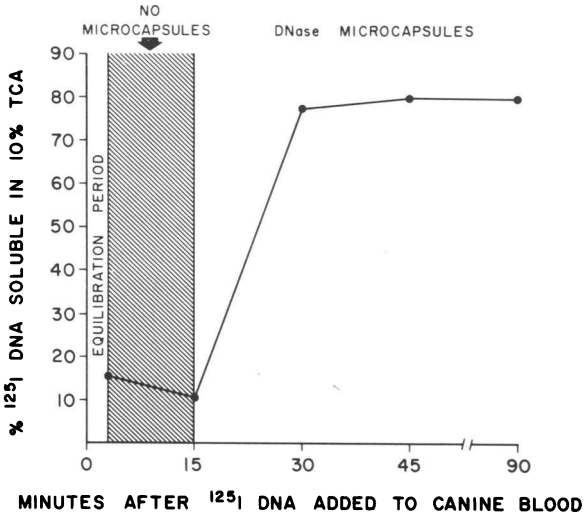


FIGURE 2 Marked increase in the percentage of TCA soluble ^{125}I-nDNA was observed 15 min after it was circulated over DNase I microcapsules in vitro.

It may be seen that the decay rate for these substances varied from animal to animal (Fig. 3). Because of the variation in individual decay rates for these substances, it was necessary for each animal to serve as his own control in experimental studies. Accordingly, in order to determine the effect of DNase microcapsules on nDNA degradation in experimental dogs, a control decay period was first carried out in each dog. The decay rate of nDNA after introduction of DNase microcapsules in each dog was then compared with its own control decay curve and statistical analysis performed on the control and experimental slopes.

In experimental and control animals, the initial pattern of decay of nDNA and nDNA: anti-DNA complexes was linear and curvilinear, respectively. In all the control animals, degradation lines were curvilinear when they were carried out to a point where 15% of the original counts were remaining in the serum of each animal. Therefore, in each experimental animal, the control portion of the slope (untreated microcapsules) was extrapolated as an interrupted line into the experimental period (DNase microcapsules) for comparison with the actual degradation curve occurring after introduction of DNase microcapsules.

Degradation of labeled nDNA by immobilized DNase in vivo (group I). Three dogs were injected with 17 μg of ^{125}I-nDNA (3.0×10^6 cpm). The studies were divided into three phases. (*a*) An equilibration period, (*b*) a microcapsule control period, and (*c*) a DNase microcapsule period. Increased decay of ^{125}I-nDNA after introduction of DNase microcapsules is evident in

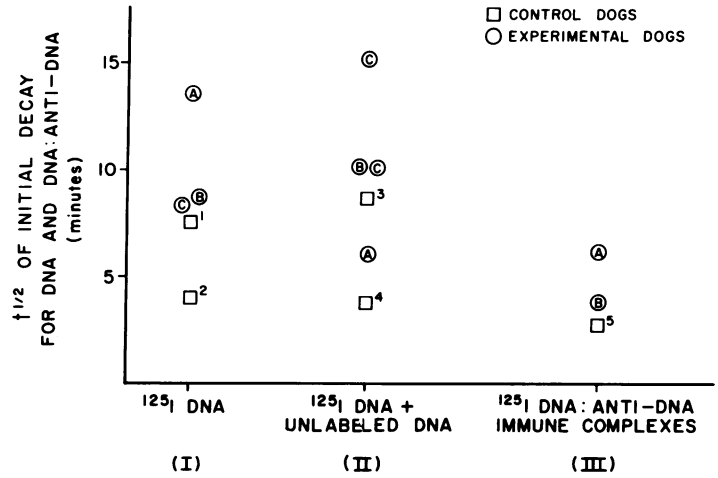


FIGURE 3 The $t_{\frac{1}{2}}$ of the initial decay rates for control animals over 3–13 min and for control periods of untreated microcapsule circulation in experimental animals over 3–10 min is given for each of the groups studied. The striking individual variation in the decay rates for these substances in vivo is demonstrated. In these control periods, the pattern of decay was linear or curvilinear for each animal studied. The more extended pattern of nDNA or nDNA:anti-DNA decay for control animals 1–5 in groups I, II, and III was curvilinear over 56, 57, 29, 25, and 17 min, respectively. These times indicate the period required for decay to reach 15% of the initial counts per minute remaining in serum.

the three animals studied compared to their own control decay patterns (Figs. 4a–c).

Degradation of larger amounts of nDNA in vivo (group II). Unlabeled nDNA, 200 and 17 μg of ^{125}I-nDNA (3.0×10^6 cpm) was infused into each of four dogs. After an equilibration period of 2–4 min, untreated microcapsules were placed in the extracorporeal circuit for approximately 5 min. DNase microspheres were then introduced with resulting increase of nDNA degradation rate (Figs. 5a–d). In dogs depicted in Figs. 5c and d, $MgCl_2$ was slowly infused at a point just before blood entry into the microcapsule chamber (total infusion volume 0.5–1 ml). A comparison of the change per unit time between the last control and first post-experimental point with the initial slopes in each case showed an increase of 43 and 97% for animals in Figs. 5a and 5b, respectively. Dogs in Figs. 5c and 5d which received the $MgCl_2$ infusion showed increases of 92 and 739%, respectively.

Degradation of nDNA in nDNA:anti-DNA complexes in vitro. Soluble nDNA:anti-DNA complexes were prepared as described in Methods. Fresh normal dog blood (100 ml) was drawn from the femoral artery and 300 U of sodium heparin added. nDNA:anti-DNA complexes in antigen excess containing 2 μg of ^{125}I-nDNA was added with $MgCl_2$ (100 mg). The blood was incubated for 15 min at 27°C and then drawn into polyethylene tubing and circulated by a roller pump at 200 ml/min through a chamber containing DNase microcapsules. Results showed a marked increase in the percentage of $(NH_4)_2SO_4$ soluble ^{125}I-nDNA 15 min after circulation of immune complexes over DNase microcapsules (Fig. 6).

Degradation of nDNA in nDNA:anti-DNA complexes in vivo (group III). Soluble nDNA:anti-DNA complexes were prepared as described in Methods. Two dogs were injected with ^{125}I-nDNA:anti-DNA (10^6 cpm) containing 2 μg of ^{125}I-nDNA. A slow infusion of 10% $MgCl_2$ (total infusion volume, 0.5–1.0 ml) was continued for the course of each procedure. After an equilibration period of 2 min, untreated microspheres were inserted into the extracorporeal circulation for approximately 7 min. DNase microspheres were then placed in the circuit which resulted in a rapid decrease in the quantity of $(NH_4)_2SO_4$ precipitable ^{125}I-nDNA (Figs. 7a and b).

Release of DNase from the microcapsules. To determine whether the observed degradation of ^{125}I-nDNA was due to DNase released from the microcapsules during the experiments, vital organs and serum of the dogs were counted for the presence of ^{131}I at the conclusion of each experiment. Results showed no significant ^{131}I in organs and serum of the dogs above background suggesting that the degradation of ^{125}I-nDNA was effected by the immobilized enzyme and not due to enzyme released from the microcapsules (Table II).

Hematologic studies on dogs undergoing extracorporeal circulation. Studies of hematocrit, leukocyte, and platelet counts were performed on dogs before and after extracorporeal circulation across the DNase micro-

capsules. Results showed no appreciable alteration in hematocrits or platelet counts during the procedure (Table III). There was, however, a reduction in leukocyte counts in all animals studied. In four animals, the decline was moderate (postperfusion values not more than 2,000/mm³ below preperfusion figures). In three animals, there were significant reductions in the postperfusion leukocyte counts. However, examination of the capsules at the conclusion of each experiment revealed no evidence of thrombotic material or significant residual cellular debris on their surface.

DISCUSSION

These foregoing studies demonstrate that nylon microcapsules which have been subjected to mild hydrolysis and glutaraldehyde treatment will bind significant quantities of DNase. Once immobilized on the nylon microcapsules, the DNase appears to retain sufficient function to accelerate the degradation of nDNA circulating free or as nDNA : anti-DNA complexes in vitro and in vivo in dogs. The observed effect of DNase on nDNA degradation could, in all likelihood, be attributed to the enzyme which was covalently conjugated to the nylon microcapsules since (*a*) no significant free ^{131}I-DNase could be detected in the tissues or serum of experimental animals at the conclusion of the experiments, (*b*) there was no significant uptake of ^{131}I recovered in the thyroid gland at the conclusion of the experiments, (*c*) no significant ^{131}I-DNase was identified in a serum sample taken at the conclusion of the in vitro nDNA degradation study.

Previous studies have established the linear or curvilinear decay pattern of DNA after it was injected into experimental animals (17). A variation in the time-course of decay was observed depending on the quantity of DNA that was administered (17). The present study shows considerable variation in the initial $t_{\frac{1}{2}}$ of nDNA decay in mongrel dogs (Fig. 3) which may be attributed, in part, to individual differences in compartmentalization, as well as the quantities of circulating nucleases and inhibitors (18–20). In view of these findings, to demonstrate the effect of immobilized DNase in degrading circulating nDNA in vivo, it was necessary for each animal to serve as his own control. Therefore, after injection of nDNA into experimental dogs, a phase of normal nDNA degradation lasting from 4 to 10 min was plotted. While the initial $t_{\frac{1}{2}}$ rate for this phase varied from animal to animal (Fig. 3), a linear degradation pattern was discernable in control and experimental animals during this period. At the end of this phase, DNase microcapsules were introduced into the extracorporeal circuits, which resulted in statistically

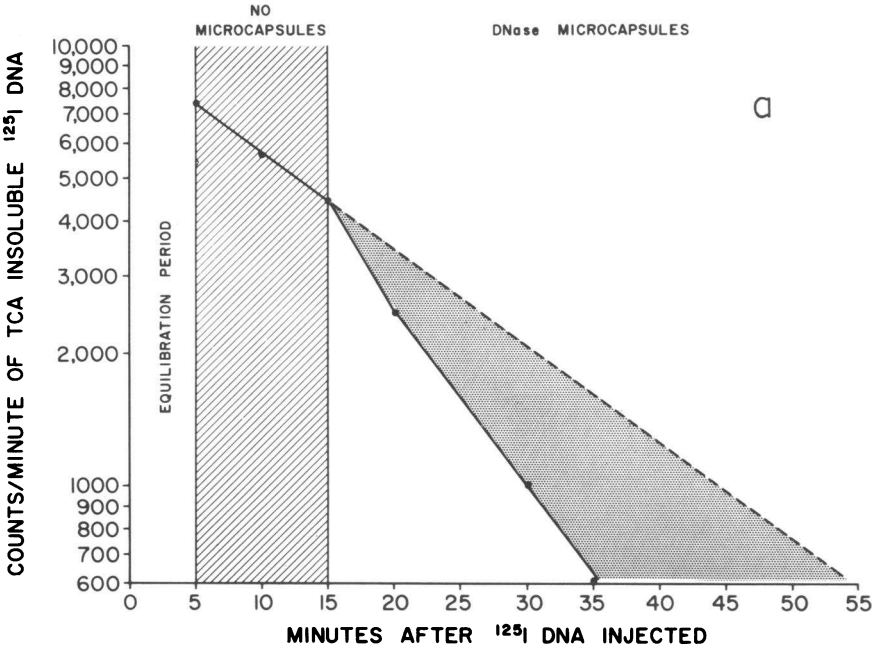


FIGURE 4 Each of three dogs was infused with 17 μg of ^{125}I-nDNA. In the three animals studied, there was an abrupt increase in the rate of ^{125}I-nDNA degradation after introduction of DNase I microcapsules into the extracorporeal circuit (uninterrupted lines). Regression analysis of dogs depicted in Fig. 4a shows the change in slope at 15 min to be significant ($P < 0.003$).

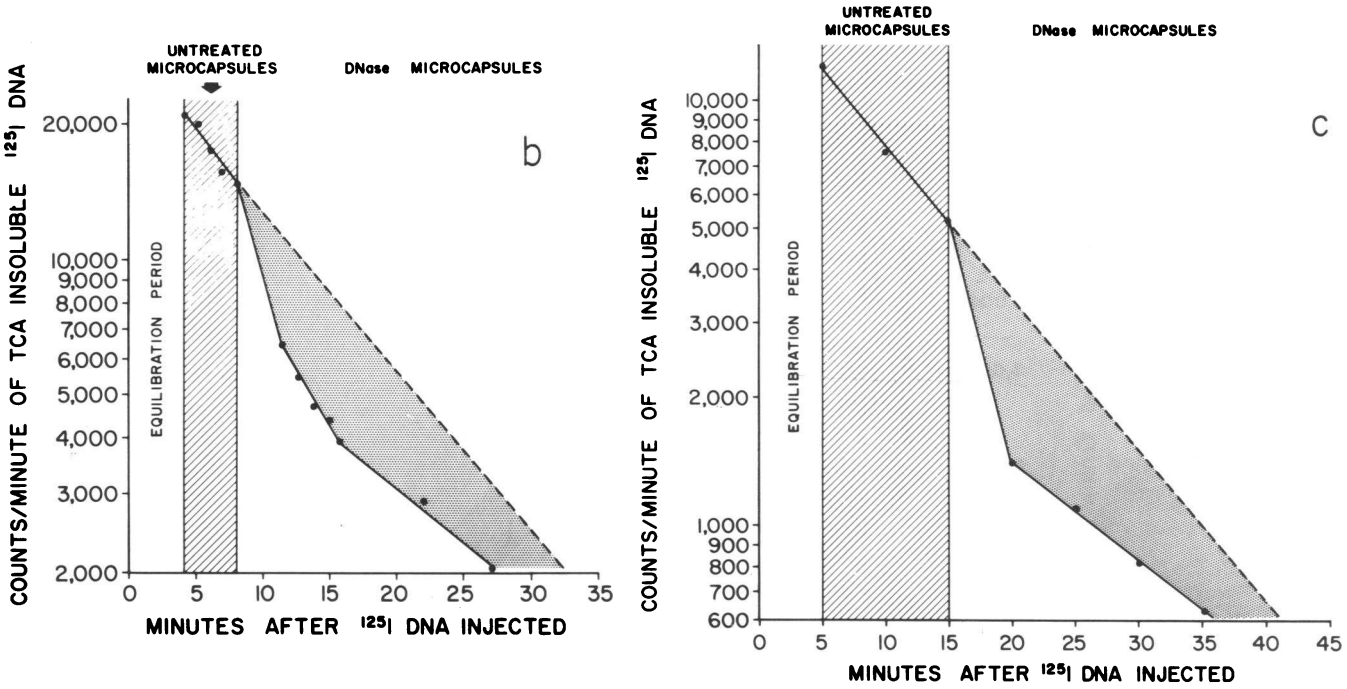


FIGURE 4 In Fig. 4b, regression lines to points taken up to 8 min and to those between 12 and 27 min reveal a difference ($P << 0.001$) indicating that the decline at 8 min is significant. In Fig. 4c, the data points after the introduction of DNase I microcapsules fall 22, 15, 13, and 9 SD below their predicted values. The probability of this occurring is $P << 0.01$, thus the drop at 15 min is significant. Interrupted lines represent extrapolations of each animal's control decay curve into the experimental period for comparative purposes.

significant changes in the slopes of nDNA degradation compared to their own initial and extrapolated control slopes in each experimental animal studied.

A reduction in postperfusion leukocyte counts was observed in all animals studied without associated alterations in hematocrits or platelet counts. This decline in leukocyte counts was probably not due to binding of leukocytes to the immunoadsorbent surface since (*a*) no significant cellular debris or thrombotic material was observed on the microcapsules and (*b*) there were no significant alterations of platelet counts, even though platelets have been observed to decline in some extracorporeal systems due to excessive adhesion to polymeric surfaces (21). The explanation of the postperfusion decline in leukocyte counts is unclear at present. However, a reduction in leukocyte counts has been noted in patients undergoing extracorporeal hemodialysis (22). This effect has been attributed to sequestration of leukocytes in extravascular pulmonary pools (22). This phenomenon appears to be transient since leukocyte counts have been observed to normalize shortly after discontinuation of extracorporeal circulation (22).

The requirement of divalent cations for the structural integrity and functional activity of DNase has been well

TABLE II

^{131}I DNase I in Tissues and Serum of Dogs

Dog	Background	Heart*	Lungs*	Kidneys*	Liver*	Spleen*	Thyroid*	Serum‡
no.								
4a	92	68	82	91	76	90	83	65
4b	92	92	81	69	75	73	78	92
4c	94	76	92	92	83	82	78	57

* 1 g of tissue was counted for 1 min. Figures represent the mean of three determinations.
‡ 0.2 ml of serum was counted for 1 min. Figure represents the mean of three determinations.

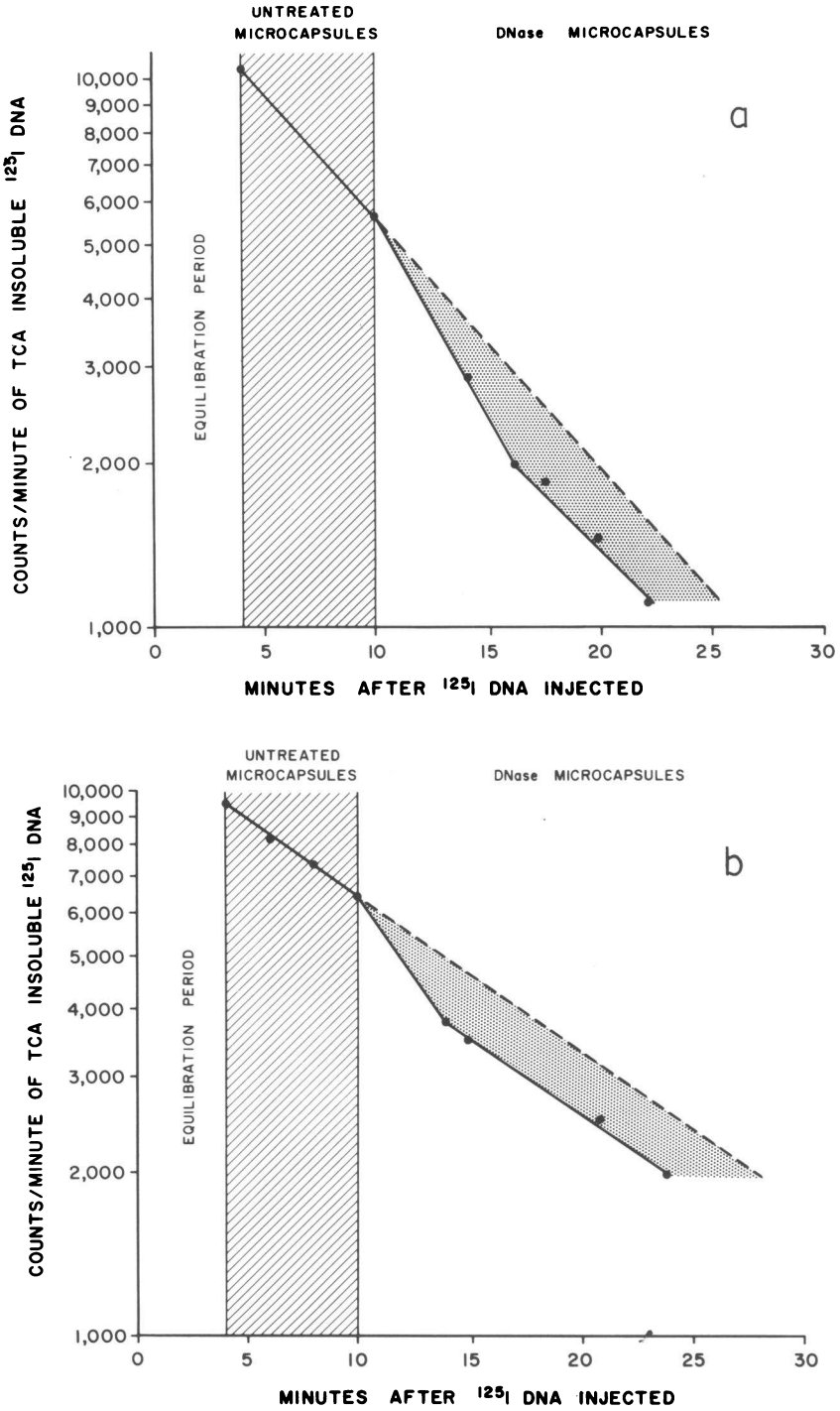
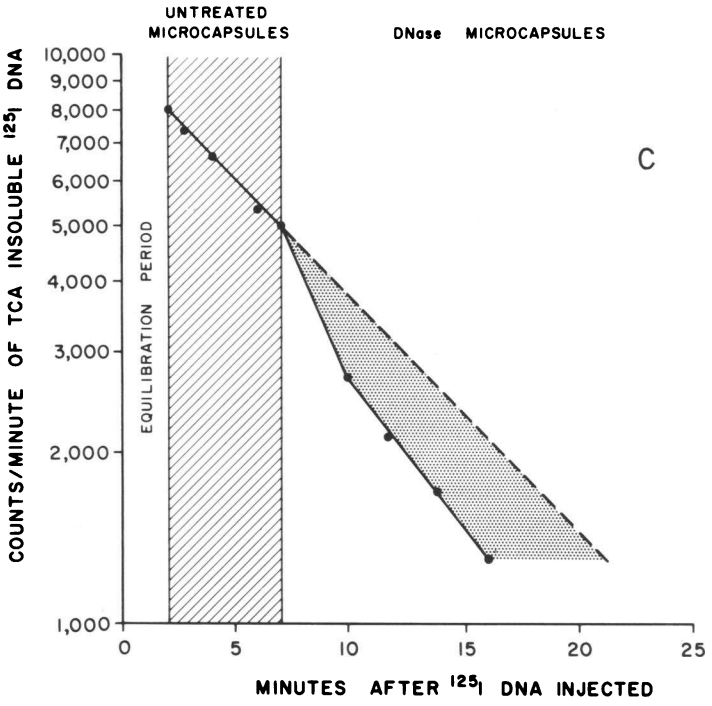
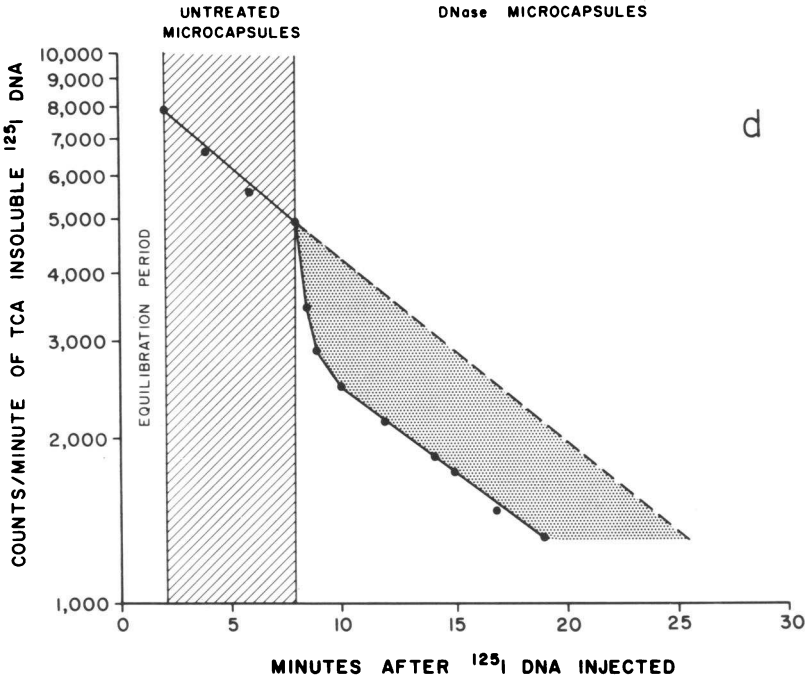


FIGURE 5 Each of four dogs was infused with 200 μg of unlabeled nDNA and 17 μg of ^{125}I-nDNA. In the four dogs studied, there was an increase in the rate of ^{125}I-nDNA degradation after introduction of DNase I microcapsules into the extracorporeal circuit (uninterrupted lines). Regression analysis of the control and experimental slopes in the dogs of Fig. 5a–d shows changes in intercepts to be significant (5a, $P < 0.02$; 5b, $P << 0.005$; 5c, $P < 0.01$; 5d, $P < 0.02$). Interrupted lines represent extrapolations of each dog's control decay curve into the experimental period.





FIGURES (Continued.)

TABLE III
Hematologic Studies

Dog	Hematocrit		Leukocyte count		Platelet count	
	Pre	Post	Pre	Post	Pre	Post
no.						
4a	37	38	6,000	5,300	147,000	162,000
4b	37	42	8,800	2,200	134,000	163,000
4c	45	43	5,100	4,200	188,000	162,000
5a	43	44	16,600	10,900	136,000	134,000
5b	45	45	9,100	7,300	146,000	144,000
7a	38	37	9,500	5,700	154,000	151,000
7b	35	34	8,800	7,500	131,000	119,000

recognized (10–13). The rate of substrate hydrolysis by DNase in the presence of magnesium is pH dependent and under certain conditions may be augmented by the addition of other metal ions such as calcium and manganese (11, 12). The present study demonstrates that immobilized DNase may degrade circulating nDNA without the infusion of additional $MgCl_2$. However, because one study showed that $MgCl_2$ infusion augmented the degradation rate of nDNA in vivo (Fig. 5d), a slow $MgCl_2$ infusion was utilized to study nDNA : anti-DNA degradation in vivo. In the latter experiments, an increased degradation rate of nDNA in the immune complexes was observed when DNase I microcapsules were placed in the extracorporeal circuit. Studies presently in progress are directed at discerning the optimal serum concentrations of magnesium and calcium consistent with maximum enzyme activity. If there exists a requirement for extra magnesium to augment the activity of the immobilized enzyme, any excessive accumulation of the ion could be dialyzed out of the plasma at the venous end of the extracorporeal circuit.

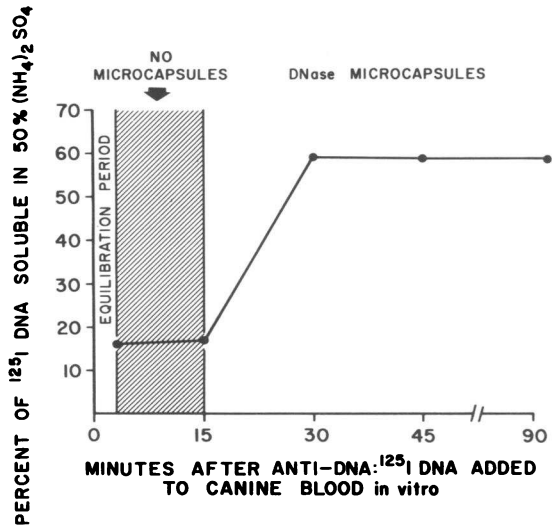


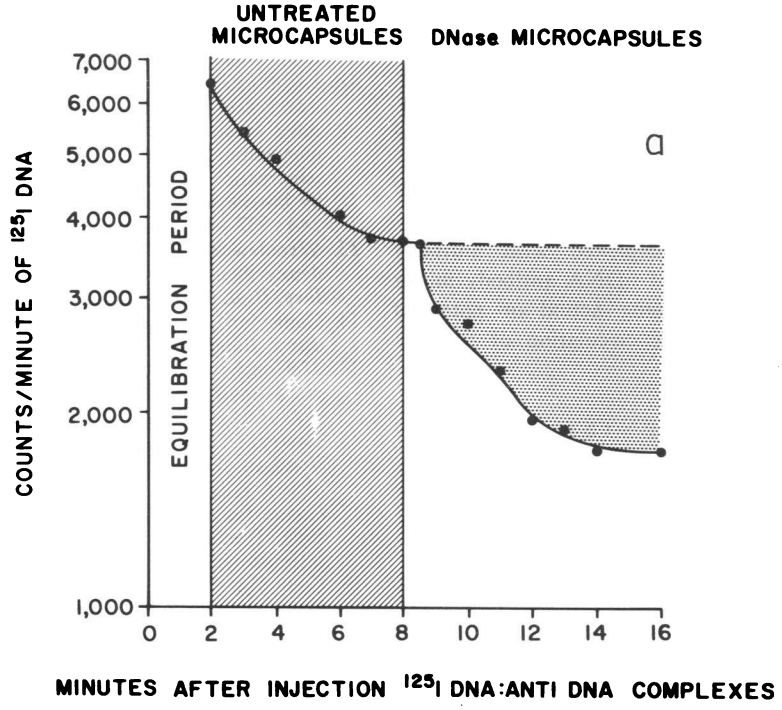
FIGURE 6 Significant increase in the percentage of $(NH_4)_2SO_4$ soluble ^{125}I-nDNA was noted 15 min after ^{125}I-nDNA : anti-DNA complexes in normal dog blood were circulated over DNase I microcapsules.

Nylon was chosen as a material for use as an in vivo immunoabsorbent because of its relative inertness and minimal host toxicity demonstrated in previous experiments (7, 8). Microcapsules were utilized because of their enormous surface area with minimum extracorporeal volume requirements. Similar microspheres have been previously used in vivo to encapsulate various proteins (7, 8). The latter systems depend to some extent on the diffusability of the membrane to permit entrance of circulating substrate. For our system where diffusion was unimportant, we added additional hexanediamine in the synthesis of the capsules to strengthen them in the face of hydrolytic treatment. During in vivo circulation, the capsules were quite mobile in their chamber. Reversal of flow in the chamber at 7- and 12-min intervals prevented clogging of the screens and facilitated blood flow. At the conclusion of the experiments, the capsules appeared quite intact structurally and were free of thrombotic material and cellular debris.

It would appear that nylon microcapsules possess many features of the optimal immunoadsorbent. These include (*a*) large surface area and minimal extracorporeal volume requirement, (*b*) capacity to bind large quantities of protein, (*c*) firm adherence of material bound to it without release into the host, and (*d*) minimal toxicity to the host's hematologic status. Thus, this material would seem to have considerable potential for application to treatment of disease in man.

It appears that the optimal approach to autoimmune disease would be to reinstitute immune unresponsiveness to the pathogenic antigen. While there are numerous methods for inducing experimental tolerance in the unprimed host, it is quite difficult to reinduce this state in an animal that is immunized (23). Since the host with autoimmune disease is immunized, the usual modalities of experimental tolerance induction would be predictably ineffective. However, promising approaches to the specific reinstitution of partial or complete tolerance in ongoing disease processes have been described (24–26).

The extracorporeal approach herein described involves an attempt to hydrolyze pathogenic circulating immune reactants. As such, it might be employed during the active phase of SLE to degrade nDNA : anti-DNA complexes or alternatively on a prophylactic basis to reduce circulating levels of nDNA. Employed in this way, the system would be used to sustain the disease in a clinically quiescent state without actually achieving a state of absolute unresponsiveness. A compromise of this nature is perhaps analogous to the prevention of symptomatic uremia with the use of intermittent hemodialysis.



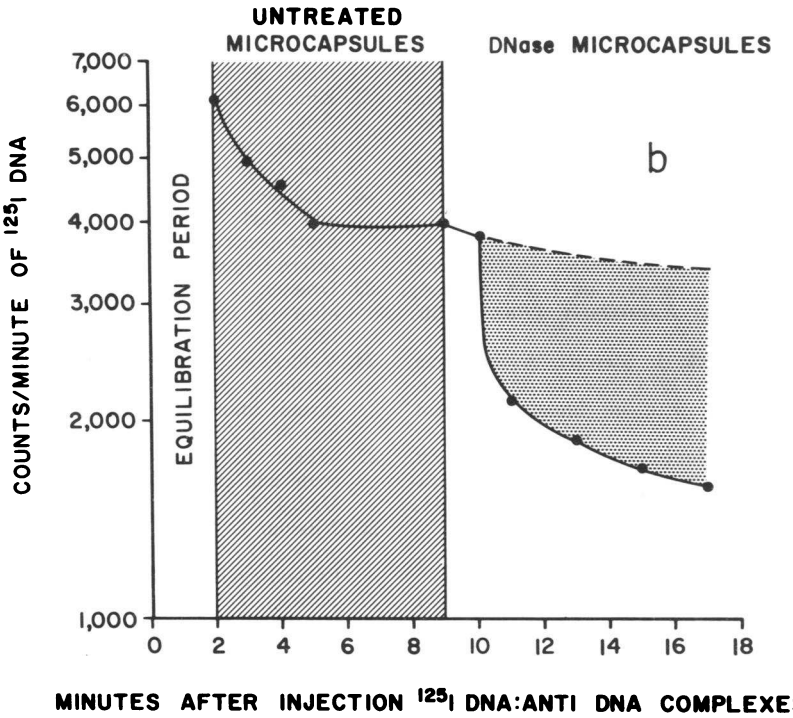


FIGURE 7 Each of two dogs was infused with ^{125}I-nDNA : anti-DNA complexes containing 2 μg of nDNA. In the two dogs studied there was an increase in the rate of nDNA degradation in the immune complexes after placement of DNase I microcapsules in the extracorporeal circulation (uninterrupted lines). In Fig. 7a, the points from 9 to 16 min are at least 12 SD below their predicted values ($P < 0.003$). In Fig. 7b, the points from 11 to 17 min are at least 25 SD below their predicted values ($P < 0.001$). Interrupted lines represent extrapolations of each animal's control decay curve into the experimental period.

Previous studies have shown the feasibility of withdrawing nDNA antibodies from the circulation of actively immunized rabbits with an immunoadsorbent composed of nDNA entrapped in agarose (5, 6). The specific withdrawal of circulating bovine serum albumin and anti-bovine serum albumin was effected with two different immunoadsorbents (27, 28). An extracorporeal system capable of effecting specific degradation of circulating nDNA alone or complexed with antibody coupled with specific removal of nDNA antibody might prove very effective in removing actual or potential pathogenic substances from the circulation of patients with SLE.

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