

# Local Immune Response in Experimental Pyelonephritis

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**ABSTRACT** Experiments using an in vitro method of assessing protein synthesis by  $^{14}\text{C}$  amino acid incorporation were designed to determine whether pyelonephritic kidneys were capable of local antibody production. Unilateral pyelonephritis was produced in rabbits by intravenous injection of *E. coli* 0-75 while one ureter was transiently occluded. The capability of protein and immunoglobulin synthesis by pyelonephritic kidneys, contralateral kidneys, normal kidneys, and spleens from normal and pyelonephritic animals was measured.

Enhanced protein and immunoglobulin syntheses by pyelonephritic kidneys were first detected by the 11th day after infection and persisted through day 120. In individual experiments the pyelonephritic kidney produced 6–170 times more soluble protein than did the contralateral, uninfected kidney. In seven experiments, IgG comprised a mean of 72% of the total protein synthesized by the pyelonephritic kidney, compared with a mean of 19% in the contralateral kidney. IgA accounted for 10 and 9%, respectively. In these experiments 0.6–17% of the synthesized IgG was precipitable by somatic antigen of the *E. coli* 0-75.

The capability of the pyelonephritic kidney to synthesize soluble protein was quantitatively similar to that of spleens from infected animals. The proportion of synthesized protein which was immunoglobulin G, however, was greater in the

pyelonephritic kidney than in the spleen. Furthermore, specific antibody synthesis by the pyelonephritic kidney persisted longer than did synthesis by the spleen of the same animal.

These studies provide evidence that in experimental pyelonephritis a significant local immune response occurs which is represented primarily by the production of IgG. Local immunoglobulin formation and specific antibody synthesis may be important factors in determining patterns of host resistance.

## INTRODUCTION

It is well established that infections of the urinary tract, especially pyelonephritis, are associated with an immune response (1–7). Antibody titers against the somatic antigen of the infecting organism are generally higher in the presence of upper urinary tract infection than when infection is limited to the bladder (2, 5, 7). Whereas such observations may be of diagnostic value, the biologic significance of circulating antibody levels remains controversial (8, 9).

Recent studies have indicated that local antibody synthesis may be of more importance than serum antibody in protecting mucous surfaces of the respiratory and gastrointestinal tracts against infection (10–12). In addition, antibody formation has been postulated in nonlymphoid organs such as the central nervous system and uveal tract in association with chronic inflammatory reactions (13–15). There is a paucity of information available on either of these aspects of the immune

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response in urinary tract infections. Cotran, using fluorescent antibody techniques, demonstrated gamma globulin-containing cells in the kidney during the course of experimental pyelonephritis (16). Furthermore, many of the plasma cells seen in the interstitial infiltrate contained specific anti-bacterial antibody. While these data demonstrate the presence of antibody, they do not allow differentiation between the local synthesis of antibody and the presence of antibody-containing cells. The present studies were designed to evaluate the capability of experimental pyelonephritic kidneys to synthesize immunoglobulins and specific anti-*Escherichia coli* antibody.

## METHODS

### A. Pyelonephritis

Unilateral pyelonephritis was produced in 2-3-kg white male New Zealand rabbits by transient ureteral obstruction and intravenous injection of *Escherichia coli* 0-75. The organisms were grown in trypticase soy broth<sup>1</sup> for 18 hr at 37°C and washed twice with sterile 0.9% saline solution. After 20 mg/kg thiamylal sodium<sup>2</sup> and ether anesthesia, the right ureter was isolated through a paraspinous incision using sterile technique. A polyethylene ligature was passed around the ureter and both ends secured loosely over a button on the skin. The following day  $1 \times 10^7$  *E. coli* 0-75 contained in 1.0 ml of saline was injected into an ear vein and the ureter was occluded by tightening the ligature. 18 hr later the ligature was removed. The same operative technique was used to study the effect of ureteral obstruction without infection.

Voided urine was obtained during the course of infection, and at sacrifice, bladder urine was aspirated for bacteriologic culture. Quantitative bacterial counts were performed on tissue homogenates at sacrifice. All *E. coli* isolated were identified as *E. coli* 0-75 by the use of specific antisera.<sup>3</sup> Tissue wedges were fixed in 10% formalin and stained with hematoxylin and eosin. Cellular infiltration and parenchymal changes were graded by an independent observer who was unaware of the results of immunoglobulin studies.

### B. Serum antibody

Serum antibody levels were determined using an agglutination technique with somatic antigen. Somatic antigen of *E. coli* 0-75 was prepared by boiling an 18 hr broth culture for 2 hr. The antigen was washed in saline and then resuspended in saline containing formalin at

a final concentration of 0.5%. Using microtiter equipment,<sup>4</sup> serial twofold dilutions of sera were made in duplicate. Antigen (50  $\mu$ l containing  $1 \times 10^8$  organisms) in saline was added to one set of sera, and an equal amount of antigen containing 2-mercaptoethanol (2-ME) in a final concentration of 0.1 M was added to the second set of sera. The plates were incubated for 2 hr at 50°C and held overnight at 4°C. Titers were expressed as the reciprocal of the highest dilution exhibiting gross agglutination.

### C. Protein synthesis

In vitro protein synthesis was carried out by the method of Smiley, Sachs, and Ziff (17).

1. *Tissue incubation.* Kidneys from animals with unilateral pyelonephritis, the contralateral unobstructed kidneys, normal kidneys, kidneys which had been obstructed but not challenged with intravenous organisms, normal spleens, and spleens from some animals with pyelonephritis were studied. Animals were bled by cardiac puncture, then sacrificed by fracture of the cervical vertebrae. The renal capsule and ureter were removed from the kidneys and surrounding adipose tissue separated from spleens. 4-6 g of kidney tissue or the spleen were cut into 3-mm pieces. Each specimen was added to 5 volumes of modified Eagle's medium lacking the amino acids contained in the <sup>14</sup>C label and containing 5% decomplexed rabbit serum. The medium had been adjusted to a pH of 7.2 by addition of saturated sodium bicarbonate and sterilized by filtration through a Millipore filter.<sup>5</sup> 4  $\mu$ c of a mixture of algal protein hydrolysate <sup>14</sup>C amino acids<sup>6</sup> (containing L-arginine, L-leucine, L-lysine, and L-valine) was added for each gram of tissue incubated. Ampicillin, 500  $\mu$ g/ml, was added to inhibit bacterial growth. Tissue incubation was carried out for 6 hr at 37°C in a 50 ml conical tube, with a glass capillary sealed to the bottom, through which a 95% O<sub>2</sub>-5% CO<sub>2</sub> gas mixture was introduced to maintain the pH at 7.4.

2. *Fractionation of soluble protein.* After incubation the mixture was centrifuged at 105,000 g for 90 min to remove insoluble particles. Casein hydrolysate amino acid mixture,<sup>7</sup> in a final concentration of 1%, was added to the supernatant, which was then dialyzed with stirring against 1000 volumes of 0.01 M Na<sub>2</sub>HPO<sub>4</sub> for 72 hr to remove unincorporated radioactivity. The dialysate was then chromatographed on diethylaminoethyl cellulose<sup>8</sup> (DEAE) using a stepwise elution method which resulted in four protein peaks (18). The concentration of protein in the eluate fractions was determined by measurement of optical density at 280 m $\mu$  on a Beckman DU spectrophotometer. The eluate from the ascending portion of the first protein peak (designated as Ia) contained most of the radioactivity of peak I and was pooled

<sup>4</sup> Cooke Engineering Company, Alexandria, Va.

<sup>5</sup> 0.45  $\mu$ , Millipore Filter Corp., Bedford, Mass.

<sup>6</sup> Schwarz Bio Research, Inc., Orangeburg, N. Y.

<sup>7</sup> Difco Laboratories, Detroit, Mich.

<sup>8</sup> Brown Co., Berlin, N.H. DEAE capacity 0.86 mEq/g.

<sup>1</sup> Baltimore Biological Laboratories, Baltimore, Md.

<sup>2</sup> Surital, Parke, Davis & Co., Detroit, Mich.

<sup>3</sup> Department of Health, Education, and Welfare, U. S. Public Health Service, NCDC Biological Reagents Section, Atlanta, Ga.

separately from the descending portion (designated as Ib). Fractions Ia and Ib were lyophilized, redissolved in 3 ml of sterile glass-distilled water, centrifuged at 1000 *g* for 30 min to remove denatured protein, and stored at  $-20^{\circ}\text{C}$  until further use. Elution peaks II, III, and IV were dialyzed with stirring against 1000 volumes of 0.01 M phosphate buffer pH 7 for 72 hr before lyophilization, then handled in the same manner as peak I. To find the total soluble protein synthesized, we determined the radioactivity in each of the five DEAE fractions and totaled it. An aliquot (0.1 ml) of each fraction was added to 1.0 ml of 0.5 M NaOH and this solution added to 15 ml of Bray's phosphor containing 40 g/liter of thixotropic gel.<sup>9</sup> Liquid scintillation counting was carried out in a Beckman L-S 259 B spectrometer. The counts per minute from each fraction were added and the total soluble protein finally expressed as counts per min per gram wet weight of tissue.

3. *Immune precipitation of immunoglobulins.* Synthesized immunoglobulin was quantitated by an immune precipitation technique using specific goat anti-rabbit IgG and goat anti-rabbit colostral IgA in antibody excess.

Rabbit IgG was purified from rabbit gamma globulin<sup>10</sup> by elution from DEAE using 0.01 M phosphate buffer, pH 7.0. Colostrum was obtained by needle aspiration of the stomach of nursing newborn rabbits. After being mixed with one-half volume of saline, the stomach aspirate was centrifuged at 7500 *g* for 60 min at  $4^{\circ}\text{C}$ . IgA was purified from the supernatant by the method of Cebra and Robbins (19). Antisera to colostral IgA and to IgG were prepared in goats by the repeated injections of antigen incorporated into complete Freund's adjuvant. The IgA antiserum was absorbed with rabbit IgG to remove the trace quantities of antibody directed against the latter globulin. Specificities of both antisera were confirmed by immunoelectrophoresis against colostrum and normal rabbit serum.

To quantitate specific immunoglobulin synthesis, precipitations in antibody excess were carried out by addition of 0.5 ml of either antiserum to 0.1 ml of each of the DEAE fractions. The samples were incubated for 1 hr at  $37^{\circ}\text{C}$  and then held at  $4^{\circ}\text{C}$  for 36 hr. The samples were then centrifuged at 7000 *g* for 15 min and the resultant precipitates washed two times with 0.9% saline solution before being dissolved in 1 ml of 0.5 M NaOH. Radioactivity of the dissolved precipitate was determined as described above.

4. *Specific antibody.* The capability of the pyelonephritic kidney to synthesize anti-*E. coli* 0-75 IgG antibody was assessed. 1 ml of *E. coli* 0-75 somatic antigen ( $2 \times 10^9$  organisms) was added to 0.1 ml of peak Ia. The mixture was incubated at  $37^{\circ}\text{C}$  for 1 hr, then refrigerated at  $4^{\circ}\text{C}$  for 24 hr. After incubation, the mixture was centrifuged at 1200 *g* for 30 min and the precipitate washed

<sup>9</sup> Cab-O-Sil, Packard Instrument Co., Inc., Downers Grove, Ill.

<sup>10</sup> Cohn fraction II. Rabbit gamma globulins, Pentex, Inc., Kankakee, Ill.

twice in 0.9% saline solution, dissolved, and counted as previously described. As a control for nonspecific absorption of radioactivity, somatic antigen prepared from a strain of *Pseudomonas aeruginosa* was employed in the same procedure.

5. *Procedural controls.* As a control for nonspecific radioactive contamination of the soluble protein synthesized (section C2), the  $^{14}\text{C}$  label was added to normal kidney tissue at the end of incubation. After dialysis against 0.01 M  $\text{Na}_2\text{HPO}_4$  for 72 hr, radioactivity in the sample reached background levels.

To ascertain that the eluate from DEAE was soluble protein, we precipitated aliquots of the five DEAE fractions by 10% trichloroacetic acid (TCA). In seven experiments on pyelonephritic kidneys 95% of the radioactivity in the five fractions was precipitated by the TCA.

As a control for nonspecific coprecipitation of radioactivity (section C3), ovalbumin and anti-ovalbumin at equivalence (3.2 mg) were added to representative DEAE fractions. Less than 2% of the radioactivity in any fraction was precipitated by this antigen-antibody complex, emphasizing the specific nature of the anti-rabbit immunoglobulin precipitations.

To confirm that the soluble protein of fraction Ia was IgG, we characterized it further on the basis of molecular size. A 0.5 ml aliquot of peak Ia was mixed with 15 mg of purified unlabeled rabbit IgG and applied to a Sephadex G-200 column<sup>11</sup> ( $90 \times 1.3$  cm) and the protein eluted with 0.1 M phosphate buffer at pH 6.8.

## RESULTS

*Pyelonephritis.* Unilateral pyelonephritis after bacterial challenge was confirmed in the previously obstructed kidneys by bacteriologic and histologic examination. Bacteriuria had been demonstrated in all animals during the course of observation although it had spontaneously cleared in some by the time of sacrifice. Five of six animals studied from 2 to 15 days after challenge had bacteriuria and positive cultures in the previously obstructed kidney (Table I). Only one of five animals studied after day 15 had positive cultures of urine and previously obstructed kidney at time of sacrifice.

On gross examination, minimal hydronephrosis was seen in some kidneys. Parenchymal changes consisted of wedge-shaped areas of involvement extending from the medulla to the cortical surface. The histological changes observed during the course of infection were similar to those previously described for this model (20). At 2, 4, and 7 days after challenge, numerous foci of inflammatory

<sup>11</sup> Pharmacia Fine Chemicals Inc., New Market, N. J.

TABLE I  
Bacteriologic, Histologic, and Immunologic Features at Time of Study

Day studied after challenge	Urine culture	Renal findings						Serum antibody†	
		Obstructed kidney			Nonobstructed kidney			Total	2-ME resistant
		Py- elone- phritis*	Culture	Mononu- clear in- filtrate	Py- elone- phritis	Culture	Mono- nuclear infiltrate		
2	Positive	+	Positive	0	0	Positive	0	0	0
4	Positive	+	Positive	0	0	Sterile	0	256	0
7	Sterile	+	Sterile	0	0	Sterile	0	1024	8
11	Positive	+	Positive	++	0	Positive	0	256	0
14	Positive	+	Positive	0	0	Sterile	0	4096	32
15	Positive	+	Positive	++++	0	Positive	0	4096	16
22	Sterile	+	Sterile	++++	0	Sterile	0	512	32
34	Sterile	+	Sterile	+	0	Sterile	0	128	32
39	Sterile	+	Sterile	++++	0	Sterile	0	2048	512
48	Sterile	+	Sterile	+++	0	Sterile	0	1024	256
120	Positive	+	Positive	+	+	Positive	+	256	256

\* Based upon histologic findings including polymorphonuclear leukocytes, mononuclear leukocytes, and varying degrees of interstitial fibrosis.  
† Reciprocal of the highest serum dilution showing macroscopic agglutination.

cells with microabscess formation were seen in the interstitial areas. In animals sacrificed 11 days or more after challenge, the infiltrate consisted primarily of mononuclear cells. The degree of infiltration varied from scattered foci of round cells to large aggregates resembling lymphoid follicles. Varying degrees of interstitial fibrosis were present.

All contralateral kidneys were normal on gross examination. Contralateral kidneys having positive cultures showed occasional small foci of inflammatory cells.

*Serum antibody.* Circulating antibody to *E. coli* 0-75 appeared by the 4th day after challenge

and persisted throughout the duration of study (Table I). The antibody present before the 12th day was almost exclusively 2-ME sensitive. Thereafter, 2-ME-resistant (IgG) antibody was present in all animals.

*Immunoglobulin synthesis.* Pyelonephritic kidneys demonstrated significant soluble protein and immunoglobulin synthesis. Data from rabbits with pyelonephritis of 14-120 days' duration are presented in Table II. In all experiments the synthesized, soluble, TCA-precipitable protein was significantly greater in the pyelonephritic kidney than in the nonpyelonephritic contralateral kidney. In individual experiments, the pyelonephritic kidney

TABLE II  
Comparison of Protein and Immunoglobulin Synthesis in Pyelonephritic and Contralateral Kidneys from Days 14 through 120

Day	Pyelonephritic kidneys					Contralateral kidneys				
	Total protein (TCA ppt)	IgG	IgA	IgG	IgA	Total protein (TCA ppt)	IgG	IgA	IgG	IgA
		<i>cpm/g</i>		<i>%</i>			<i>cpm/g</i>		<i>%</i>	
14	16,770	9,530	2,300	57	14	1,530	190	110	12	7
15	57,620	36,000	2,720	62	5	3,870	360	290	9	8
22	27,020	25,600	3,500	95	13	1,770	220	370	12	21
34	12,350	8,200	1,400	66	11	2,020	550	60	27	3
39	113,000	83,500	19,000	74	12	670	380	180	57	27
48	65,000	48,400	7,300	74	11	2,370	120	120	5	5
120	31,490	17,280	2,200	55	7	4,780	1,320	315	28	7
Mean	46,180	32,660	4,770	72	10	2,380	450	210	19	9

TABLE III  
*Protein and Immunoglobulin Synthesis by Pyelonephritic and  
 Contralateral Kidneys at Early Intervals\**

Day	Pyelonephritic kidneys					Contralateral kidneys				
	Total protein	IgG	IgA	IgG	IgA	Total protein	IgG	IgA	IgG	IgA
		cpm/g		%		cpm/g		%		
2	8,300	180	160	2	2	17,600	830	770	5	4
4	11,360	640	360	6	3	5,800	210	180	4	3
7	3,900	200	220	5	6	6,000	230	290	4	5
11	8,000	1,930	430	24	6	2,990	270	95	9	3

\* The mean  $\pm$  1 sd of the total soluble protein, IgG and IgA synthesized by obstructed kidneys without infection (Table IV) was as follows: total protein  $6,490 \pm 3,370$  cpm/g, IgG  $610 \pm 440$  cpm/g, and IgA  $320 \pm 300$ .

demonstrated 6–170 times more protein synthesis than did the contralateral kidney. The mean increase was 19-fold ( $P < 0.001$  based upon comparison of the standard error of the means by use of Student's *t* test).

IgG synthesis by pyelonephritic kidneys ranged from 14 times greater in the day 34 experiment to 400 times greater in the day 48 experiment, with a mean 73-fold increase by pyelonephritic over contralateral kidneys ( $P < 0.001$ ). Considerable variation in IgG synthesis was present within the pyelonephritic group, but this variation did not correlate with duration of pyelonephritis. Despite this variation in individual experiments, IgG consistently comprised greater than 50% of the total soluble protein which was synthesized. In these seven experiments, 72% of the total protein synthesized by pyelonephritic kidneys was IgG. In contrast, only 19% of the protein synthesized by contralateral kidneys was IgG.

The increase in IgA synthesis by pyelonephritic kidneys paralleled the increase in total protein synthesis but was less than the increase in IgG synthesis. In individual experiments, the increase in IgA synthesis ranged from 7-fold to 105-fold with a mean increase of 22-fold over that by the contralateral kidney ( $P < 0.001$ ). IgA accounted for 10% of synthesized protein in pyelonephritic kidneys and 9% in contralateral kidneys. IgG and IgA together accounted for 82% of the total protein produced by pyelonephritic kidneys and 28% of that produced by contralateral kidneys.

Immunoglobulin synthesis correlated with the degree of mononuclear cell infiltration (Tables I and II). Pyelonephritic kidneys which were judged to have the most mononuclear cells (days

15, 22, 39, and 48) also demonstrated the greatest immunoglobulin synthesis. The pyelonephritic kidneys studied at 14, 34, and 120 days after challenge had only scattered foci of round cells and demonstrated the least immunoglobulin synthesis.

To determine when a significant increase in immunoglobulin synthesis first could be detected, we studied animals with pyelonephritis of less than 14 days' duration (Table III). In individual experiments, levels of synthesis which varied by more than 2 sd from the mean synthesis by a control group were considered as significant. No significant IgG or IgA synthesis was noted in pyelonephritic kidneys studied at the 2nd, 4th, and 7th days of infection; however, there was a significant increase in IgG but not in IgA synthesis at the 11th day. Significant mononuclear infiltration was seen in microscopic sections of this kidney (day 11). In contrast, pyelonephritis of shorter duration demonstrated an acute inflammatory response characterized primarily by polymorphonuclear leukocytes and microabscess formation.

Very little immunoglobulin was synthesized by normal kidney tissue (Table IV). IgG and IgA together accounted for less than 10% of the total soluble protein which was synthesized. Kidneys from animals which had had transient ureteral obstruction without intravenous bacterial challenge showed a slight but significant increase in total protein and IgG synthesis when compared to normal kidneys.

The capability of the pyelonephritic kidney to synthesize immunoglobulins was comparable to, and in some experiments even greater than, the capability of either normal spleen or the spleen from pyelonephritic animals (Table IV). The

TABLE IV  
Total Soluble Protein and Immunoglobulins G and A Synthesized by  
Pyelonephritic Kidneys and Control Tissues

Tissue	No. of animals	Total protein	IgG		IgA		IgG		IgA	
			cpm/g ± 1 SD				%			
Normal kidney	6	2,650 ± 950	90 ± 40	40	120 ± 40	40	3.5	4.5		
Obstructed kidney without infection	3	6,490 ± 3,370	610 ± 440	440	320 ± 300	300	10	5		
Pyelonephritic kidney (day 14-120)	7	46,180 ± 35,450	32,640 ± 26,660	26,660	4,770 ± 4,500	4,500	72	10		
Contralateral kidney (day 14-120)	7	2,380 ± 1,420	450 ± 410	410	210 ± 120	120	19	9		
Spleen (normal)	3	73,300 ± 4,450	10,780 ± 6,230	6,230	8,470 ± 3,790	3,790	15	12		
Spleen (pyelonephritis)	5	73,230 ± 31,260	26,300 ± 16,210	16,210	9,290 ± 3,450	3,450	36	13		

IgG synthesized by pyelonephritic kidneys accounted for a mean of 72% of the total soluble protein. In contrast, IgG comprised 36% of the protein synthesized by spleens from pyelonephritic animals and only 15% of that produced by normal spleens. IgA synthesis by pyelonephritic kidneys was comparable to that of both normal spleens and spleens from pyelonephritic animals.

**Immunoglobulin distribution.** The distribution of IgG and IgA in newly synthesized soluble protein fractions from DEAE is shown in Table V. In fractions Ia and Ib, IgG was eluted almost free of IgA. IgA, which was eluted primarily in fraction IV, was not eluted free of IgG. Although IgG was present in fractions III and IV, 70% of the total IgG recovered was eluted in fractions Ia, Ib, and II.

When peak Ia was added to unlabeled rabbit IgG and eluted from Sephadex G-200 the peak of radioactivity coincided with the major peak of unlabeled protein (Fig. 1). This confirms on the basis of molecular size that the soluble protein in peak Ia was IgG.

TABLE V  
Distribution of Immunoglobulins in Soluble Protein  
Fractions Eluted from DEAE Cellulose

Immuno- globulin	DEAE fraction				
	Ia	Ib	II	III	IV
IgG	75 ± 13	77 ± 14	87 ± 10	40 ± 12	44 ± 10
IgA	2 ± 2	2 ± 1	7 ± 8	8 ± 7	41 ± 20

Expressed as per cent of newly synthesized total protein precipitated from each fraction by goat anti-IgG and anti-IgA. Each value represented is the mean per cent ± SD of seven experiments on pyelonephritic kidneys.

**Specific antibody synthesis.** To determine whether the IgG synthesized by pyelonephritic kidneys represented antibody directed against the infecting strain of *E. coli* 0-75, we agglutinated *E. coli* somatic antigen with peak Ia. Specific anti-*E. coli* antibody first was detected at day 11, concurrently with the increase in IgG synthesis (Table VI). In individual experiments specific antibody comprised 0.6-17.3% of the total IgG synthesized. Antibody was not detected in the soluble protein synthesized by contralateral kidneys. Antibody production by spleens of pyelo-

TABLE VI  
Specific IgG Antibody to *E. coli* 0-75

Day	IgG*	<i>E. coli</i> *	Pseudo- domonas*	"Speci- fic" <i>E. coli</i>	% Speci- fic anti- <i>E.</i> <i>coli</i>
Pyelonephritic kidney					
4	61	28	28	0	0
7	127	21	21	0	0
11	526	56	19	37	7.0
14	2,420	67	27	40	1.7
15	11,310	1,130	530	600	5.3
22	15,925	2,430	231	2,199	13.8
34	3,787	96	72	24	0.6
39	38,518	4,037	847	3,190	8.3
48	29,895	5,718	642	5,176	17.3
120	8,960	1,318	70	1,248	13.9
Pyelonephritic spleen					
4	6,790	279	186	93	1.4
11	17,120	960	64	896	5.2
14	4,851	2,156	147	2,009	41.4
48	3,213	432	324	108	3.4
120	9,940	518	434	84	0.8

\* Radioactivity precipitated from peak Ia expressed as cpm/g tissue in the original incubation mixture.

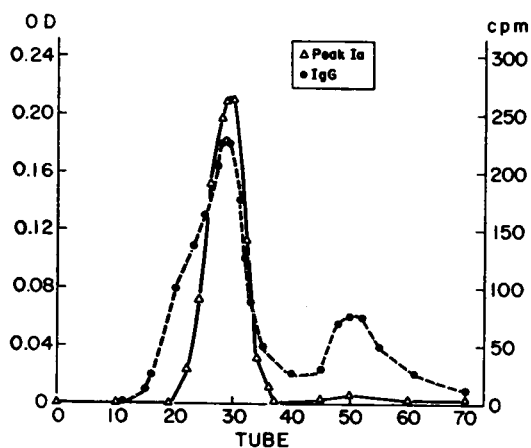


FIGURE 1 Elution pattern of DEAE-cellulose fraction Ia and purified rabbit IgG on Sephadex G-200.

nephritic animals was first apparent the 4th day after infection. Although antibody synthesis by kidneys appeared later in the course of infection than did synthesis by spleens, enhanced specific antibody production by the pyelonephritic kidneys persisted longer than did synthesis by spleens from the same animals.

#### DISCUSSION

In the present studies the normal uninfected kidney was found to synthesize only minimal amounts of immunoglobulin, an observation that supports the view of Askonas, Humphrey, and Porter that the normal kidney is not a significant site of gamma globulin synthesis (21, 22). In contrast, we have demonstrated a significant immune response by the pyelonephritic kidney. This is considered to represent an example of the development of immunocompetence in association with a mononuclear infiltrate in a normally nonlymphoid organ. The proportion of immunoglobulin to total protein synthesized by the pyelonephritic kidney was 62–100%, a proportion which is comparable to that reported by others for lymph nodes of hyperimmunized animals (23, 24). The principal immunoglobulin produced by the pyelonephritic kidney is IgG, although all animals studied synthesized some IgA. IgM synthesis was not quantitated. However, this immunoglobulin could not have predominated after the 14th day, since a majority of total protein synthesized is accounted for by IgG and IgA.

A local immune response can occur as the con-

sequence of either of two processes. First, certain organ systems such as the gastrointestinal and respiratory tracts normally contain immunocompetent cells (25, 26). Local production of immunoglobulin which is principally secretory IgA and specific IgA antibody has been demonstrated (12, 27–32). Furthermore, studies have demonstrated that protection against reinfection of the respiratory tract correlates better with the presence of local antibody than with the presence of circulating antibody (11). In addition, secretion of immunoglobulin has been postulated to account for the presence of IgA and free secretory "piece" in human urine (33).

The second process by which local antibody synthesis might occur involves the migration of immunocompetent cells into a nonlymphoid organ. Several lines of investigation have supported this hypothesis. Morgan demonstrated higher titers of neutralizing antibody in the central nervous system than in the serum after experimental poliomyelitis in monkeys (13). In studies on experimental uveitis, Witmer demonstrated that significant amounts of antibody were produced by the uveal tract and in an occasional animal, there was a higher antibody titer in the aqueous fluid of the involved eye than in the blood (14). Silverstein postulated that the cellular events occurring within the uveal tract during uveitis were comparable to the sequence of events taking place in a lymph node during an antibody response (15). Cotran, using fluorescent antibody techniques, demonstrated gamma globulin-containing cells in the kidney during the course of experimental pyelonephritis (16). Many of the plasma cells seen in the interstitial infiltrate contained specific antibacterial antibody. However, the above studies have not enabled differentiation between the local production of antibody and the local accumulation of antibody produced at distant sites. More recently, Smiley, Sachs, and Ziff have clearly demonstrated significant local immunoglobulin synthesis, predominantly IgG, by synovial tissue from patients with rheumatoid arthritis (17). The present studies which demonstrate not only the production of IgG but also the synthesis of specific anti-*E. coli* antibodies by pyelonephritic kidney clearly establish that antibody can be synthesized within a nonlymphoid organ such as a kidney during the course of the inflammatory response.

The paucity of IgA synthesized suggests that this immunoglobulin is of less significance in the kidney than in the respiratory or gastrointestinal tracts.

Studies of immunoglobulin synthesis within the kidney may contribute to a further understanding of the source of antibodies found in urine. Previous investigators have demonstrated antibody activity in urine associated with low molecular weight gamma globulins, IgG and IgA (34-36). Vosti and Remington demonstrated that the anti-O antibody in the urine of previously infected patients was exclusively IgG (37). Some investigators have proposed that the immunoglobulins present in urine are derived from plasma proteins which reach the urine by an undefined mechanism (36, 38-40). Our studies would suggest that antibody in the urine, particularly in association with pyelonephritis, may be the result of local synthesis.

Finally, these studies which demonstrate the capability for local antibody synthesis within the pyelonephritic kidney may elucidate some of the controversy regarding the role which antibody may play in both experimental and clinical pyelonephritis.

Studies in experimental animals have demonstrated that immunity may play a significant role in the pathogenesis of pyelonephritis (41-45). Pyelonephritis produced with *Escherichia coli* or *Proteus mirabilis* was associated with the development of circulating agglutinins and resistance to both hematogenous and retrograde reinfection with the same organism (43, 44). The studies of Arana, Kozij, and Jackson suggested that while resistance to reinfection could be demonstrated in experimental retrograde pyelonephritis, the protection achieved after immunization or previous infection appeared to be independent of the level of serum antibody (45). Recent studies in our laboratory have confirmed that resistance to reinfection followed prior infection but that the protection could not be directly related to the level of either circulating IgG or IgM antibody.<sup>12</sup> Likewise in clinical pyelonephritis, the persistence of active infection in the presence of high levels of circulating antibody has been demonstrated (5, 7, 8). The phenomenon of local antibody synthesis might account for this apparent discrepancy.

<sup>12</sup> Smith, J. W., T. E. Miller, J. A. Barnett, and J. P. Sanford. Unpublished observations.

Persistent bacteriuria may be the result of infection with microorganisms which do not elicit a significant immune response such as *Klebsiella pneumoniae*, or the protective effect of locally synthesized antibody may be nullified in the presence of obstruction (9, 43, 46).

Thus, these experiments clearly demonstrate that with the development of a chronic inflammatory response, a nonlymphoid organ such as the kidney is capable of immunoglobulin synthesis. Furthermore, the local synthesis of antibody within the kidney may account for the protection against reinfection with homotypic organisms.

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#### REFERENCES

1. Kaarsalo, E., A. Kasanen, B. Laurent, O. Piironen, and V. Raunio. 1962. Occurrence of *E. coli* agglutinins in the sera of patients with coli pyelonephritis. *Ann. Med. Internae Fenniae*. 51: 31.
2. Winberg, J., H. J. Andersen, L. A. Hanson, and K. Lincoln. 1963. Studies of urinary tract infections in infancy and childhood. I. Antibody response in different types of urinary tract infections caused by coliform bacteria. *Brit. Med. J.* 2: 524.
3. Kunin, C. M., R. Deutscher, and A. Paquin, Jr. 1964. Urinary tract infection in school children: an epidemiologic, clinical and laboratory study. *Medicine*. 43: 91.
4. Ehrenkranz, N. J., and M. J. Carter. 1964. Immunologic studies in urinary tract infections. I. The hemagglutinin response to *Escherichia O* antigens in infections of varying severity. *J. Immunol.* 92: 798.
5. Percival, A., W. Brumfitt, and J. de Louvois. 1964. Serum-antibody levels as an indication of clinically inapparent pyelonephritis. *Lancet*. 2: 1027.
6. Hauschka, M. S., E. Neter, and M. I. Rubin. 1965. Urinary tract infection in children. *Am. J. Diseases Children*. 109: 238.
7. Vosti, K. L., A. S. Monto, and L. A. Rantz. 1965. Host-parasite interaction in patients with infections due to *Escherichia coli*. II. Serologic response of the host. *J. Lab. Clin. Med.* 66: 613.
8. Williamson, J., H. Brainerd, M. Scaparone, and S. P. Chueh. 1964. Antibacterial antibodies in coli-



- form urinary tract infections. *Arch. Internal Med.* 114: 222.
9. Sanford, J. P., and J. A. Barnett. 1965. Immunologic responses in urinary-tract infections. *J. Am. Med. Assoc.* 192: 587.
  10. Burrows, W., and L. I. Ware. 1953. Studies on immunity to Asiatic cholera. VII. Prophylactic immunity to experimental enteric cholera. *J. Infect. Diseases.* 92: 164.
  11. Smith, C. B., R. H. Purcell, J. A. Bellanti, and R. M. Chanock. 1966. Protective effect of antibody to para-influenza type 1 virus. *New Engl. J. Med.* 275: 1145.
  12. Bellanti, J. A. 1968. Role of local gamma-A-immunoglobulins in immunity. *Am. J. Diseases Children.* 115: 239.
  13. Morgan, I. M. 1947. The role of antibody in experimental poliomyelitis. III. Distribution of antibody in and out of the central nervous system in paralyzed monkeys. *Am. J. Hyg.* 45: 390.
  14. Witmer, R. H. 1955. Antibody formation in rabbit eye studied with fluorescein-labeled antibody. *A.M.A. Arch. Ophthalmol.* 53: 811.
  15. Silverstein, A. M. 1964. Ectopic antibody formation in the eye: Pathologic implications. In *Immunopathology of Uveitis*. A. E. Maumenee and A. M. Silverstein, editors. The Williams & Wilkins Company, Baltimore. 83.
  16. Cotran, R. S. 1963. Retrograde proteus pyelonephritis in rats. Localization of antigen and antibody in treated sterile pyelonephritic kidneys. *J. Exptl. Med.* 117: 813.
  17. Smiley, J. D., C. Sachs, and M. Ziff. 1968. In vitro synthesis of immunoglobulin by rheumatoid synovial membrane. *J. Clin. Invest.* 47: 624.
  18. LoSpalluto, J., J. Chegoriansky, A. Lewis, and M. Ziff. 1960. Chromatographic properties of gamma globulin: behavior of serum gamma macroglobulins. *J. Clin. Invest.* 39: 473.
  19. Cebra, J. J., and J. B. Robbins. 1966. Gamma-A-immunoglobulin from rabbit colostrum. *J. Immunol.* 97: 12.
  20. Heptinstall, R. H., and R. H. Gorrill. 1955. Experimental pyelonephritis and its effect on the blood pressure. *J. Pathol. Bacteriol.* 69: 191.
  21. Askonas, B. A., J. H. Humphrey, and R. R. Porter. 1956. On the origin of the multiple forms of rabbit gamma-globulin. *Biochem. J.* 63: 412.
  22. Askonas, B. A., and J. H. Humphrey. 1958. Formation of specific antibodies and gamma-globulin *in vitro*. A study of the synthetic ability of various tissues from rabbits immunized by different methods. *Biochem. J.* 68: 252.
  23. Helmreich, E., M. Kern, and H. N. Eisen. 1962. Observations on the mechanism of secretion of gamma-globulins by isolated lymph node cells. *J. Biol. Chem.* 237: 1925.
  24. Ralph, P., M. Becker, and A. Rich. 1967. Immunoglobulin synthesis in a cell-free system. *Cold Spring Harbor Symp. Quant. Biol.* 32: 277.
  25. Crabbé, P. A. and J. F. Heremans. 1966. The distribution of immunoglobulin-containing cells along the human gastrointestinal tract. *Gastroenterology.* 51: 305.
  26. Rossen, R. D., C. Morgan, K. C. Hsu, W. T. Butler, and H. M. Rose. 1968. Localization of 11 S external secretory IgA by immunofluorescence in tissues lining the oral and respiratory passages in man. *J. Immunol.* 100: 706.
  27. Tomasi, T. B., Jr., and S. Zigelbaum. 1963. The selective occurrence of gamma-1-A globulins in certain body fluids. *J. Clin. Invest.* 42: 1552.
  28. Tomasi, T. B., Jr., E. M. Tan, A. Solomon, and R. A. Prendergast. 1965. Characteristics of an immune system common to certain external secretions. *J. Exptl. Med.* 121: 101.
  29. South, M. A., M. D. Cooper, F. A. Wollheim, R. Hong, and R. A. Good. 1966. The IgA system. I. Studies of the transport and immunochemistry of IgA in the saliva. *J. Exptl. Med.* 123: 615.
  30. Butler, W. T., R. D. Rossen, and T. A. Waldmann. 1967. The mechanism of appearance of immunoglobulin A in nasal secretions in man. *J. Clin. Invest.* 46: 1883.
  31. Felsenfeld, O., W. E. Greer, and A. D. Felsenfeld. 1967. Cholera toxin neutralization and some cellular sites of immune globulin formation in *Cercopithecus aethiops*. *Nature.* 213: 1249.
  32. Douglas, R. G., Jr., R. D. Rossen, W. T. Butler, and R. B. Couch. 1967. Rhinovirus neutralizing antibody in tears, parotid saliva, nasal secretions and serum. *J. Immunol.* 99: 297.
  33. Bienenstock, J., and T. B. Tomasi, Jr. 1968. Secretory gamma-A in normal urine. *J. Clin. Invest.* 47: 1162.
  34. Merler, E., J. S. Remington, M. Finland, and D. Gitlin. 1963. Characterization of antibodies in normal human urine. *J. Clin. Invest.* 42: 1340.
  35. Hanson, L. A., and E. M. Tan. 1965. Characterization of antibodies in human urine. *J. Clin. Invest.* 44: 703.
  36. Turner, M. W., and D. S. Rowe. 1967. Antibodies of IgA and IgG class in normal human urine. *Immunology.* 12: 689.
  37. Vosti, K. L., and J. S. Remington. 1968. Host-parasite interaction in patients with infections due to *Escherichia coli*. III. Physicochemical characteristics of O-specific antibodies in serum and urine. *J. Lab. Clin. Med.* 72: 71.
  38. Franklin, E. C. 1959. Physicochemical and immunologic studies of gamma globulins of normal human urine. *J. Clin. Invest.* 38: 2159.
  39. Vaughan, J. H., R. F. Jacox, and B. A. Gray. 1967. Light and heavy chain components of gamma globulins in urines of normal persons and patients with agammaglobulinemia. *J. Clin. Invest.* 46: 266.
  40. Berggård, I., and H. Bennich. 1967. Fc fragment of immunoglobulin G in normal human plasma and urine. *Nature.* 214: 697.
  41. Weyrauch, H. M., M. L. Rosenberg, A. D. Amar, and M. Redor. 1957. Effects of antibiotics and vacci-

- nation on experimental pyelonephritis. *J. Urol.* **78**: 532.
42. Braude, A. I., and J. Sieminski. 1961. The influence of endotoxin on resistance to infection. *Bull. N. Y. Acad. Med.* **37**: 448.
43. Sanford, J. P., B. W. Hunter, and L. L. Souda. 1962. The role of immunity in the pathogenesis of experimental hematogenous pyelonephritis. *J. Exptl. Med.* **115**: 383.
44. Hunter, B. W., L. L. Akins, and J. P. Sanford. 1964. The role of immunity in the pathogenesis of experimental retrograde pyelonephritis. *J. Exptl. Med.* **119**: 869.
45. Arana, J. A., V. M. Kozij, and G. G. Jackson. 1965. The immunologic status of the host and pyelonephritis: A study of retrograde *Escherichia coli* urinary infection in rats. *J. Immunol.* **94**: 337.
46. Carter, M. J., and N. J. Ehrenkranz. 1966. Immunologic studies in urinary tract infections. II. Correlation of hemagglutinin response with bacteriuria following catheterization. *J. Lab. Clin. Med.* **67**: 13.