Agar Gel Diffusion Studies with Sera and Urinary Proteose from Patients with Certain Renal Diseases.* (26109)

JACOB M. LEVINE, BILL P. MADUROS AND ALBERT SUTTON
(Introduced by S. Howard Armstrong, Jr.)
Hektoen Institute for Medical Research, Cook County Hospital, Chicago, Ill.

Isolation and characterization of a proteose in urines of patients with certain active renal diseases has been reported(1). We are investigating whether this carbohydrate-rich proteose might have antigenic properties and be implicated in an autoimmune process in renal disease. The demonstration of reactions by the agar diffusion technics between sera and urinary proteose from patients with certain renal disease is reported here.

Methods and procedures. Urinary proteose was prepared according to the method previously reported(1). The proteose containing filtrate was brought by boiling to half the volume of the original urine, and served as antigen.† Double diffusion agar studies were carried out by the Korngold modification of the Ouchterlony technic(2) and with a modification of the Oakley-Fulthorpe technic(3). The modification was addition of 3.75 g % of ammonia-free glycine to the 2 concentrations of agar employed. Difco Noble Agar was used and no preservative added. Two ml of proteose solution were added to 5.0 ml of antigen agar and the serum layered over the clear zone agar. The tubes were sealed with modeling clay and incubated at 27°C for periods up to 30 days. Patients from the Nephritic Research Clinic of Hektoen Inst. for Medical Research who had been followed for from 6 months to 3 years and who had been diagnosed clinically and with renal biopsies were selected for this preliminary study.

Results. One hundred and eighty-two tests were performed with 44 sera and 43 urinary proteose preparations. Thirteen sera and 14 urinary proteose reacted. The type of reaction observed is illustrated in Fig. 1 and relationship of patient diagnosis to reaction is summarized in Table I. The diagnosis of glomerulonephritis was established by clinical evaluation and renal biopsy, while diagnoses of Kimmelstiel-Wilson disease, pyelonephritis and nephrosclerosis rest on clinical evidence and the usual laboratory findings. Only the predominant pathologic diagnosis is listed; many of the biopsies also revealed concomitant interstitial nephritis. One patient with chronic glomerulonephritis and systemic lupus erythematosus and on steroid therapy failed to react in autologous system but did cross react with the proteose and serum of a patient with active glomerulonephritis. The urinary proteose of a patient with biopsy evidence of chronic membranous glomerulonephritis and also on steroid therapy cross reacted with the serum of a patient with biopsy evidence of chronic glomerulonephritis. The sera and urinary proteose of a third patient on steroid therapy at time of study and with renal biopsy evidence of chronic glomerulonephritis and acute focal interstitial nephritis, failed to react in autologous or cross systems.

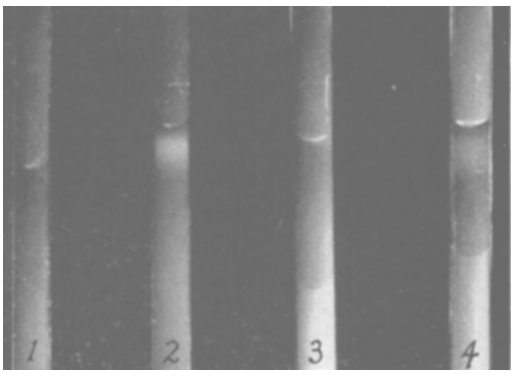

FIG. 1.
1. T.G.*/R.McK.* (neg)
2. T.G./E.S.† (pos)
3. E.R.*/J.L.‡ (neg)
4. E.R./E.R. (pos)

* Chronic glomerulonephritis.
† " " on steroid therapy.
‡ Nephrosclerosis.

* Supported by grant from Nat. Inst. Health.

† Solubilization of both albumins and globulins on boiling with proteose between pH 5 and 6 has recently been observed; antigen thus prepared cannot be considered free from other proteins.

TABLE I. Relationship of Diagnosis to Reactions.

Sera	Urinary proteose	Positive reactions
Glomerulonephritis	Glomerulonephritis	21
"	Glomerulonephritis + systemic lupus erythematosus	1
Glomerulonephritis + systemic lupus erythematosus	Glomerulonephritis	1
Kimmelstiel-Wilson + pyelonephritis	"	6
Idem	Kimmelstiel-Wilson + pyelonephritis	1
"	Nephrosclerosis*	1
Nephrosclerosis*	Kimmelstiel-Wilson + pyelonephritis	1
"	Glomerulonephritis	3
"	Nephrosclerosis*	1

* This patient also had parathyroid adenoma, positive urine culture at one time, and urinary sediment contained red blood cell and white blood cell casts. No renal biopsy is available.

Attempts to obtain reactions between orosomucoid‡ and positive and negative sera failed. Seven positive and 11 negative sera did not react with orosomucoid (100 mg % in phosphate buffer at pH 6.9) either before or after boiling. Tests between 2 normal sera, 2 sera from patients with pneumonia and 12 positive reacting urinary proteose were negative. Four patients with nephrosclerosis also failed to react.

The clinical and laboratory findings listed for each patient were those present on the day blood was drawn and urinary proteose or urine treated as for proteose was prepared. Table II illustrates occurrence of various factors in reactors and non-reactors. Proteinuria and significant urinary infection were more frequent in positive reacting patients than in non-reactors and apparently the sera and urinary proteose from patients with the nephrotic syndrome gave highest evidence of reaction and interreaction.

Discussion. In those patients with glomerulonephritis and pyelonephritis, it is interesting to speculate whether or not the cross reactions are due to the antigenicity of the protein derivatives from the interstitial component of the disease. The findings of Goodman, Greenspan and Krakower(4) that in the dog, renal tubular epithelial cells possess antigens in common with the epithelial cells of the renal glomerulus, and of Baxter and Goodman(5) that the renal medulla is likewise capable of producing a nephrotoxic antiserum, tend to support this view. Failure of the 3 patients on steroid therapy to react in autologous system while 2 did cross react raises the question whether steroid therapy prevents or attenuates the autoimmune mechanism as measured by these methods. Liu and McCrory(6), using the Ouchterlony double diffusion agar technic, tested sera from patients with glomerulonephritis and the nephrotic syndrome against whole extracts of human kidney, liver and lung. They reported an overall higher incidence of reactors in patients with the nephrotic syndrome. We have found a higher percentage of reactions with sera and urinary proteose from patients with the nephrotic syndrome. The reported reactions of sera and proteose in man as evidenced by double diffusion agar technics have their counterparts in the work of Witebsky, Rose and Shulman(7), suggesting the possibility of an autoimmune process much the same as the abnormal thyroglobulin in thyroiditis.

TABLE II. Occurrence of Factors in Reactors and Non-reactors.

					Antistreptolysin-O titers			Urinary infection		
		Symptoms	Abnormal urinary protein	Abnormal urinary red blood cells	Normal	Elevated	Unknown	+	?	Nephrotic syndrome
		%								
Sera	+	48	81	71	72	23	5	42	5	33
	0	30	72	62	69	23	8	31	4	20
Urinary proteose	+	33	100	75	61	33	6	44	3	58
	0	27	70	63	61	30	9	26	3	29

+ = Reactors. 0 = Non-reactors.

‡ Kindly supplied by Dr. Richard J. Winzler.

Summary. Visible precipitin bands have been demonstrated by double diffusion in agar studies with human urinary proteose and nephritic sera. These reactions were observed in autologous and cross systems. It appears that sera and urinary proteose obtained from patients with the nephrotic syndrome are the most likely to react.

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Received July 26, 1960. P.S.E.B.M., 1960, v105.

Effect of *Sterculia Foetida* Oil on Uptake of Water by the Avian Egg Yolk.*† (26110)

A. R. DOBERENZ, D. L. SCHNEIDER, A. A. KURNICK, M. G. VAVICH AND A. R. KEMMERER

Departments of Agricultural Biochemistry and Poultry Science, University of Arizona, Tucson

Addition of sterculic acid(1,2), *Sterculia foetida* oil(1), malvalic acid(2), or cottonseed oil(3) to the diet of laying hens, causes pink discoloration in cold storage eggs. Schaible and Bandemer(4) showed that the pink color was imparted by a complex of iron with conalbumin. Evans, *et al.*(5) using paper electrophoresis reported that protein migrated from the white to the yolk during storage. This suggests changes in the egg which allow passage of iron and conalbumin across the vitelline membrane(4). The purpose of this investigation was to determine the effect of *Sterculia foetida* oil on the water passage into intact yolks *in vitro*.

Experimental. A preliminary experiment was designed to study the effect of "clutch size" on water uptake by the yolk. In Experiment I a group of 65 randomly selected S. C. White Leghorn hens were housed in individual wire cages and fed a practical laying diet *ad libitum*. Eggs were collected daily at 3:00 P.M. for 21 consecutive days and stored in the refrigerator at 7°C overnight. The next morning the eggs were tested for water uptake by the method of Orru(6). The intact yolk was placed in a tared beaker and weighed. Deionized water was added and the contents incubated at 37.0 ± 1.0°C for 3 hours. Following incubation the liquid was removed from the beaker with the aid of an aspirator and blotted with filter paper. The beaker and its contents were weighed and the increase in weight was considered to be water uptake.

On the basis of the clutch size data (Table I), hens from Exp. I averaging 2, 3, and 4 day clutches were divided randomly into 4 lots of 8 or 9 birds each for Exp. II. Housing, laying diet, collection of eggs, and method for determination of water uptake by egg yolks were similar to procedures employed in Exp. I with the following exceptions: Gelatin capsules containing supplements of *Sterculia foetida* oil‡ or corn oil§ were administered daily. Hens from Lots 1 and 2 received a supplement of 0.30 g of corn oil daily, while Lots 3 and 4 received a mixture of 0.15 g *Sterculia foetida* oil and 0.15 g corn oil. The

* Supported in part by a grant from Mead Johnson & Co., Evansville, Ind.

† Preliminary report presented in *Fed. Proc.*, 1960, v19, 221.

‡ Extracted from the whole ground nut of the Java Olive tree, *Sterculia foetida*.

§ Mazola Oil, Corn Products Co., New York.