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Serum Globulins in Experimental Nephritis. Effect of Conditioning Factors.* (24821)

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It has been shown that Masugi's experimental nephritis can be enhanced and evolution towards specific clinical forms determined with the aid of conditioning factors such as saline-drinking water and/or unilateral nephrectomy(1,2). The present paper is concerned with the effect of these conditioning factors upon various electrophoretic fractions of serum globulins in normal and nephritic rabbits.

Materials and methods. A. *Control:* Thirty-four albino rabbits of either sex weighing 2800-3200 g, were separated into 4 groups as follows: 16 were controls; 7 were submitted to unilateral nephrectomy; 7 were maintained on saline-drinking water; 4 were unilaterally nephrectomized and one month subsequently were given saline-drinking water. All animals were bled at various intervals during following 6 months. Total serum proteins were determined with biuret method of Reinhold(3). Tiselius moving boundary electrophoresis determined various serum protein fractions. The results were averaged for each group and mean values and standard deviation deter-

mined (S.D. = $\sqrt{\frac{\sum(X^2)}{N-1}}$). B. *Glomerulonephritis* was induced with duck antirabbit-kidney serum prepared according to method previously described(2). Pools and amount of sera provoked clinical and pathological symptoms of glomerulonephritis in all experimental animals. However, in contrast with the kaleidoscopic but relatively mild symptoms of controls, the unilaterally nephrectomized group uniformly showed acute glomerulonephritis with early death accompanied by non-edematous (dry) uremia, while saline treated animals developed either subacute edematous or chronic polyuric glomerulonephritis. Clinical findings were substantiated by histologic studies(2). All animals were bled at varying intervals before and after nephrotoxic injection. The results of electrophoretic determinations, obtained between 7th and 21st day following nephrotoxic injection, were averaged and differences from control baseline expressed in standard deviation units.

Results. Table I shows results of 44 electrophoretic determinations averaged for each group of normal and preconditioned rabbits. Conditioning procedures do not alter the electrophoretic pattern of serum as compared to controls. Total mean values shown in extreme right column were used as control baselines for each electrophoretic protein fraction.

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TABLE 1. Electrophoretic Fractionation of Globulins in the Serum of Normal and Preconditioned Rabbits (mg/100 ml Serum).

Globulin fraction	Normal control	Unilaterally nephrectomized	Saline drinking	Unilateral nephrectomy and saline	Total mean $\pm$ S.D.
Total alpha globulins	1.01	1.09	1.04	1.00	1.03 $\pm$.19
Alpha-1	.21	.28	.22	.21	.23 $\pm$.09
" -2	.45	.44	.45	.44	.45 $\pm$.09
" -3	.35	.36	.37	.35	.35 $\pm$.09
Total beta globulins	.86	.90	.88	.84	.87 $\pm$.22
Beta-1	.56	.62	.63	.62	.59 $\pm$.16
" -2	.29	.28	.25	.23	.28 $\pm$.15
Gamma globulins	.64	.70	.66	.60	.65 $\pm$.24

Fig. 1 summarizes results of 25 electrophoretic determinations performed between 7th and 21st day following injection of nephrotoxic serum. The columns show average deviations from control values as expressed in standard deviation units (S.D.). Shaded area covers 2.6 S.D. (e.g., $P = .01$).

The dose of nephrotoxic serum did not induce significant alteration in serum globulin values of control rabbits. However, unilaterally nephrectomized rabbits increased alpha-3 globulin fraction, while saline-drinking animals increased alpha-2 serum globulin component following nephrotoxic injection.

A typical normal electrophoretic pattern of rabbit serum is shown in Fig. 2. The additional intermediate fraction situated between alpha-2 and beta-1, has been conventionally called alpha-3. It was present in all 69 electrophoretic determinations.

Fig. 3 shows electrophoretic patterns of a control and of 3 preconditioned rabbits 3 weeks after intravenous injection of similar doses of nephrotoxic serum. Alterations of

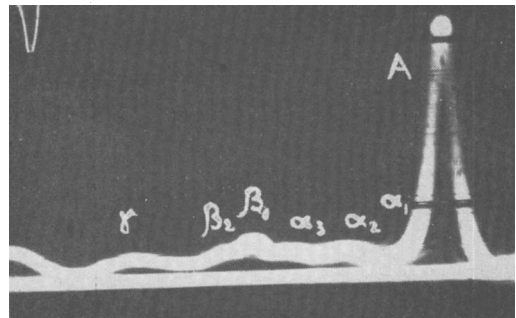


FIG. 2. Moving boundary electrophoretic pattern of normal rabbit serum.

serum protein patterns are in agreement with average deviation shown in Fig. 1.

Discussion. From above results it may be concluded that nephrotoxic sera, in doses that do not influence the electrophoretic serum globulin pattern of control rabbits, may induce significant alterations when given to animals preconditioned with saline and/or uninephrectomy. When nephrotoxic serum was given to saline preconditioned animals, a significant increase of alpha-2 serum globulin was noticed. Considering that these animals developed chronic glomerulonephritis with polyuria and hyposthenuria(1,2), the increase of alpha-2 serum globulins reproduces experimentally a pathologic condition already reported in chronic nephritic patients(4,5). Alpha-2 globulin increase could be caused, according to Eiselt and Hrabane(4), by adsorption of lipids secondary to hyperlipemia which is common in this disease. However, since it appears in chronic forms of both human and experimental nephritis, the possibility of increased serum hypertensinogen (also an alpha-2 euglobulin) should not be dis-

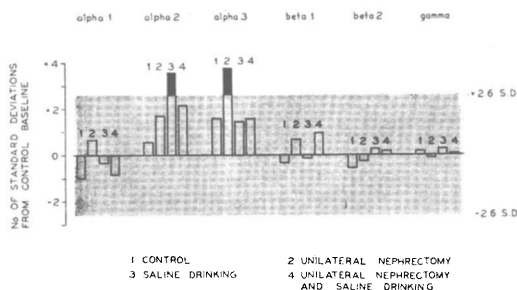


FIG. 1. Deviation from control baseline of various serum globulins during experimental nephritis induced with antirabbit kidney duck serum in rabbits: 1. normal; 2. uninephrectomized; 3. saline-drinking; 4. uninephrectomized and saline-drinking.

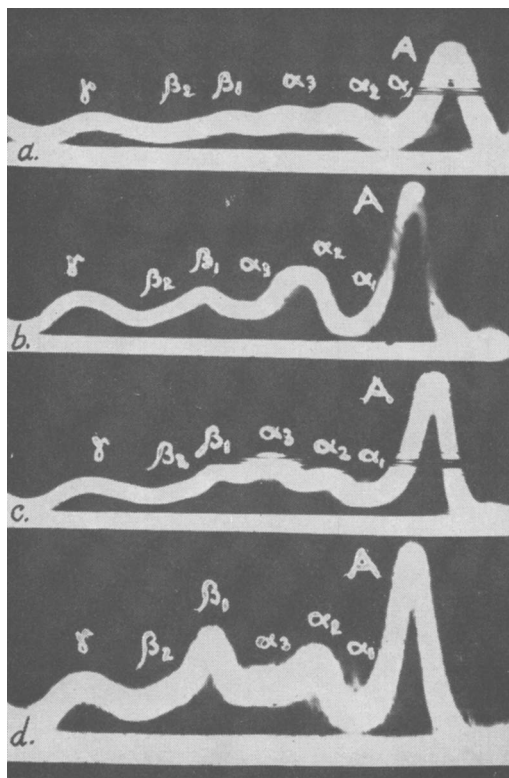


FIG. 3. Alteration of serum electrophoretic pattern during experimental nephritis in rabbits: (a) control; (b) saline-drinking; (c) uninephrectomized; (d) uninephrectomized plus saline.

counted. The conditioning effect of salt, demonstrated in chronic forms of experimental nephritis(1,2), confirmed observations of Barker and Robinson in humans(6).

When nephrotoxic serum was given to animals preconditioned by surgical removal of one kidney, the alpha-3 globulin fraction was significantly increased. The animals exhibited severe nephritis-nephrosis lesions presumably because the residual kidney received the same amount of nephrotoxic antibodies absorbed by 2 kidneys in controls(2). The association between nephritis-nephrosis lesions and increased alpha-3 globulin can be considered another experimental parallel with the disease in humans. Instead of the alpha-3 fraction which does not exist in human serum, the

neighboring alpha-2 and beta globulins increased in adult patients with mixed nephritis-nephrosis(5,8), and in children with lipoid nephrosis(5). The alpha-3 globulin found as constant component of rabbit serum in above determinations was not reported in the work of Ellis using paper electrophoresis. The Figures suggest that the intermediary fraction was included in neighboring alpha and beta globulins(7).

Summary. 1. Experimental glomerulonephritis following administration of antirabbit kidney duck serum was induced in normal and preconditioned rabbits. 2. Significant alterations in serum globulins were recorded only in preconditioned animals. a) Rabbits preconditioned with 0.9% saline-drinking water developed clinical symptoms of chronic glomerulonephritis and showed significant increase of alpha-2 serum globulin fraction. b) Animals preconditioned by uninephrectomy showed severe nephritis-nephrosis lesions associated with significant increase of serum globulin fraction situated between alpha-2 and beta globulin (alpha-3 globulin) in the moving boundary electrophoretic patterns.

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