6. Kaplan, A. S., *Virology*, 1955, v1, 377.

7. Mogabgab, W. J., Green, I. J., Dierkhising, D. C., Phillips, I. A., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 654.

8. Mogabgab, W. J., Green, I. J., Dierkhising, D. C., *Science*, 1954, v120, 320.

9. ———, *J. Lab. Clin. Med.*, 1954, v44, 899.

Received February 10, 1959. P.S.E.B.M., 1959, v101

Metabolism of Acid Mucopolysaccharides of Rabbits with Serum Sickness.* (24820)

GERALD S. BERENSON (Introduced by R. A. Coulson)

Dept. of Medicine, Louisiana State University School of Medicine, New Orleans

The clinical description of serum sickness by Von Pirquet and Schick(1), aroused considerable interest. More recently, Gregory and Rich(2) demonstrated that serum sickness can be produced readily in rabbits and can serve as a useful experimental model to study phenomena of hypersensitivity. In studies with rabbits, lesions of connective tissue structures are observed, similar to those associated with the group of diffuse diseases of human connective tissue, as lesions associated with polyarteritis nodosa. One factor that seems common to the human diseases, experimental serum sickness, and related hypersensitivity states is the similar pathologic involvement of matrix or ground substance of connective tissue. This similarity is the basis for considering an etiologic relation between hypersensitivity and diseases of connective tissue. Although such relation remains to be established, the inflammatory response of components of connective tissue to disease due to hypersensitivity is provocative. For purposes of studying this inflammatory response, biochemical technics were used to investigate components of the matrix of connective tissue. The effect of serum sickness on acid mucopolysaccharides (MPS) of skin and cartilage of rabbits was observed.

Methods and materials. Six groups of 4 to 6 rabbits, each of approximately 2.6 kg weight, were studied. The rabbits were offered water and laboratory chow freely. Serum sickness was induced in one-half or more of each group by 2 intravenous injections of horse serum (10 ml/kg) given 2 weeks apart (3). From each group 2 rabbits were selected: one demonstrating most clinical evidence of serum sickness, judged by sedimentation rates and changes in weight, was matched approximately by weight with a normal rabbit. Rabbits were sacrificed 6 days after second injection of horse serum, when maximal lesions from serum sickness are expected(3). Examination of gross and histologic sections† confirmed the presence of pathologic lesions of serum sickness. During period of observation after second injection of horse serum to sick animals, the pair selected from each group (one animal with serum sickness and its control) received subcutaneous injections (50 μc) of C^{14} carboxyl-labeled sodium acetate‡ in 0.15 *M* solution 3 times a day. One series of 3 pairs received the isotope for 3 days (450 μc) and another 3 pairs for 5 days (750 μc) before sacrifice. Timing of administration of labeled acetate was chosen for comparison with other observations in normal animals(4). MPS, hyaluronic acid and chondroitin sulfate, were isolated from skins of rabbits by methods previously described(5), and chondroitin sulfate was isolated from cartilage(6) obtained from various sites of body, such as ears, trachea and sternal cartilages. The amount of MPS isolated was determined by weighing or estimated from solutions by uronic acid deter-

* Supported by grants from Am. Heart Assn. and Nat. Heart Inst.

† The author appreciates these examinations by Dr. E. P. Benditt, Univ. of Chicago and Dr. H. C. McGill, Louisiana State Univ. School of Med.

‡ Appreciation is extended to Dr. J. Meyers, Vet. Admin. Hosp., New Orleans, for assistance in preparing the acetate.

minations(7). In addition, glycogen was isolated from skeletal muscle and liver of individual rabbits and fractionated with alcohol (8) to yield preparations with optical rotation $[\alpha]_D^{23}$ of $+200°$. Radioactivity of C^{14} in MPS and glycogen was determined by microcombustion of samples, collected as $BaCO_3$ and counted in open window, gas-flow counter. Radioactive counts were corrected to "infinite thickness" and to equivalent weights of rabbits. Analyses of data from normal animals in these and other experiments (4) yielded average variation of approximately $\pm$ 18% (range, 1 to 47%) for a single animal. These experiments include triplicate determinations for each category studied.

Results. Total amount of MPS that could be isolated from skin or cartilage was essentially the same for each group of animals. Radioactivity of hyaluronic acid from skin was slightly higher for rabbits with serum sickness [avg. 3800 (3050-4180)] than for controls [avg. 3005 (2900-3165)] in the series that received isotope for 5-day period. Similarly, radioactivity of chondroitin sulfate from skin of rabbits with serum sickness [avg. 1603 (1330-1785)] was slightly greater than observed for controls [avg. 1324 (902-1600)] after receiving C^{14} acetate for 5 days. No apparent effect was observed for the series with 3 days of isotope administration and no differences of isotope incorporation were observed for chondroitin sulfate of cartilage in either series. In glycogen samples, a considerably greater incorporation of isotope in diseased animals was demonstrated. Increase of radioactivity in glycogen was noted particularly in samples from liver and in animals that received isotope for 5 days. The average of the group with serum sickness was 2080 (1440-2720) and 192 (171-211) for the control group. A 2-fold increase of comparative radioactivity for sick animals was observed for muscle. Smaller but definite differences in the same direction were found in the series receiving isotope for 3 days. In addition, total amount of glycogen that could be extracted from livers of rabbits with serum sickness was approximately one-half that for control animals.

Discussion. The magnitude of differences observed, is only slightly greater than the limit of error for such experiments. Since isolation and study of radioactivity of these compounds require a relatively large quantity of tissue, skin and cartilage were considered most suitable for study; however, selection of tissue may have limited the detection of changes of MPS. Serum disease produces diffuse pathologic changes, with small lesions throughout various connective tissue structures and tissues studies probably represent an admixture of tissue with and without lesions. These studies primarily measure incorporation of an isotope into MPS, without a quantitative estimate of the MPS pool. Although it would be desirable to know effect of serum sickness on rate of synthesis of MPS of the body or in a uniform lesion, incorporation alone is not an absolute measure of synthesis. The isolation under comparable conditions of equal amounts of MPS from tissues of normal and diseased animals and the slight variance of isotope labeling, suggest that serum sickness does not produce significant changes in the over-all metabolism of MPS.

Contrary to studies of MPS, a rather remarkable effect was observed on liver glycogen and, to a lesser extent, on muscle glycogen. That changes occurred in glycogen is a further manifestation of the generalized illness. Such changes in glycogen could be interpreted as nonspecific, being similar to those that accompany starvation(9,10).

Summary. Studies of incorporation of C^{14}-labeled acetate into MPS, hyaluronic acid and chondroitin sulfate from skin and chondroitin sulfate from cartilage of rabbits with and without serum sickness, indicated little difference effected by serum sickness. Specific activities of skin MPS were possibly increased. Greater incorporation of the isotope was observed in liver and muscle glycogen from rabbits with serum sickness. Further study of other hypersensitivity reactions and of tissues more specifically involved by such reactions need investigation. Such experiments, currently in progress utilizing the local Shwartzman lesion, do indicate a considerable increase of metabolic activity of the MPS.

The author is very grateful for cooperation of Dr. Albert Dorfman, in whose laboratory these experiments were begun.

1. Von Pirquet, C. Frh., Schick, B., *Serum Sickness*, Translated by B. Shick, Williams and Wilkins Co., Baltimore, 1951.

2. Rich, A. R., Gregory, J. E., *Bull. J. Hopkins Hosp.*, 1943, v72, 65.

3. Wissler, R. W., Smull, K., Lesh, J. K., *J. Exp. Med.*, 1949, v90, 577.

4. Schiller, S., Mathews, M. B., Goldfaber, L., Ludowieg, J., Dorfman, A., *J. Biol. Chem.*, 1955, v212, 531.

5. Schiller, S., Mathews, M. B., Jefferson, H., Ludowieg, J., Dorfman, A., *ibid.*, 1954, v211, 717.

6. Mathews, M. B., Roseman, S., Dorfman, A., *ibid.*, 1951, v188, 327.

7. Dische, Z., *ibid.*, 1947, v167, 189.

8. Stetten, D. Jr., Boxer, G. E., *ibid.*, 1944, v155, 231.

9. Boxer, G. E., Stetten, D. Jr., *ibid.*, 1944, v155, 237.

10. Fruton, J. S., Simmonds, S., *General Biochemistry*, Ch. 19, p454. John Wiley and Sons, N. Y., 1953.

Received December 22, 1958. P.S.E.B.M., 1959, v101.

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

CONSTANTIN V. TEODORU, ABRAHAM SAIFER AND HARRY FRANKEL
Isaac Albert Research Inst., Jewish Chronic Disease Hosp., Brooklyn, N. Y.

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 determined $\left(\text{S.D.} = \sqrt{\frac{\Sigma(X^2)}{N-1}} \right)$. 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.

* This investigation was supported in part by Research Grant from U.S.P.H.S.