# SYNTHESIS OF ALPHA - 2 MACROGLOBULIN IMMUNOLOGIC DISEASE

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REPORT OF A SUMMER MEDICAL RESEARCH PROJECT
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**Introduction:**

In immunologic disease, characterized by antibodies directed against body protein, quantitative and qualitative changes are noted in the serum proteins. Acute glomerulonephritis serves as an example of this group of conditions. Here changes can be detected in serum proteins by use of simple techniques, e.g., electrophoresis on paper. As more refined methods become available, different types of protein (1) are identified, then characterized. Since the identification of $\alpha$ - 2 macroglobulin, its physiologic role has been studied (2, 3).

Experimental work has involved the production of immunologic renal disease in rats. Antibodies to rat glomeruli are produced in rabbits by sequential stimulation with antigenic rat kidney tissue. Rabbit serum containing antibody to glomerular protein of the rat kidney is finally collected (4). This serum injected intravenously into a rat will produce gross proteinuria in five hours, also changes in serum proteins. This type of experimental nephrosis in rats causes an increase in macroglobulin, which can be detected by starch gel or acrylamide gel electrophoresis (5). Experimental nephrosis produced in this manner is remarkably similar in its serologic and urinary manifestations to naturally occurring glomerulonephritis (6).

Recently the relative increase in slow migrating macroglobulin (Beaton's S $\alpha$ - 1M) (7) in anti rat kidney serum nephrosis was associated with the appearance of a new slow migrating $\alpha$ - 2 macroglobulin (S $\alpha$ - 2M) not seen in healthy adult rats (5, 7, 8). It was our intention to demonstrate that this increase in macroglobulin was due to protein synthesis, and not to destruction or release of pre-formed protein. We also hoped to distinguish between the macroglobulins, and show which ones were actively synthesized. Further, it was hoped that the slow migrating $\alpha$ - 2 macroglobulin seen in AKS nephrosis should be isolated, and antibody to it prepared. This would permit quantitation and further characterization by immunoelectrophoresis.

The incorporation of radioactive glucosamine in glycoprotein can be demonstrated by counting the radioactivity of serum proteins. Moscarello has used this method to demonstrate increased incorporation of glucosamine, and consequently increased protein synthesis, in aminonucleoside and antikidney serum nephrosis (9, 10). With slightly different results we repeated the experiment using scintillation methods. We attempted to demonstrate what fractions of serum protein were synthesized by means of autoradiographs of electrophoretic patterns obtained on starch and acrylamide gels.

**Results:**

(1) Isolation of Slow Migrating Macroglobulin:

Rats suffering from anti rat kidney serum nephrosis for four days, and showing proteinuria (4 plus with sulfosalicylic acid) after five hours, were exsanguinated by heart puncture. Lipemic serum was obtained.

The serum was separated on starch gel by electrophoresis. Results consistent with those obtained by DeWolfe (6) were obtained. Concurrently rat serum from animals suffering from anti rat kidney serum nephrosis for two weeks was separated by starch gel and acryl-

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amide gel electrophoresis. Here the differences observed after four days of illness were exaggerated. The differences observed included:

- gross lipemia of the serum
- decreased serum albumen concentration
- marked increase in the slowly migrating fractions
- an additional fraction slower than those present in normal rat serum.

Now that the extent of the changes in serum proteins had been established, a preparative separation of 20 mls of lipemic rat serum was attempted. Separation of the serum proteins was obtained by electrophoresis-on a starch block for three days. By use of a distribution curve, obtained by sampling the block and determining the protein concentration, the block was divided into six fractions, separating the serum protein into pre-albumen, albumen, alpha-1, alpha-2, beta, and gamma fractions. These fractions were separated from the starch by centrifugation, and concentrated by ultra-filtration under negative pressure at 8° C. Starch gel and acrylamide gel electrophoresis of these fractions showed the separation of macroglobulin to be poor. Isolation of electrophoretically pure $\alpha 2$ macroglobulin was not achieved, so no antigenic protein was available for the stimulation of antibody production in rabbits. Time precluded further work on this experimental method.

An attempt was made to separate macroglobulin by a shorter technique. Franklin's method (11) of ultracentrifugation was used, but a satisfactory separation of macroglobulin was not achieved.

(2) Synthesis of Slow $\alpha$ 2 Macroglobulin:

From earlier studies by Moscarello et al (9) it has been shown that in experimental anti kidney serum nephrosis there is an increase in protein synthesis, evidenced by a 80 – 100% increase in the specific activity of serum protein labeled with glucosamine – 1 – 14C. In the present study, as preliminary step in demonstrating increased synthesis of $\alpha$ – 2 macroglobulin and slow $\alpha$ – 2 macroglobulin, whole protein fractions were counted by liquid scintillation methods.

Rats under nembutol anesthesia were injected via the jugular vein with 1 cc of anti rat kidney serum. Controls received 1 cc of normal rabbit serum. After varying lengths of time glucosamine – 1 – 14 C (1 ml M 13.1 $\mu$c) was injected by the same route into experimental and control animals. After two hours the rats were exsanguinated by cardiac puncture. Before death all rats were checked for proteinuria. Activity in the trichloroacetic acid soluble and insoluble fractions of the serum was calculated.

Results showed a variable but significantly increased incorporation of glucosamine – 1 – 14C into the serum proteins of nephrotic rats. The results disagree with Moscarello's 80 – 100% increase in specific activity by demonstrating only a 0 – 16% increase in specific activity ($\mu$c/mg) at two hours.

This result may be explained by considering the variations in response with the amount (mg) of glucosamine injected. Perhaps the normal rate of depletion of serum glucosamine in health is high enough to allow incorporation of all the material injected, and larger amounts must be used to demonstrate differences in specific activity in disease. Indeed Moscarello injected more sugar.

As the activity was incorporated rapidly in the protein, experiments were carried out on rats nephrotic for seventeen hours by injecting activity one hour before exsanguination. Now an increase in the specific activity of 300% was noted. With this result we draw closer to Moscarello's findings. Time did not permit further experiments to determine the affect of injection timing or the response to increased doses of glucosamine.

Starch gel and acrylamide gel electrophoresis were done on radioactive protein from nephrotic rats and control rats. Autoradiographs, using Kodak No Screen X-ray film, were made to determine which proteins were actively synthesized. It was expected that a great deal of the activity would appear in the slow alpha – 2 macroglobulin, and so demonstrate its active synthesis in disease. After three days, then two weeks, of exposure no activity was seen on the film. Presently film is being exposed for six weeks. The technique of autoradiography on small radioactive amounts of serum proteins is yet to be perfected.

**Summary:**

(1) Serum proteins were separated by acrylamide gel, starch gel, and starch block electrophoresis, then by ultracentrifugation, in an attempt to isolate slow alpha – 2 macroglobulin produced in experimental nephrosis.

(2) A new method for counting the radioactivity of serum protein by liquid scintillation was devised.

(3) Incorporation of glucosamine – 1 – 14C in serum proteins in experimental anti kidney serum nephrosis was demonstrated. By this means we have demonstrated active synthesis of serum proteins.

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