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Serum mucoproteins : with special reference to the role of mucoproteins in rheumatic fever

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SERUM MUCOPROTEINS WITH SPECIAL REFERENCE TO THE ROLE OF MUCOPROTEINS IN RHEUMATIC FEVER

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Introduction

The differential diagnosis of rheumatic fever has long been a problem. The majority of cases may be relatively easy to diag-nose on the basis of histories and physicals; however there are cases which are borderline. In fact, rheumatic fever and the collagenous diseases seem to fuse one into another; some cases show typical findings of two different diseases. This is also true of some of the arthritides. These facts have made diagnostic laboratory tests highly desirable and much sought for.

Further, since rheumatic fever tends to be prolonged over weeks and months, a laboratory aid in following the progress of the disease and the effectiveness of therapy is desirable, For, even though a patient may be clinically well, the disease may be active and cessation of therapy may result in acute exacerbation. Thus we have two problems in connection with rheumatic fever: the diagnosis, and a check for adequacy and for the appropriate time for termination of therapy.

A Comparison of Tests Used in Rheumatic Fever

To date satisfactory solutions to neither of these problems have been found. There is no specific diagnostic test nor is there a reliable test of rheumatic activity of a known case under therapy. There are tests, however, which are of sufficient value to be use-ful in diagnosis and in following the effectiveness of therapy. Among the most valuable are the antistreptolysin O titer and the erythrocyte sedimentation rate. Newer tests, which are still in the experimental stage, are tests for C-reactive protein, serum hyaluronidase inhibitor and serum mucoprotein levels. These are of

little value in diagnosis but may be useful in following progress.

Each of these has its drawbacks and its advantages. Anti-streptolysin O titer is simple, and the reagents needed are more readily available than those used in other antibody titrations. Especially is this true since streptolysin is available commercially. (1). Titration for the antibody to streptokinase is an example of a complicated procedure, while that for hyaluronidase antibody involves an antigen that is very difficult to prepare and unavailable commercially. Anti-desoxyribonuclease tests depend on the use of desoxyribose nucleic acid which is difficult to procure, and the percentage of patients with rheumatic fever who give a positive test is lower than for other tests. Anti-streptolysin O requires only washed rabbit red cells. The highest dilution of serum that totally inhibits lysis of red cells by one unit of streptolysin O is the end point.

The drawbacks are, first, that patients do not all respond with significant increases of antibody. However if other antibody titers are run, the per cent of patients responding to one or another approaches 100 per cent (1). A second difficulty is that antistreptolysin O is not specific for rheumatic fever but gives a positive test in recent streptococcal infections. Third, antibiotic therapy lowers the overall antibody levels. Anti-streptolysin O is a diagnostic test and is of no value in following rheumatic fever therapy.

The most popular test for use in following rheumatic fever is the erythrocyocyte sedimination rate. Such tests are essential because, as has been stated before, the signs and symptoms of

rheumatic fever are extremely variable, often presenting as another disease; and rheumatic activity very often is subclinical and clinically undetectable. Yet the therapy in subclinical periods is as important as in the obviously active cases. The erythrocyte sedimentation rate, however, is often negative in subclinical cases and may be negative in clinically active cases. Yet it is simple and easy to perform.

C-reactive protein, since it is present only when there is an active disease process (1, 2, 3) and since it is elevated when rheumatic fever is active, may be useful in following therapy. However in convalescence it falls to normal when subclinical activity may still be present. Also, the most specific C-reactive protein test involves an antibody obtained by injecting C-reactive protein into rabbits. This is a difficult and highly technical procedure and the quantities obtained are meager. The alternative is to use the P-polysaccharide from the pneumococcus, but this reduces the accuracy of the test (2, 4). Since it is absent in normal individuals, it is diagnostic in a negative manner. C-reactive protein is present in many diseases and is nonspecific.

Nonspecific serum hyaluronidase inhibitor falls to normal and subnormal levels during convalescence from rheumatic fever. The level stays subnormal often for months and may be correlated with a period of increased susceptibility to rheumatic fever or may have been low to start with, indicating the person to have been susceptible before acquiring rheumatic fever (5). However, it falls too early to be of value in following the disease since the disease may be active subclinically. Also, since it ~~is present~~ in normal

serum, many individuals are borderline.

Mucoprotein offers the advantage of staying elevated for variable periods of time after clinical evidence of rheumatic fever has disappeared. This will be discussed more fully later.

Other tests include total leukocyte count, Weltman serum coagulating reaction, measurement of serum component, the bactericidal activity of the blood versus bacillus subtilus, a serum precipitation reaction with a quaternary annonin salt, and a diphenylamine color reaction with serum. None of these has gained significant acceptance.

It is obvious, then, that there is no satisfactory test for the diagnosis of rheumatic fever or for the following of the clinical course of the disease (1, 6, 7).

Even though serum mucoprotein is not completely satisfactory as a determination of activity of disease in rheumatic fever, it is the author's purpose to evaluate it. This will include a brief presentation of the historical background of mucoprotein, its chemical nature as far as is known, its differentiation from some other acute phase reactants, a brief comparison of their relative values as tests in rheumatic fever, and some of the theories of the origin, regulation and function of mucoproteins.

Historical Review

The first mention of mucoprotein was made by Zenetti in 1879 (8, 9). He then isolated it from blood serum in 1903. In 1907 Bywaters isolated "seromucose" from horse serum.(10). He used ox, horse, dog, and cat blood (11). He also showed that the

"albumose" of other workers to be the same as "seromucoid"* (12). In 1921 Wolff (13) separated "albumose" with phosphomolybdic acid and observed that it was elevated in fevers, especially in typhoid fever. Rimington (14) in 1923 isolated carbohydrates from serum proteins and calculated the emperical formula to be $C_{12}H_{23}O_{10}N$. He analyzed and found it to contain glucosamine and mannose. It was found in both the serum albumin and globulin fractions and probably included other acute phase reactants as well as mucoproteins, as did the substances isolated by the earlier workers listed above. He suggested that this substance might function in immune mechanisms. In 1934 Goiffon (15) developed a method of determining the tyrosine in trichloroacetic acid filtrate. He found that the tyrosine bore a constant relationship to the rest of the material present. This discovery proved to be very important in simplifying procedure with mucoproteins by subsequent workers. Hewitt (16, 17) in 19 7 isolated what he thought was a new protein, calling it "seroglycoid," but it was a mucoprotein. It was high in

*Horse and ox blood were treated with 0.1% Na citrate or oxalate and 0.1% formaldehyde diluted with 2-3 vol. H_2O made acid to litmus with H_2SO_4, heating with steam to boiling, the ppt. filtered and washed, evaporated to small vol., dialyzed, acidified with AcOH and the crude seromucoid pptd. by alc. It is purified from coloring matter by repeated treatment of the H_2O extract with SO_2 and pptd. with alc., and, finally, from nucleoprotein by treatment with AcOH and NH_4OH as many times as either reagent produces a ppt. The pure substance pptd. by alc. and dried at 100-110° is a pale yellow powder, difficultly sol. in hot H_2O; yield 5-15 g. pure product from 1 L. blood; reaction with glyoxalic acid intense; treated with KOH and Pb acetate it gives no PbS; gives reactions for glucoproteins in general; does not reduce Fehling's sol.; 2 preps analyze to give the following in %; C 47.92 and 47.32, H 6.85 and 6.84, N 11.75 and 11.43, S 1.70 and 1.81, ash 1.77 and 1.10, carbohydrate equivalent to 23.3% glucose; by B_2Cl and NaOH on products of hydrolysis with 5% HCl, pentabenzoylglucosamine, m. 213°, is produced. Horse blood varies from 0-0.26% mucoid, a dog's blood from 0.284-0.964%, and a ca 's blood gives 1.136% (11).

carbohydrate and was not coagulated by heat or precipitated by 2% trichloracetic acid. In 1939 he published his attempt at analysis and found this new protein to contain galactose, mannose and acetylhexosamine in the carbohydrate portion. He believed that "serum mucoid" was a breakdown product of this new protein (18). Waldschmidt, Leitz and Mayer (19) in 1939 published a test* for cancer using the polarograph and a sulfsalicylic acid filtrate. This filtrate contains mucoprotein which is elevated in cancer. Rimington in 1940 (20) improved the method for isolating "seromucoid" and analyzed it. He found 10.7% carbohydrate, consisting of N-acetylglucosamine, galactose, and mannose in equimolecular proportions, 3% tyrosine, 1% tryptophine, and 2.3% cystine. Using heat, trichloroacetic acid or alcohol filtrates, Alber (21) in 1940 worked on the polarographic tests for cancer again. He found increased levels of the substance involved (mucoprotein) in cancer but also very strong in inflammatory processes. He believed that negative results excluded carcinoma. Mayer (22) in 1942, still searching for a cancer test, used EtOH precipitation of protein free serum and found a substance which was mucoid-like and was elevated in cancer. It contained glucosamine and cystine and

*Tabulated clinical data on cancer diagnosis by means of the polarograph on serum after a variety of treatments leads to the following method as most specific. The serum (0.5cc.) is dild. with 1 cc. of H_2O kept at 100° for 10 min., deproteinized by addn. of 1.5 cc. of 25% sulfosalicylic acid, filtered through hardened paper and 0.5 cc. of the filtrate mixed with 5 cc. of a soln. contg. 0.02675 g. Co $(NH_3)_6CL_3$, 10 cc. of N NH_4Cl and 20 cc. of M NH_3 per 100 cc. The increase of the polarographic wave above normal is the diagnostic sign. Fevers are often accompanied by false positives. (19).

nitrogen. The search for a cancer test was continued by Winzler and Burke in 1944 (23) using rats and rabbits. They tested for "proteoses," using polarographic and chemical methods. Proteose was found to be non-dialyzable, cystine-containing material, heat stable, precipitable by 70% saturated ammonium sulfate, alcohol or tungstic acid but not by 20-30% trichloroacetic acid or sulfosalicylic acid. The "proteose" was elevated in cancer, pyogenic infections, pneumonia, cystitis, severe injuries, and advanced pregnancy. Sex, strain, age, diet, liver cirrhosis and regeneration of liver had no effect. Malignancies caused elevated levels while benign tumors did not. They concluded that this substance was identical to the "index of polypeptidemia" and polarographic filtrate wave observed by others listed above. Rimington (24) in 1948, using ox blood, separated "seromucoid" into two fractions, one being high in carbohydrate which he called a glycoprotein, and a protein similar or identical to those involved in immune mechanisms.

The substances isolated by the above workers are all crude and contain mixtures of proteins. This will be mentioned later in this paper. Winzler et al. and Winzler and Burke (8, 23) concluded, on the basis of the methods used, that they all primarily involved mucoproteins.

As was noted above, several of these authors thought they were finding a new substance and thus several different terms were applied to these serum mucoproteins. This symbolized the whole field up to 1948 when Winzler et al. (8) did their work. It was a confusion of methods as well as terms. Work was sporadic and little was done either to use the results practically in clinical situations or to understand better the physiology of disease.

An attempt was made in 1945 by Meyer (25) to bring order out of the chaos. This was the first, and to date, the only review of the subject as a whole. The result was primarily to bring under one heading all the work on mucopolysaccharides, mucoid, and glycoproteins, not only on serum but also those on all tissues, secretions and animals. He stated that there was no accepted terminology, hence the reviewer had the problem of making his own definitions and classification. Because of the diversity of methods and authors this was very difficult. He classified serum mucoprotein (a term not in use at that time) as seromucoid and seroglycoid. Actually these terms probably included several other serum acute phase reactants as well, since it included elements in both albumin and globulin fractions. There was little done further to clarify this roiley area of "mucoids" and glycoproteins until Winzler et al. in 1948 isolated the substance which they believed to be the same as that isolated by the above authors. To this they applied the term, mucoprotein.

Determination and Isolation of Mucoprotein

Winzler et al. (8) in determining mucoproteins, used modifications of the above methods.* They had decided, on the basis of the similarities of methods and results, that the authors listed in the introduction were all working with the same substance. They

*To 2 ml. of serum or plasma are added 8 ml. of 0.75 M perchloric acid and the mixture shaken. In exactly ten minutes the precipitated proteins are filtered off through a Whatman No. 50 filter paper. To 5 ml. of the filtrate is added 1 ml. of 5 per cent phosphotungstic acid in 2N HCL. In 15 minutes the precipitated mucoproteins are centrifuged down and washed once more with phosphotungstic acid. (8)

also used electrophoresis for isolation. They determined the tyrosine, carbohydrate, nitrogen and protein components of mucoprotein. It is to be noted that the tyrosine, carbohydrate ratio is about 3.7 both in normal serum and in serum from cancer patients.

TABLE I*

PLASMA MUCOPROTEIN LEVELS IN NORMAL AND CANCER PATIENTS

	No. of cases	Tyrosine mg.%	Carbohydrate mg.%	Nitrogen mg.%	Protein mg.%	Ratio CHO/T mg.%
Normal	10	3.38±0.27	12.6±1.1	5.6±0.6	86.7±9.5	3.69±0.23
Cancer	10	8.53±0.7	33.0±2.6	22.8±1. 2	22.8±15.5	3.85±0.30

In isolating mucoprotein they used 500 ml. of 1.8 M perchloric acid of 0.6 M sulfosalicylic acid, added while stirring. This was filtered through a Whatman No. 5 filter, started two to five minutes after protein precipitation. The precipitate was then dialyzed to remove acid and mucoprotein precipitated by saturating the dialysate with ammonium sulfate at a pH of 4. The material was exhaustively dialyzed against distilled water and dried by lyophilization. They isolated 20 mg./100 ml. of plasma.

This procedure produced a white, fluffy, slightly hygroscopic, water-soluble material which formed a slightly turbid solution. It gave a strong Molish reaction and positive reactions to the usual amino acid and protein tests.

Samples examined electrophoretically showed three demonstrable components. (See Fig. 1.) The chemical composition of similar samples is given in Table II. The authors felt that all three electrophoretic components were mucoproteins. Mucoproteins were found

*This is Table I from Winzler et al., J. Clin. Invest., 27:609, 1948, (8).

to be low in nitrogen, a finding borne out by most other authors.

TABLE II*

CHEMICAL COMPOSITION OF NORMAL HUMAN PLASMA MUCOPROTEINS

Component	Method	Gm./100 gm.*x
Ash	Ignition in air	2.8
Nitrogen	Digestion and Nesslerization	7.0
Amino nitrogen (per cent of total N)	Van Slyke nitrous acid method	4.3
Protein	Biuret method (18)	58.0
Carbohydrate	Orcinol reaction (as mannose-galactose) (19)	15.1
Hexosamine	Acetylacetone method (13)	11.9
Hexuronic acid	Carbazole method (23)	neg.
Lipid	Hot alcohol extraction	12.9
Cholesterol	Liebermann-Burchard reaction	neg.
Phosphorus	Molibdivanidate method (24)	0.064
Sulfur	Gravimetric	1.3
Cystine	Polarographic (25)	0.5
Methionine	McCarthy-Sullivan method (26)	2.1
Tyrosine	Phenol reagent (20)	4.2
Tryptophane	Erlich reagent (27)	1.8

*Average of ten preparations
x On moisture-free basis

Since this, Winzler and coworkers have published a series of papers on the same subject (26, 27, 28, 29). Their findings are as follows. Mucoproteins as shown by electrophoresis have three distinct components. In disease (cancer and pneumonia) the mucoprotein fractions which they call MP-1 (or M-1) are consistently elevated, M-2 is often elevated, while M-3 is usually not altered (27). Mucoproteins isolated chemically, added to serum, increase the M-1 fraction but not the others on electrophoresis. Adding M-1 to serum increases the alpha-globulin fractions (26, 28) which shows that mucoprotein is in this fraction.

*This is Table IV from Winzler et al., J. Clin. Invest., 27:609, 1948, (8).

MP-1 has a mobility of -6.4 x 10-5 and an isolectric point of pH 2.3 and is the largest in amount of the three components. MP-2 has a mobility of -2.3 x 10-5 and an isoelectric point of pH 3.4. MP-3 is hard to separate from albumin and has an isoelectric point of pH 4.3. Table III gives the chemical analysis of serum samples in health and disease. It can be seen that, though the amounts vary, the proportions are relatively the same. This indicates that, though there is an increase in disease, there is no alteration of the normal substance. M-1 may equal, never exceeds and is often less than the amount of $alpha_1$-globulin. M-1 and M-2 totaled exceed $alpha_1$-globulin. M-1 and chemically determined mucoprotein tyrosine rise and fall together. The chemical composition of these two is in general quite similar, as shown by Tables IV and V; so these two are probably the same substance. M-2 may not be a mucoprotein (28).

TABLE III*

ANALYSIS OF MUCOPROTEIN IN HEALTH AND DISEASE

Preparation	Source	Yield mg./100 ml. plasma	Nitrogen	Glucosamine	Carbohydrate	Tyrosine
14	Human normal	38	8.1	10.1	15.6	3.9
44	Human cancer	100	7.5	8.9	16.3	3.9
55	Human normal	28	9.0	9.1	15.7	4.3
56	Human normal	32	7.1	8.1	13.6	3.4
Pooled *	Human normal	—	8.3	9.8	14.3	3.6
Salt mat X	Human normal	72	7.25	7.6	13.2	3.1

*Five separately isolated lots were pooled for this sample.
X Contained 31% albumin by electrophoresis, assuming equal refractive increments per g. of albumin and MP-1.

*This is Table I from Mehl, et al., Proc. Soc. Exper. Biol. & Med., 72:106, 1949, (27).

TABLE IV*

ANALYTICAL VALUES FOR ELECTROPHORETICALLY ISOLATED M-1

Tyrosine	7.6 mg.%
Carbohydrate	34.4 "
CHO/T	4.5 "
Nitrogen	16.2 mg.%
Glucosamine	28.5 "
CHO/G	1.2 "

TABLE V**

ANALYTICAL VALUES OBTAINED ON PERCHLORIC ACID FILTRATES FROM SERA OF 3 PATIENTS--GAM, BUR, AND HUM

Sample	Tyrosine mg.%	CHO mg.%	CHO/T	N mg.%	Protein (Bierut) mg.%	Glucos-amine mg.%	CHO/G
GAM ca.	9.7	52.0	5.3	23.5	240.0	33.5	1.55
BUR pneumo.	10.8	55.0	5.1	27.5	270.0	33.5	1.64
HUM pneumo.	5.7	27.5	4.8	14.5	-----	21.0	1.31
Normal	---	----	3.6	----	-----	----	1.27

Concentrations are expressed as mg./100 cc. of original serum and were obtained by the methods previously described. Average values of the ratios of carbohydrate:tyrosine (CHO/T) and carbohydrate: glucosamine (CHO/G) obtained on mucoprotein isolated from normal human plasma are given for comparison.

Waldron and Woodhouse (30) point out that each of the basic methods of isolating mucoprotein gives a different hexose/nitrogen ratio so that each method produces a different mixture of proteins. They analyzed for amino acids. They found phenylalanine, leucine,

*This is Table III from Mehl, et al., Proc. Soc. Exper. Biol. & Med., 72:110, 1949, (28).

**Ibid., Table I.

serine, glycine, aspartic acid, glutamic acid, histidine, lysine, argenine, cysteine, tryptophane and tyrosine.

Raymond and D'Eshougnes (31), using paper electrophoresis, state that mucoproteins are in the alpha$_2$-globulin fraction, in contrast to the above workers. They say that glycoproteins are in the alpha$_1$-fraction. This may be due to the problem of terminology which was mentioned earlier. Jackson et al. (32) agree, basing their beliefs also on the fact that alpha$_2$-globulin has the highest polysaccharide content as well as agreement of mucoprotein levels and alpha$_2$-globulin pattern on electrophoresis.

Winzler and coworkers (26) found the mucoprotein levels, using the mucoprotein tyrosine method,* to be 2.7 mg.% average±0.05 with a range of 1-4 mg.%; while in cancer patients it ranges from 2-12 mg.%, averaging 6.1±0.13 mg.%.

Winzler et al. (8) believe that the serum polysaccharide studied by Seibert and collaborators (32) included the mucoproteins and probably was only mucoprotein.

The Differentiation of Mucoprotein from other Acute Phase Reactants

C-reactive protein is easily distinguishable from mucoprotein because it is present only in disease states (1, 2, 4, 7). It is serologically distinct from other normal serum proteins (1, 7),

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*Determination of mucoprotein tyrosine is done because of the ease of determining tyrosine and because the tyrosine/mucoprotein ratio is constant. They dissolved mucoprotein in 6.5 ml. of 1/5 saturated sodium carbonate, adding 1 ml. of Folin's phenol reagent and, after one hour, reading the color development with a red filter on a Klett-Summerson colorimeter. A standard containing 0.05 mg. of tyrosine is simultaneously treated. (26)

including mucoproteins. It requires calcium ions for precipitation (7).

Nonspecific serum hyaluronidase was thought possibly to be identical with mucoprotein, its activity being just another manifestation of the same substance. This was thought because the two are usually elevated in the same diseases and tend to migrate together on electrophoresis. However fractions of human serum separated electrophoretically and chemically show no increase in non-specific hyaluronidase activity as compared to untreated serum or mucoprotein-poor fraction (34). There is also considerable difference in the level of mucoprotein and hyaluronidase inhibitor in certain diseases, most notable of which is lipoid nephrosis (9, 34, 35). In glomerulonephritis the levels tend to follow one another, but in lipoid nephrosis hyaluronidase inhibitor is elevated while mucoprotein is subnormal. Also hyaluronidase inhibitor tends to be subnormal during convalescence from most diseases, while mucoprotein tends to be elevated (1, 7, 35, 36).

Serum nonglucosamine polysaccharides are acute phase reactants. Those of Siebert et al. (33) are probably mucoproteins. However, Kelley (37), determining serum polysaccharides by the tryptophane method (first hydrolyzing the polysaccharides to sugars and then combing them with tryptophane), treats them as a separate acute phase reactant. He is probably justified because the method is totally different from that involved in determining mucoproteins. Also the nonglucosamine polysaccharides are elevated in Sydenham's chorea; whereas mucoproteins are not. However, the hydrolysis involved hydrolyzes any proteins present to amino acids, so

mucoprotein is probably hydrolyzed to amino acids and its carbohydrate portion to sugars; hence it may constitute at least part of the serum nonglucosamine polysaccharides. These polysaccharides are elevated consistently in rheumatic fever.

Serum hexosamine, although part of mucoprotein, is also part of other serum proteins and is in each of the mucoprotein fractions as determined by Winzler et al. (8). Since M-2 and M-3 are probably not mucoprotein and often not elevated in rheumatic fever and other diseases, the part that mucoprotein hexosamine plays in total serum hexosamine is doubtful and probably not important (38). Further, hexosamine is not always elevated in rheumatic fever; whereas mucoprotein is. Hexosamine is usually absent in convalescence; whereas mucoprotein is usually still elevated (36, 39).

Mucoproteins in Rheumatic Fever

It is agreed by all authors writing on the subject that mucoproteins are markedly and consistently elevated in rheumatic fever (1, 7, 9, 32, 36, 40, 41, 42, 43, 44). They all likewise state that mucoproteins are nonspecific and of no value diagnostically, except Kushner et al. (41). These workers attempted to correlate mucoprotein and gamma-globulin levels in order to diagnose diseases since they are elevated, normal, or decreased in different diseases. They concluded that even this combination was of limited value. It is generally agreed that mucoprotein levels would be useful in following the clinical course of rheumatic fever. Changes in mucoprotein level are well correlated with changes in the clinical picture in a given case of rheumatic fever (7), the level in general fairly well indicating the severity of the disease.

In order to evaluate mucoprotein in disease it is necessary to find the normal values. Winzler et al. (34) found a level of 2.2 mg.% mucoprotein tyrosine in normal adults. This agrees remarkably well with Kelley et al. (9) who found 2.34 mg.% in adults aged 18-33 and 2.49 mg.% in children aged 1-15. See Tables VI and VII. Table VI also shows the statistically analyzed values obtained in various diseases including rheumatic fever.

TABLE VI*

SUMMARY OF SERUM MUCOPROTEIN-TYROSINE LEVELS IN NORMAL ADULTS AND CHILDREN WITH CERTAIN DISEASES

Group	Age Range	No. of Cases	Mean-SEM
Normal young adults	18-33	50	2.34±0.05
Normal children	1-15	75	2.49±0.06
Lipoid nephrosis	2-13	12	1.59±0.14
Acute poliomyelitis	2-15	24	3.93±0.29
Convalescent poliomyelitis	3-16	20	2.77±0.14
Acute osteomyelitis	1½-14	6	4.49±0.29
Pneumonia nonbacterial	2½-11	10	5.10±0.26
Streptococcus pharyngitis	5-24	11	2.87±0.20
Active rheumatic fever	5-33	20	6.00±0.27
Inactive rheumatic fever	5-21	42	2.75±0.11
Sydenham's chorea	5-12	17	3.00±0.20

TABLE VII**

SERUM MUCOPROTEINS IN PATIENTS WITH RHEUMATIC FEVER

Group	No. of Cases	Age Range	MUCOPROTEIN TYROSINE(mg/100cc) Mean	S.E.M.	Range
Active rheumatic fever	91	3-21	8.51	±0.31	3.4-19.9
Convalescent rheumatic fever	53	¾-18	2.46	±0.07	1.5- 3.6
Inactive rheumatic fever	38	5-19	2.88	±0.13	1.8- 5.5
Sydenham's chorea	26	4-15	3.27	±0.18	2.0- 4.9
Sick controls	120	¼-33	5.19	±0.24	2.6-14.3
Normal children	75	1-15	2.49	±0.06	1.9- 4.5
Normal young adults	50	18-33	2.34	±0.05	1.9- 4.3

*This is Table I from Kelley et al., Pediatrics 5:824, 1950 (9)

**This is Table I from Kelley et al., Pediatrics 12:608, 1953, (36)

It can be seen that in rheumatic fever values from 100-300% above normal are obtained. Kelley et al. (9) used the methods of Winzler modified to use the Evylin photoelectric colorimeter. Serum proteins were precipitated with perchloric acid, mucoproteins precipitated from the filtrate with phosphotungstic acid, and tyrosine content estimated by the intensity of the molybdenum blue color developed with the Folin-Ciocalteau phenol reagent. This is valid since tyrosine, carbohydrate, and protein bear a constant ratio to each other in mucoprotein (8).

In the course of experiments it was found that mucoproteins did not return to normal as rapidly as other acute phase reactants in rheumatic fever (1, 7, 36, 44). It often stayed above normal for weeks or months after all clinical manifestations of rheumatic activity were normal, including erythrocyte sedimentation rate (Figs. 2, 3, 4, 5).

C-reactive protein disappears promptly from serum as soon as apparent rheumatic activity ceases. Hyaluronidase inhibitor returns promptly to normal or subnormal values. This continued elevation was thought possibly to be caused by prolonged "life" of mucoproteins in the serum, over that of other acute phase reactants. It is to be noted, however, that the mucoprotein curves vary a great deal from case to case, the shapes depending little on the initial elevation or effectiveness of therapy as indicated by the clinical picture or laboratory procedures. Also in short, acute, infectious diseases such as pneumonia the mucoprotein level returns uniformly and much more rapidly to normal, even though initial levels and duration of acute phases are of comparable length. In

rheumatic fever, moreover, mucoproteins tend to fall at first under hormonal therapy, ACTH or cortisone, but they soon plateau at levels above normal, staying there even though other indices of rheumatic activity return rapidly to normal. This occurs definitely but less markedly in cases treated with salicylates and rest. This level continues elevated in spite of therapy and in some cases may even rise, while erythrocyte sedimentation rate and C-reactive protein fall to normal (7, 36). The mucoprotein levels in the two situations fall at about the same rate. It is the more rapid fall in the erythrocyte sedimentation rate and temperature in cases treated with hormones over that in conservative therapy that makes the greater difference (Figs. 2, 6, and 7). In some cases the mucoproteins continue to rise till the patient succumbs to the disease while other indices are normal (Figs. 8, 9, 10). Thus it is apparent that the persistent elevation of mucoprotein is caused by persistent production of mucoprotein rather than slow loss or decay.

These facts also suggest that mucoproteins may indicate persistent rheumatic activity in spite of therapy and that this activity is not indicated by conventional tests or C-reactive protein.

It has been further noted that if therapy is discontinued before serum mucoprotein levels return to normal, even though all other evidence of rheumatic activity has disappeared, there is usually a clinical "rebound." If treated till mucoprotein is normal (7, 36, 44), there is seldom any "rebound." The basic information on "rebound" is given in Table VIII. A statistical analysis of data concerning "rebound," both laboratory and clinical, is

TABLE VIII*

DATA CONCERNING OCCURRENCE OF "REBOUND" AFTER WITHDRAWAL OF HORMONE THERAPY IN PATIENTS WITH ACUTE RHEUMATIC FEVER

Group of Patients	No. of Pts.		MUCOPROTEIN LEVEL* Initial	At time of Tapering Dose	At discontinuing Therapy	DURATION OF THERAPY DAYS Until Tapering	Until Discont.
No.		Mean	9.0	5.4	4.6	21.9	36.5
Rebound	26	SEM	0.45	0.32	0.26	3.02	3.28
		Range	4.0-14.4	2.5-10.2	1.9-7.8	4-63	10-82
Laboratory		Mean	10.3	6.7	5.6	35.5	52.2
Rebound	24	SEM	0.71	0.31	0.38	4.86	7.04
Only		Range	5.2-19.9	4.1- 9.8	3.3-9.9	7-95	16-115
		Mean	11.6	8.4	7.5	13.0	20.9
Clinical	11	SEM	0.95	0.90	0.65	1.68	3.75
Rebound		Range	5.0-15.0	4.8-12.0	5.2-10.6	7-23	7-41
All cases Not showing Clinical Rebound	50	Mean	9.6	6.0	5.1	28.4	44.0
		SEM	0.41	0.24	0.23	2.95	3.72
		Range	4.0-19.9	2.5-10.2	1.9- 9.9	4-95	10-115

*Expressed as mg.% Mucoprotein Tyrosine

TABLE IX**

SIGNIFICANCE OF DIFFERENCE OF MEANS OF VARIOUS GROUPS COMPARED TO MEANS OF "NO REBOUND" GROUP

Group	n		MUCOPROTEIN LEVEL Initial	At Taper	At D.C.	DURATION OF THERAPY (DAYS) Until Taper	Until D.C.
Lab Rebound Only	48	t=	1.54	2.95	2.17	2.37	2.02
		p=	>.05	<.01	<.05	<.05	<.05
			Not significant	Very significant	Significant	Significant	Significant
Clinical Rebound	35	t=	2.35	3.15	4.14	2.58	3.13
		p=	<.05	<.01	<.01	<.05	<.01
			Significant	Very significant	Very significant	Significant	Very significant

*This is Table II from Kelley et al., Pediatrics 12:615, 1953, (36)

**Ibid, Table III.

TABLE X*

SIGNIFICANCE OF DIFFERENCE OF MEANS OF GROUPS WITH "CLINICAL REBOUND" AND "NO CLINICAL REBOUND"

	MUCOPROTEIN LEVEL			DURATION OF THERAPY (DAYS)	
	Initial	At taper	At D. C.	To taper	To D. C.
n	59	59	59	59	59
t	1.94	2.58	3.48	4.54	4.37
p	$>.05$	$<.05$	$<.01$	$<.01$	$<.01$
	Not significant	Significant	Very significant	Very significant	Very significant

given in Tables IX and X. (Laboratory rebound is recurrence of laboratory findings such as erythrocyte sedimentation rate without clinical rebound. Clinical rebound involves return of clinical evidence of rheumatic activity and always includes laboratory rebound. Rebound occurs by definition, once the elements under consideration have become normal and then recurred on cessation of therapy.) As seen in Table VIII, there is much overlap, so that a specific level cannot be designated at which rebound will occur if therapy is discontinued.

There is a statistically significant difference in initial level in clinical rebound but not for laboratory rebound. The differences in mucoprotein level at tapering of hormonal dose and at discontinuance are all significant for laboratory and clinical rebound. Table X gives the differences between cases with clinical and cases with only laboratory rebound or no rebound combined. It is apparent that the higher the mucoprotein level is initially, at

*This is Table IV from Kelley et al., Pediatrics 12:615, 1953, (36)

tapering of the dose or at discontinuance of therapy, and the shorter the period of therapy, the more likely rebound is to occur.

Mucoproteins behave in general in polycyclic rheumatic fever as they do in monocyclic cases, rising and falling with each cycle.

The altered serum protein levels which are often seen in rheumatic carditis and which invalidate the erythrocyte sedimentation rate do not affect mucoprotein levels (8)(Fig. 11).

Causes of Altered Mucoprotein Levels

Mucoproteins are known to be lower in normal females than in normal males (45). In the first post partem month, maternal mucoprotein is elevated, while the baby's mucoprotein is below normal. Thereafter both rapidly approach normal (46).

Mucoprotein is elevated in acute rheumatic fever (1, 7, 9, 32, 36, 41, 42, 43, 44, 45) and in convalescent rheumatic fever (1, 32, 36, 42, 44). It is normal in inactive rheumatic fever and in Sydenham's chorea (1, 9, 32, 36, 42, 43, 44, 45). Mucoprotein is elevated in rheumatoid arthritis, disseminated lupis erythematosis, scleroderma (7, 41). It is greatly reduced in lipoid nephritis (9, 35, 45). It is elevated in bacterial and viral pneumonia and in bacterial and viral infections in general (7, 9, 26, 41), including acute poliomyelitis, osteomyelitis and tuberculosis (9, 41). Streptococcal pharyngitis gives a slight evanescent elevation (9).

Such nonspecific stimuli as chronic chilling and hyper-immunization cause elevation of mucoprotein (47), while the Arthus phenomenon, acute chilling, sterile ferric chloride abscess, electrocautery, mock operations, anesthesia (47) and sterile turpentine abscesses (45) do not change it.

Cardiovascular diseases in general leave the mucoprotein level normal, while acute myocardial infarction elevates it (4, 45). Cancer in general elevates mucoprotein levels while myeloma usually causes a decrease.

Endocrine factors such as hypopituitrism and Addison's diseases, whow decreases of mucoprotein levels in 70-90% of cases. All thyroid hormonal disturbances show decreases in 25% of the cases, and in 30% of diabetic cases (45). Thyrotoxicosis causes decreased serum mucoprotein levels (41), while cortate injection also causes a decrease. Adrenalin causes a rise in mucoproteins while adrenalectomy leaves its serum levels normal (47).

Connective tissue hexosamine in orbital tissue falls with age. Thyroidectomy stops this change while thyroxin administration re-establishes it. In hypophysectomized rats thyroxin does not have this effect (40).

ACTH causes an elevation of mucoprotein in normal animals and hypoglycemic children (44). Van Leeuwen et al. (48) state that hormonal therapy has little effect on mucoprotein level. However they place mucoprotein in alpha$_2$-globulin and say that alpha$_1$-globulin is lowered by hormonal therapy. Kelley and coworkers (36, 44) state that in rheumatic fever hormonal therapy has a transient suppressing effect or no effect at all on mucoprotein levels (which they believe to be alpha$_1$-globulin) at first; then it plateaus in spite of hormone therapy (Figs. 3, 7). At least it is obvious that hormonal factors play a role in regulating mucoprotein serum levels. The nature of the role is unknown.

Extra hepatic biliary obstruction causes a rise in mucoprotein in the serum (41, 45) as does fatty liver, florrid cirrhosis, Laenecc's cirrhosis with infections, and hepatic metastases; whereas chronic hepatic disease other than parenchymal (41), infectious hepatitis, homologous serum jaundice (45) and portal cirrhosis cause a fall of mucoprotein below normal. Subacute and chronic parenchymal hepatic disease (41) gives variable results from increased to normal to decreased mucoprotein levels in the serum.

It is obvious that mucoprotein levels are very non-specific and of little diagnostic value.

Some believe that mucoproteins may be produced in the liver (7, 45, 46). This is because of the low mucoprotein level in newborn infants with immature liver and because of the rise in serum mucoprotein in obstructive liver disease and fall in serum mucoprotein in hepatocellular disease or diseases which cause liver destruction.

Thus there are two suggested factors in regulating blood mucoprotein levels--unknown factors which are associated with cellular proliferation or degenerative processes which, in inflammatory or neoplastic diseases or trauma, increase mucoprotein levels (9, 45); and hepatic and endocrine factors which lower mucoprotein levels when impaired. These two are factors well-substantiated by the above.

Physiologic Role of Mucoprotein

It has been suggested that mucoproteins play a role in the non-specific defense of the host against any stress. It has been noted that some serum polypeptides have an antibacterial effect. Injection of mucoproteins isolated from gastric and salivary mucosa suppresses gastric motility (9). There is very little suggested on the physiologic role of mucoprotein.

Conclusions

Research on mucoproteins, though of considerable duration, until fairly recently has had little direction or purpose. Further, the field of acute phase reactions, and especially mucoproteins, has been confused from the start by the wide variety of methods and by the disagreement concerning definitions and terminology. Even late in 1954 some workers were isolating mucoproteins and calling them by other terms. Because of the variety of methods it is difficult to compare the results of many authors. When a given method is used, however, results are fairly constant, enough so that the results are of practical value and meaningful as a test. The best methods are probably those of Winzler et al. (8) or its variation as used by Kelley, et al. (9). These involve determining tyrosine, and, since tyrosine bears a constant ratio to the carbohydrate and protein in mucoprotein, the mucoprotein can be determined.

Jackson, et al. (32) and Van Leeuwen et al. (48) believe muco - proteins to be an alpha$_2$-globulin fraction. Evidence is given that they migrate as a globulin, and a high degree of correlation between mucoprotein levels and alpha$_2$-globulin exists. Evidence is also

given that alpha$_2$-globulin has the highest polysaccharide content.

Winzler et al. (8, 26, 27) and Greenspan (45) state that mucoproteins are in the alpha$_1$-globulin fraction. Winzler et al. give very conclusive evidence which has already been presented in this paper. They used a Tiselius cell electrophoresis apparatus and analyzed samples separated chemically as well as electrophoretically. It is the author's opinion that these later workers give by far the best evidence for their opinion and that the mucoproteins are in the alpha$_1$-globulin fraction.

Mucoproteins fall in endocrine deficiencies and if lipoadrenal extract or cortate is injected. They rise if adrenalin is injected in normal animals or humans. Yet on hormone therapy in rheumatic fever, using ACTH or cortisone, there is a preliminary effect of falling of the mucoproteins and then a leveling off well above normal even though no evidence of disease is present by conventional methods. This indicates that there is some part of rheumatic activity which hormonal therapy is unable to suppress.

The determination of mucoprotein levels in serum is in general of little diagnostic value because it is altered in so many conditions and is non-specific. Diagnostically C-reactive protein is better since, although it is also non-specific, if absent in a case displaying clinical evidence of rheumatic fever, it may be concluded that the patient does not have it. However, for following the course of rheumatic fever or the effect of therapy, mucoprotein is probably the best test devised. This is because it follows closely changes in rheumatic activity and because it stays elevated even though all other clinical and laboratory evidence of rheumatic

activity may be gone in cases where rebound is likely to occur when therapy is stopped too soon.

Furthermore, mucoprotein levels are independent in themselves of sedimentation rate, cephalin-flocculation test, albumin-globulin ratio, the thymol turbidity test, the zinc sulfate flocculation and other serum elements most commonly used in clinical laboratories. It is not altered, as is the sedimentation rate, by heart failure. It measures fairly accurately changes in the alpha-globulin, which, before this, has been impractical for clinical purposes (45).

Because mucoprotein determination is fairly simple, it is within reach of any reasonably well-equipped clinical laboratory. One technician can easily run forty mucoprotein determinations simultaneously. If a few are run, this can be done simultaneously with other work since samples do not have to be processed immediately and at no stage is precise timing essential (36). The reagents involved keep well, are easily procured, and no unusual equipment is needed.

Since mucoproteins are altered by liver or endocrine diseases, their unsuspected presence can give wrong results in a case of rheumatic fever. Incidentally, mucoprotein levels can be used to differentiate hepatocellular disease and obstructive disease in case of jaundice since the level is down in the former and elevated in the latter. It is at least as valuable as any other test currently available for this. For the same reason mucoprotein determination can be used to tell whether hepatomegaly is due to cirrhosis or neoplastic diseases (45).

In general there are two main factors influencing serum mucoprotein levels. One is hepatic and endocrine functions which lower mucoprotein when impaired, and the other consists of proliferative and degenerative processes which, when active, raise mucoprotein levels.

Knowledge of the function of mucoprotein is very limited. It is supposed by some to be part of the host defense mechanism.

It can be concluded that there is much work to be done in this field. Aside from the implications as a laboratory test, the possibilities for understanding disease processes and the physiology of defense mechanisms by further study in this field are challenging.

There needs to be considerably more work on defining mucoproteins and a greater agreement on terminology.

Summary

The needs for new and better tests for diagnosis and follow-up of rheumatic fever are discussed. A brief summary of the history of research on mucoproteins is given.

The relative virtues and deficiencies of the various tests available for rheumatic fever are discussed.

Mucoproteins are discussed as to identity and makeup. They are found in alpha$_1$-globulin fractions and have a high carbohydrate component. They contain most of the amino acids and several sugars. The mucoprotein and tyrosine bear a constant relationship to each other. Because of the ease of determining tyrosine, this procedure is used by most workers.

Mucoproteins are differentiated from C-reactive protein, nonspecific hyaluronidase inhibitor, serum nonglucosamine, polysacc-

harides and serum hexosamine--all being acute phase reactants.

The place of mucoproteins in rheumatic fever is discussed. Mucoproteins follow fairly well the clinical picture of a given case not under hormone therapy except that even on traditional therapy it lags somewhat so that it reaches normal after all other manifestations are normal. This is accentuated in hormonal therapy since manifestations of disease drop rapidly and mucoprotein level plateaus and stays elevated much longer. If therapy is stopped before mucoprotein level is normal, clinical rebound is likely to occur.

Other causes of altered mucoprotein levels are given. The possible physiologic roles of mucoprotein are listed. These are largely conjectural.

Discussion of the lack of agreement on mucoprotein data by workers in this field is made. The value and weakness of mucoprotein determination as a test in rheumatic fever and diagnosis in other disease is discussed.

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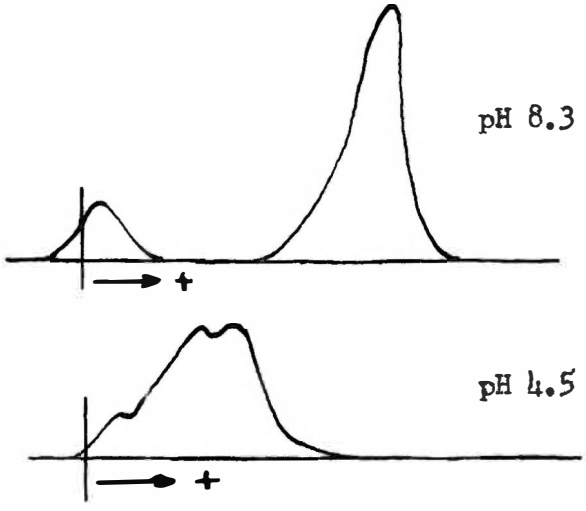

Fig. 1. Electrophoretic Patterns of Mucoproteins Isolated by the Perchloric Acid Method
Winzler et al. J. Clin. Invest. 27:609 1948 (8).

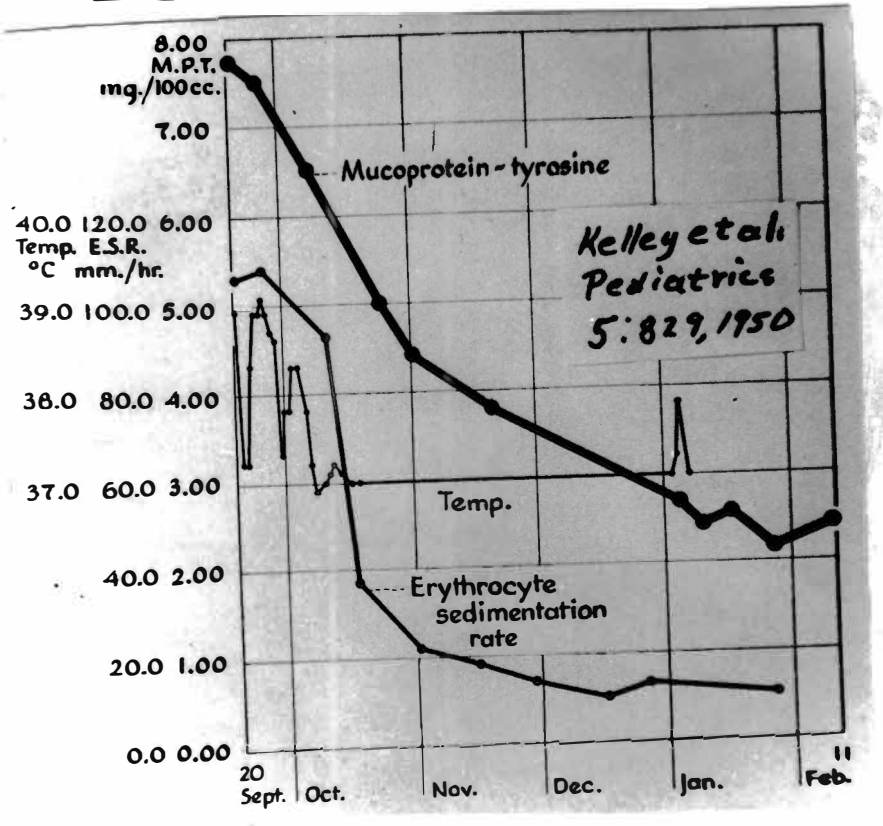

Fig. 2. Correlation of Serum Mucoprotein-Tyrosine Level, Erythrocyte Sedimentation Rate and Temperature Curves in a Patient with Rheumatic Fever

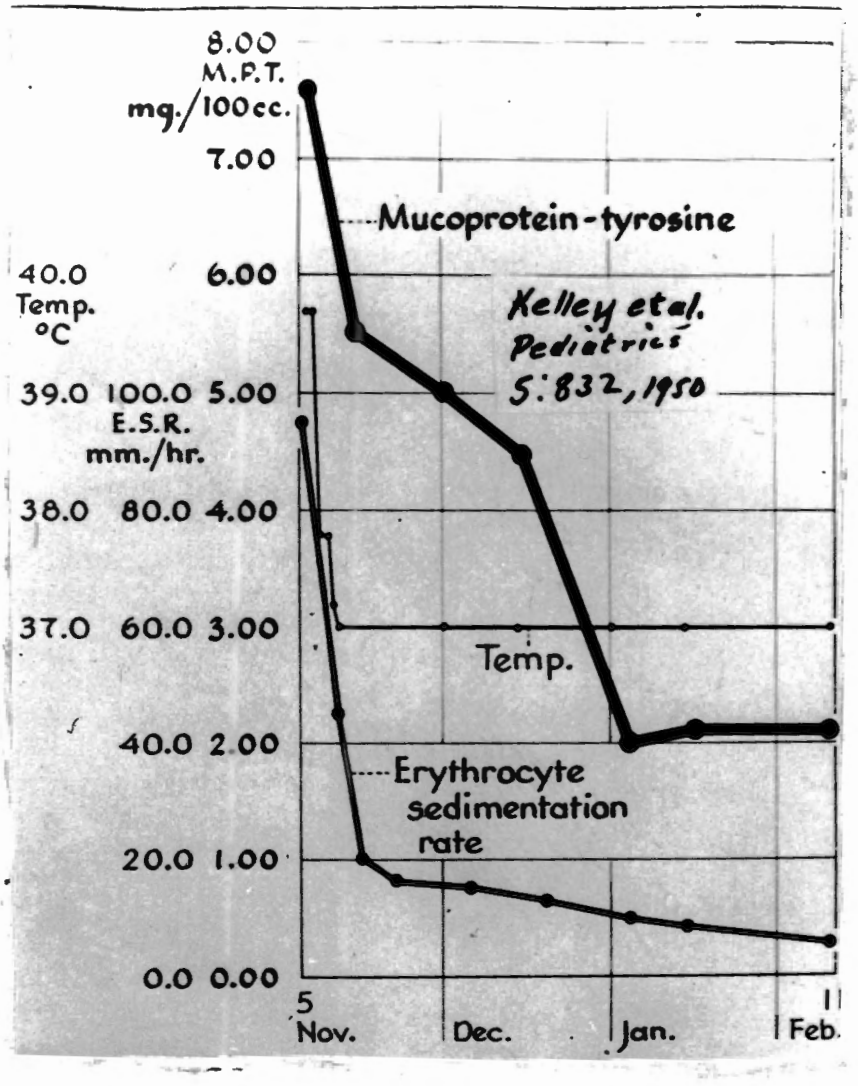

Fig. 3. Correlation of Serum Mucoprotein-Tyrosine Level, Erythrocyte Sedimentation Rate and Temperature Curves in a Patient with Rheumatic Fever

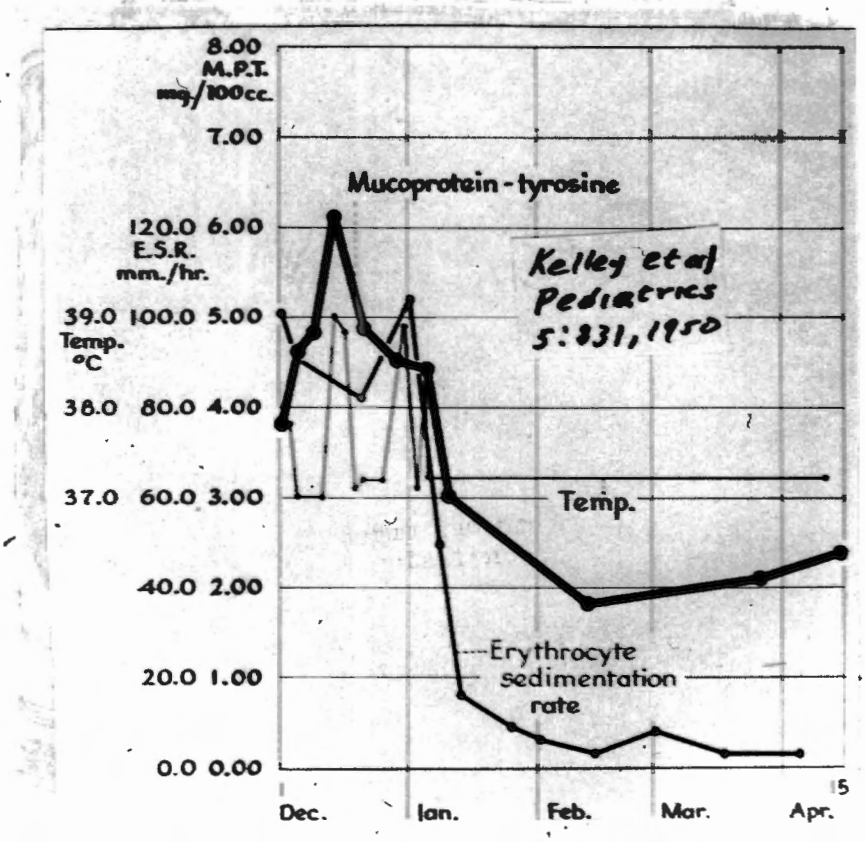

Fig. 4. Correlation of Serum Mucoprotein-Tyrosine Level, Erythrocyte Sedimentation Rate and Temperature Curves in a Patient with Rheumatic Fever

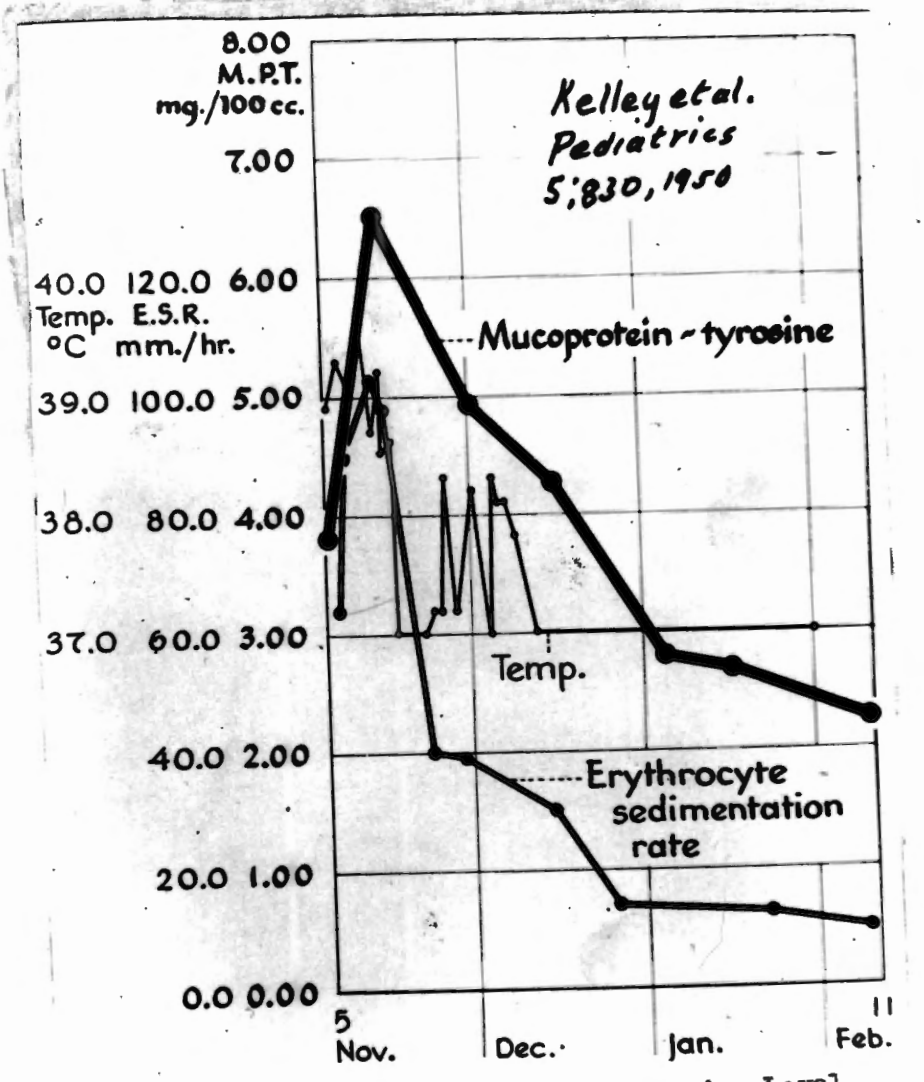

Fig. 5. Correlation of Serum Mucoprotein-Tyrosine Level, Erythrocyte Sedimentation Rate and Temperature Curves in a Patient with Rheumatic Fever

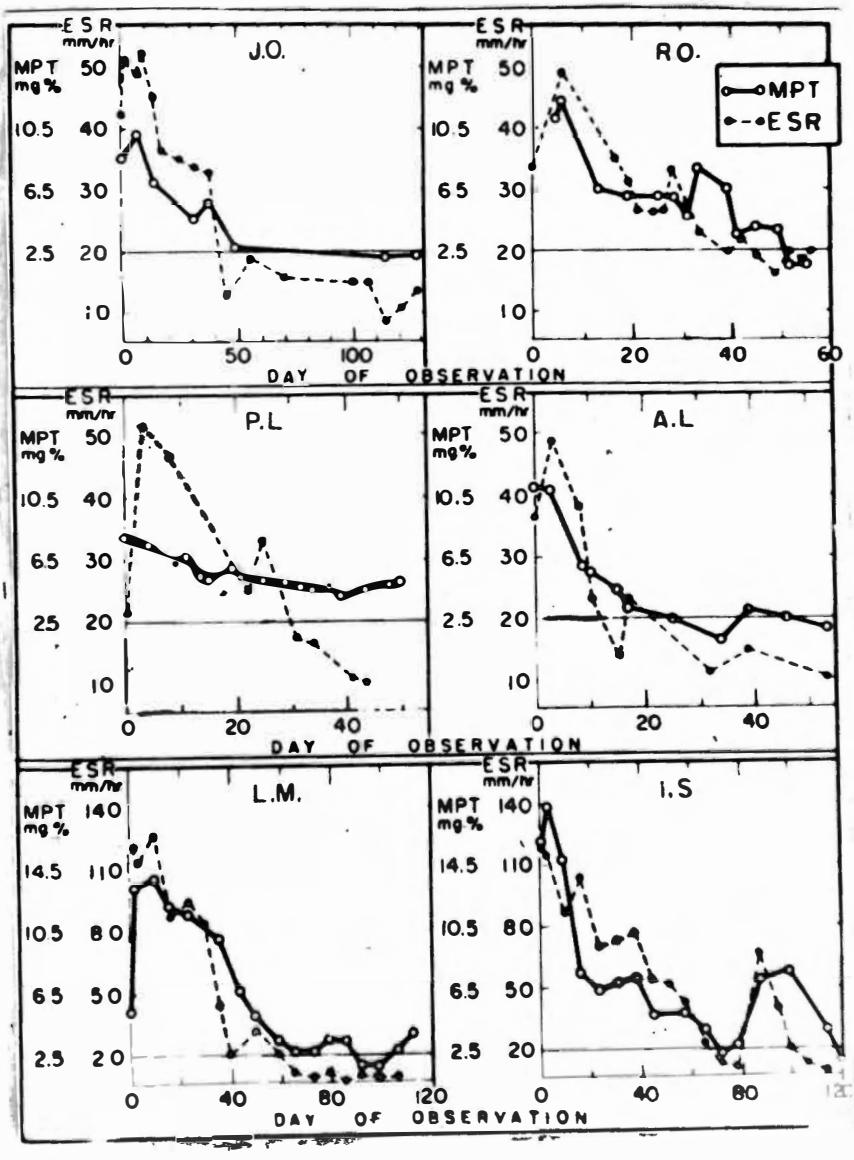

Fig. 6. Comparison of Mucoprotein Concentration and Erythrocyte Sedimentation Rate in Acute Rheumatic Fever Treated With Bed Rest and Salicylates

Kelley et al. Pediatrics 5:824, 1950. (9).

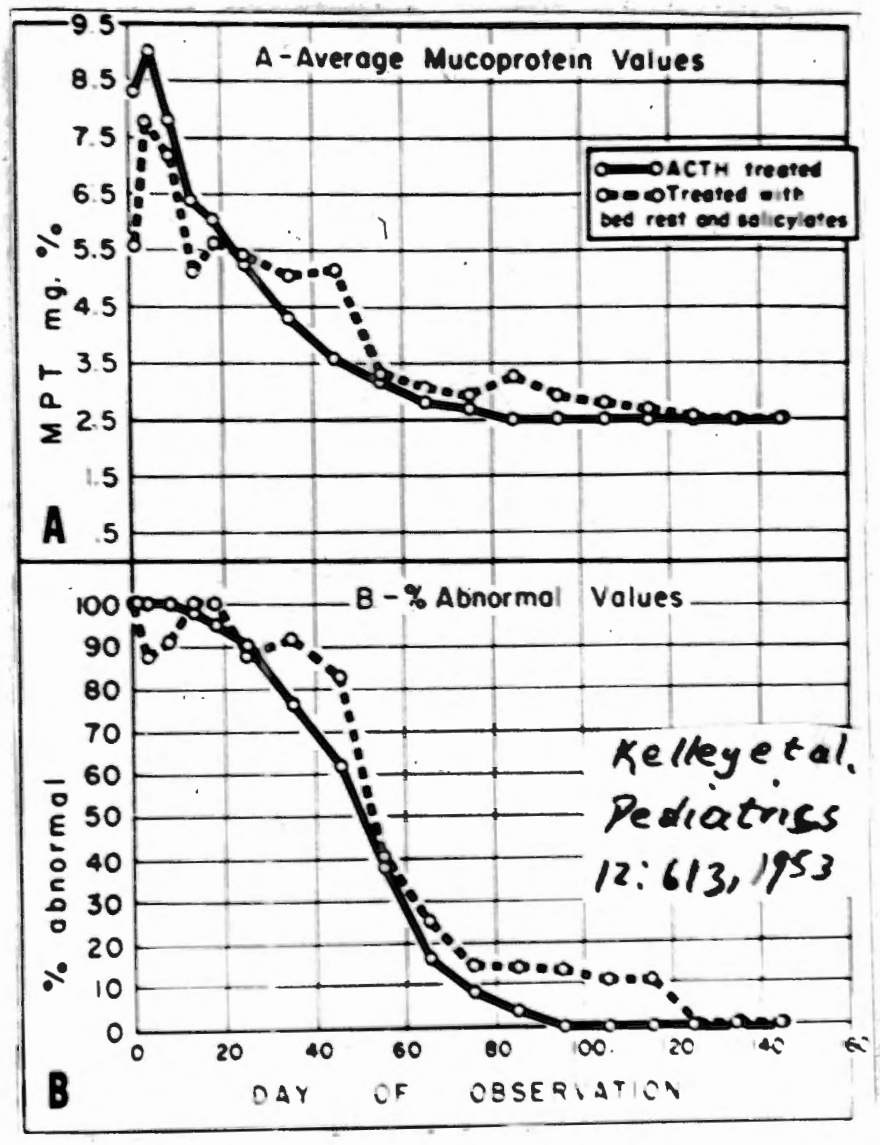

Fig. 7. Mucoprotein Concentrations During ACTH and Salicylate Therapy. A. Two curves compare averages in concentrations in patients treated with ACTH and those treated with only salicylates and bed rest. Striking similarity in curves is apparent. B. Two curves compare per cent of patients with abnormally high mucoprotein concentrations during treatment with ACTH or salicylates. Again curves are surprisingly similar.

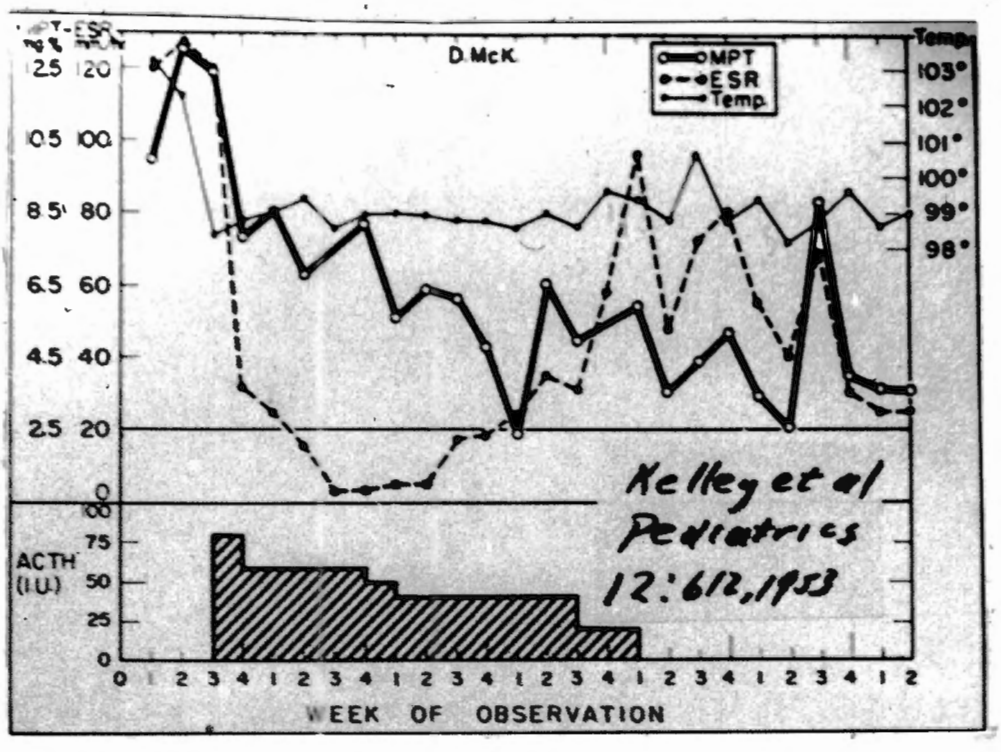

Fig. 8. Mucoprotein and Sedimentation Rate in Rheumatic Fever Treated with ACTH. During hormone therapy mucoprotein lagged behind sedimentation rate in relation to N.

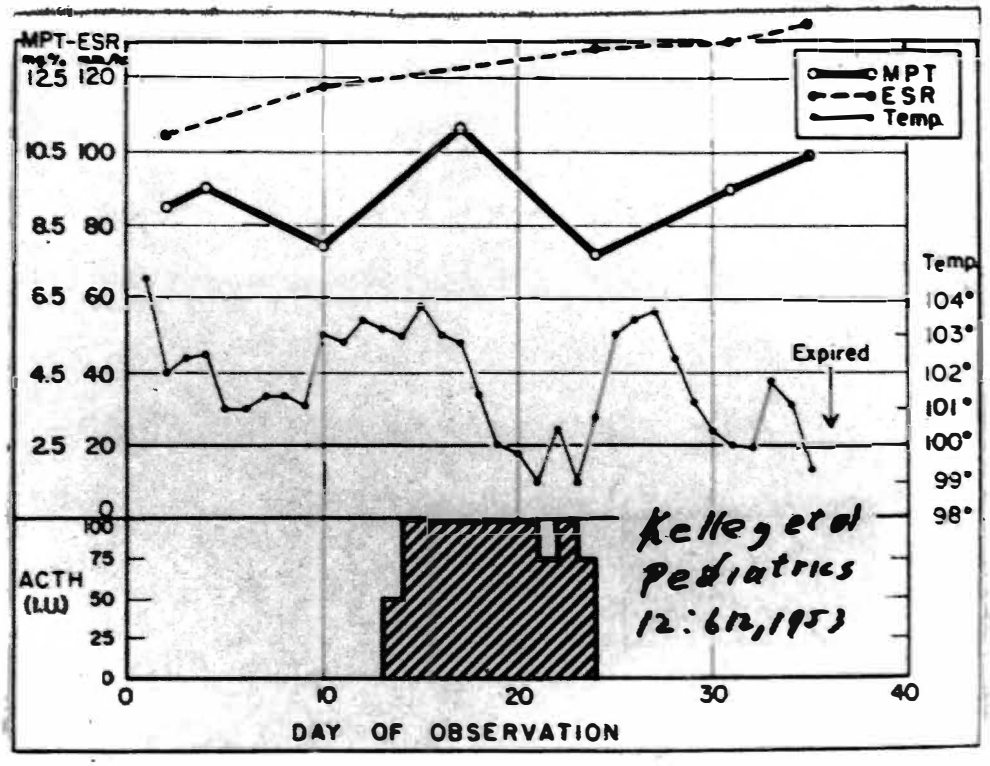

Fig. 9. Mucoprotein and Sedimentation Rate in Patients in Whom ACTH Treatment was Without Beneficial Effect. Note that in spite of treatment both sedimentation rate and mucoprotein concentration remained elevated during entire course of disease.

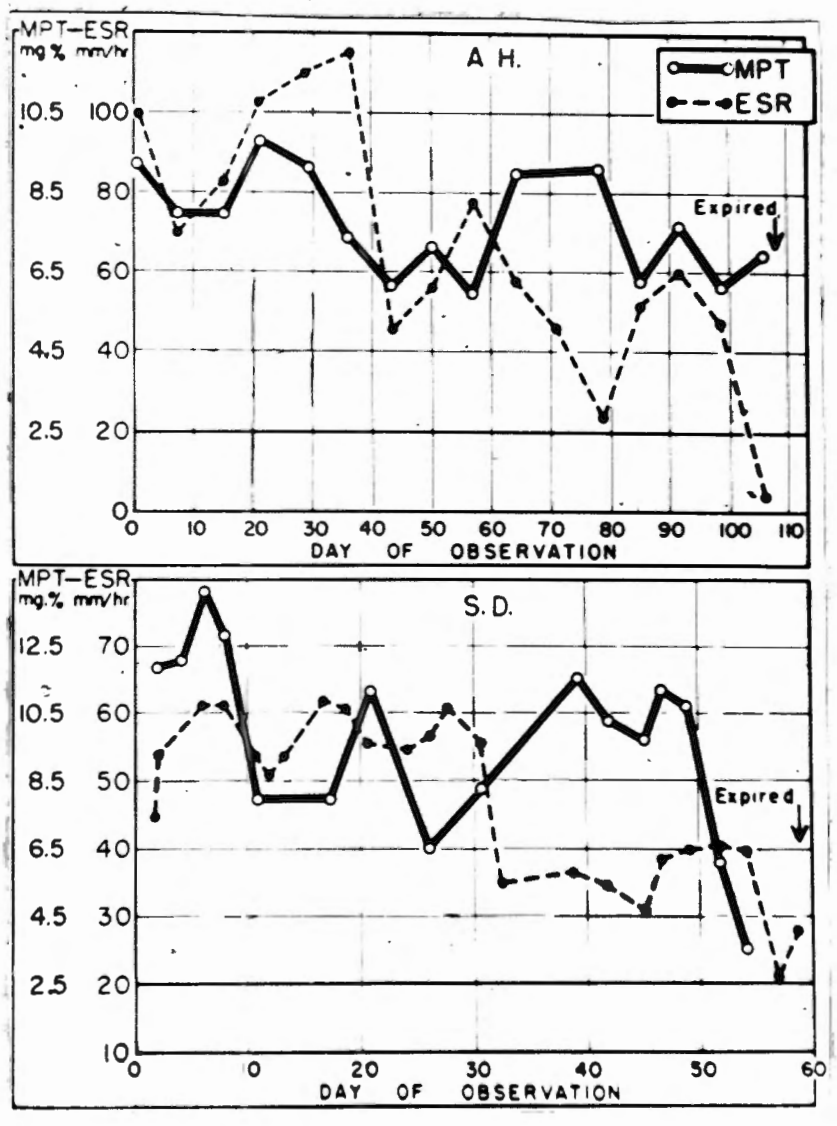

Fig. 10. Comparison of Erythrocyte Sedimentation Rate and Mucoprotein in Patient Having Prolonged Rheumatic Activity Terminating Fatally

Kelley, et al. Pediatrics 5:824, 1950 (9).

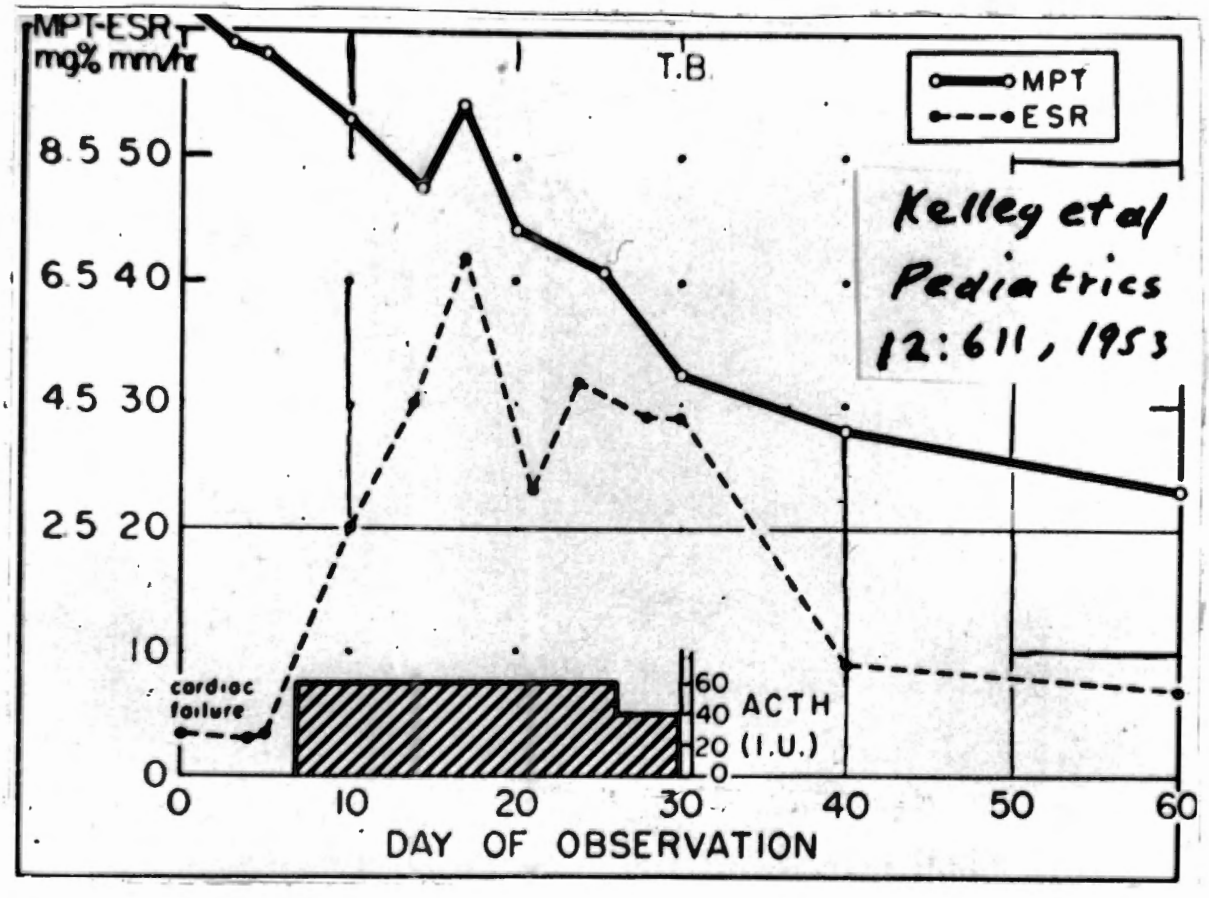

Fig. 11. Changes in Mucoprotein Concentration Compared to Changes in Erythrocyte Sedimentation Rate in Patient with Cardiac Failure due to Acute Rheumatic Carditis. Mucoprotein concentration indicates activity of disease process not reflected by sedimentation rate during period of cardiac failure.

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