
AMERICAN RHEUMATISM ASSOCIATION

PROCEEDINGS OF THE THIRD INTERIM SCIENTIFIC SESSION

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Dr. William D. Robinson, President of the American Rheumatism Association, took the chair at the opening meeting of the third interim scientific session held on November 30, 1956, at the Clinical Center, National Institutes of Health, Bethesda, Maryland.

Dr. Floyd S. Daft, Director of the National Institute of Arthritic and Metabolic Diseases, welcomed the members of the American Rheumatism Association, and Dr. Robinson expressed the deep appreciation of the Association for the opportunity to hold the interim meeting with such fine facilities and to renew the very valuable interchange between the National Institute of Arthritic and Metabolic Disease and the American Rheumatism Association.

Abstracts of sixteen papers and the discussions thereon are printed below, together with the titles of ten papers presented by title only.


It has been demonstrated by Watson, Rothbard and Vananen (1954) that the injection of rabbits with acid-soluble rat-tail collagen will induce complement-fixing antibody which will interfere with normal collagen fibre reconstitution in vitro.

The present study was designed to determine the effect of such an antiserum on the formation of collagen fibres in tissue culture. Chick embryo dermal explants were grown in liquid medium on formvar films in sealed Maximow slides. Antiserum to homologous (chicken) collagen, produced in rabbits by immunization with acid-soluble collagen, was added to these cultures of chick embryo dermis according to various experimental protocols. Control cultures to which normal rabbit serum was added were included in all experiments.

Light microscope observations revealed the presence of a clumped amorphous precipitate in the experimental cultures, visible both in the living unstained cultures and in fixed and stained specimens. It had many of the tinctorial properties generally considered to be characteristic of collagen. The cytoplasmic granules prominent in fibroblasts in serum-containing cultures were also to be found to have many of the tinctorial properties of collagen.

Electron microscope study of these preparations showed that the anticollegen serum markedly affected fibrogenesis, resulting in few normal fibres and the formation of fibres lacking the well-defined form and periodicity of normal collagen. This was in contrast to the effect of normal serum, which enhanced the production of normal collagen fibres. The amorphous precipitate was also seen with the electron microscope as masses of varying size and shape often adherent to fibres and cell processes and sometimes associated with bizarre banded structures. These observations were interpreted as additional evidence for the presence of an antibody to collagen in the sera used, and hence for the antigenicity of collagen; and as further evidence for the probable participation of the described cytoplasmic granules of the fibroblast in collagen fibrogenesis.

Discussion.—DR. EDWARD E. FISCHEL (New York, N.Y.): I should like to ask Dr. Robbins whether two other types of control were done, as these appear necessary before concluding that the interesting effects observed were specifically due to the action of anti-collagen serum.

Since prolonged immunization is necessary to obtain the anti-collagen serum, the suggestion arises that trace contaminants of other chicken tissue components might have acted as the effective antigen. Therefore, instead of normal rabbit serum, the serum of rabbits immunized with chicken serum, or chicken tissue other than chicken collagen, might serve as a control.

The effect on the tissue cultures might also have resulted from an unassociated antigen-antibody system which, because it fixes complement or may interfere with protease activity, etc., may inhibit the growth of tissue cultures. Since fresh serum is frequently necessary for the growth of tissues, this possibility should be tested. A control might be set up in which an unrelated antigen-antibody system was allowed to react on the tissue culture.

Dr. Robbins: Our interpretation of the need for prolonged immunization was that this substance is a rather weak antigen.

Antiserum to chicken serum was not used in these tissue cultures. Because of the manner in which the collagen antigen is prepared it seems unlikely that significant amounts of serum components remain. This possibility has been tested in vitro for rat collagen and rat collagen antiserum prepared by methods identical with those used in this study (Watson and others, 1954). In these experiments rat collagen failed to fix complement with antiserum to rat serum, and rat serum did not fix complement with antiserum to rat collagen.

We have not studied the possible effect of an unrelated antigen and its antibody in these cultures. However, an unrelated antibody alone (anti-bovine albumin) has been
tested and found to be without effect. Because of the marked interference with collagen fibre formation which the anti-collagen serum exhibits, it appears likely that there has been a specific combination of antibody with a component or components of the normal fibre. If the precipitate seen were that of an unrelated antigen and antibody, one would nevertheless expect to see normal numbers of collagen fibrils. This is a worthwhile control experiment which we have planned to do, but have not yet done.

**DR. HARRY BARTFELD (New York, N. Y.):** I should like to ask Dr. Robbins whether the changes that he described were seen in pathological slides in various collagen diseases, and whether he did any sensitized sheep cell agglutination or similar tests, with the anti-collagen serum.

**DR. ROBBINS:** I do not know of any pathological changes in human sections which could be said to resemble these phenomena. Of course, the changes that we observed were at a cellular and electron-microscopic level, which are difficult to compare with observations on differentiated tissue studied at low magnifications in pathological sections. We do not believe that our observations have any direct bearing on any disease process at this time.

Sheep red cell agglutinins (heterophil) appear in many of these sera and can be absorbed out with sheep red cells.

**DR. MELVIN H. KAPLAN (Boston, Mass.):** Did the collagen preparation used for immunization contain specifically glycoprotein in appreciable amount. I ask this because immunization of animals with connective tissue suspensions invariably leads to the production of antibodies which react with those constituents of the ground substance which are associated histologically with reticulin and basement membrane, and which do not react detectably with collagen bundles, at least as revealed by immunofluorescent methods. Since glycoprotein has an effect on the reconstitution of collagen fibrils, it seems to me conceivable that antibodies directed to glycoprotein might similarly interfere with the process of fibril formation.

**DR. ROBBINS:** Young collagen fibres take a strong PAS stain, and therefore presumably contain, or have associated with them, considerable amounts of polysaccharide. Mature collagen apparently contains about 0.5 per cent. carbohydrate (Beek, 1941). Antigens prepared by the method used in this study have given faintly positive Molisch and PAS reactions, indicating that they probably contain a small amount of polysaccharide.

It is possible that the polysaccharide is the antigenic component (or functions as a papiement); this has not been further investigated with respect to chicken collagen and anti-chicken collagen serum in tissue culture.

**DR. MORRIS ZIFF (New York, N. Y.):** Is anything known about the appearance of cytoplasmic granules in fibroblasts in human diseases where collagen is said to undergo degeneration?

**DR. ROBBINS:** I don't know of any observations on the cytoplasmic granules of fibroblasts in either normal or diseased human tissues. However, Gersh and Catchpole (1949) have described them in rat fibroblasts and they are almost certainly present in human fibroblasts.

**REFERENCES**


**Biosynthesis of Mucopolysaccharides by Normal Human Synovial Cells in a Simplified Medium.** By C. WILLIAM CASTOR, Jr. Ann Arbor, Mich.

Acid mucopolysaccharides of the connective tissue ground substance and synovial fluid "mucin" have assumed increasing importance in view of the possible abnormality of these substances in rheumatic disease states. Fragmentary data on mucopolysaccharide biosynthesis have been obtained from microbiologic systems. Data from in vitro cultures of mammalian cells are difficult to interpret because of the complexity of the medium used.

In the present study roller-tube cultures of knee synovium from a 4-month-old male foetus, a 14-year-old male, and a 67-year-old male were grown for periods ranging from 6 weeks to 6 months. The medium used was 80 per cent. chemically defined (Eagle's basal medium) and 20 per cent. human serum, in contrast to previously reported media employing chick embryo extract, amniotic fluid, and serum mixtures. The pattern of culture behaviour was noted through frequent microscopic observations, and stained cover-slip cultures assisted in studying cell morphology. Medium was removed from cultures at appropriate intervals and found to contain newly-formed mucopolysaccharide believed to be hyaluronic acid. The evidence for hyaluronic acid in the culture medium consisted of demonstrating turbidity with acidified albumin which could be prevented by previous treatment of the used culture medium with either testicular or bacterial hyaluronidase. Hexosamine analyses, done by a modified Sundblad technique, also indicated the presence of mucopolysaccharide. The data indicate that synovial tissue of widely varying ages, supported by a simplified medium, is capable of in vitro synthesis of hyaluronic acid.

**Discussion:** DR. KARL MEYER (New York, N. Y.): I noticed that you said there was no other report of unequivocal hyaluronic acid synthesis in tissue culture. I recollect two papers in which synthesis of hyaluronic acid by fibroblasts in tissue culture was demonstrated, one by Grossfeld, Meyer, and Godman (1955), the other by Kling, Levine, and Wise (1955). Kling as well as our group used embryo extract with one exception. In some experiments mentioned in the Table of our paper, hyaluronic acid, or rather a substance giving reactions of hyaluronic acid was produced by fibroblast grown in a completely synthetic medium, namely, Parker medium 866.

We have also shown by isolation of hyaluronic acid that not more than approximately one-tenth of the total hyaluronic acid isolated in growing cultures could have come from the medium used (Bassett and Meyer, 1956; Grossfeld, Meyer, and Godman, 1956). There is, of course, some hyaluronic acid present in the embryo extract. Such experiments were done with rat subcutaneous tissue, with embryonal human long bone, with membranous bone, and with some other tissues. In the experiment with Parker's medium, it was noticed that the cultures after a few transfers die out. Before that they stop producing hyaluronic acid as determined turbidimetrically.

I should like to make another comment. If I understood you correctly, the fluids with the medium you used

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sometimes failed to give a significant turbidity. We also have observed, in at least one instance, failure of the production of turbidity with cultures which on isolation contained a fraction containing significant amounts of uronic acid and hexosamine. We believe that this fraction represents a low molecular hyaluronic acid or an oligosaccharide.

Dr. Castor: With regard to the turbidimetric method, the medium which we used was 20 per cent. human serum and 80 per cent. synthetic diluent with approximately 1 per cent. albumin. This was acidified with a 1 M acetic acid buffer solution of pH 4.2. In the control medium, that is, the initial medium which has not supported in vitro cell growth, there was no detectable turbidity.

The procedure followed for turbidimetric measurements was to divide a given sample into two tubes; in one a buffer was placed and in the other buffer containing 50 turbidity reducing units (TRU) of hyaluronidase. These tubes were incubated at 37° C for 30 min. and then acidified with the 1 M acetic acid buffer to develop turbidity. The difference in optical density between the hyaluronidase-treated and the buffer-treated sample was then plotted against a standard curve of hyaluronate which had been run by the same turbidimetric method.

Dr. Thomas G. Kantor (New York, N.Y.): In studies with Dr. Robert Ward and Dr. George Pappas, we found essentially the same condition as Dr. Castor's with a synthetic medium employing thirty components of amino acids, enzymes, and vitamins. There was a non-dialysable hexosamine-containing material produced by chick fibroblast cultures over a period of 2 weeks. We found, however, that, in relation to the turbidimetric method, although hexosamine would increase over a 2-week period in almost all the cultures, whether we got a turbidity or not depended on whether the material was dialysable or not. I wonder if some of Dr. Castor's materials were dialysable and whether those materials which lost a lot of hexosamine by dialysis would give you as much turbidity as would those which lost little or none.

Dr. Castor: In answer to your question regarding the dialysability of this material, some of the samples were dialysed and the turbidogenic material was not appreciably dialysable. The reason for the difference between the two is that the values calculated by the hexosamine method and by the turbidimetric method on a given sample cannot be settled at this time. Conceivably, the reason for the lower value obtained by the hexosamine method may be the degradation of the standard hyaluronate used in preparing the standard curve. Perhaps the standard curve material may produce less turbidity per unit of hexosamine than the material synthesized by the culture medium. On the other hand, a non-hexosamine-containing mucopolysaccharide in the culture medium may explain this difference.

Dr. Albert Dorfman (Chicago, Ill.): I am a little surprised that the values for hexosamine did not indicate a higher mucopolysaccharide content than that obtained by the turbidimetric method. In such a complex medium there are undoubtedly compounds other than hyaluronic acid which contain hexosamines. The turbidimetric method, although quantitative in pure solutions of hyaluronic acid, is not reliable in crude tissue extracts.

Dr. Morris Ziff (New York, N.Y.): The question arises whether the composition of the medium influences the type of mucopolysaccharide synthesized. Also I wonder whether, if you cultured cells from other sources than synovial membrane, you would obtain chondroitin sulphate plus hyaluronic acid or hyaluronic acid alone.

Dr. Charles Ragan (New York, N.Y.): Dr. Meyer forgot to mention the culture of subcutaneous tissue fibroblasts. These have produced chondroitin sulphate C as well as hyaluronic acid. The disturbing thing to me is that when, all these various tissues are isolated from different in vivo sources, they act alike and look alike in vitro. They may be undergoing a process of de-differentiation.

REFERENCES

Studies on Hyaluronic Acid Synthesis by Human Synovial Tissue Slices. By K. Lemone Yielding, Gordon M. Tomkins, and Joseph J. Bunim, Bethesda, Md.

Studies on synovial fluid suggest that certain changes in the structure or metabolism of hyaluronic acid are characteristic of rheumatoid arthritis. Such findings have prompted this study of the synthesis of hyaluronic acid by articular tissues. Human synovial tissue slices were incubated for periods up to 3 hours in Kreb-Ringer's phosphate buffer containing uniformly labelled glucose-14C. After incubation, the tissue was extracted with sodium acetate pH 9. The extract was acidified and ethanol added to yield a precipitate, which, after thorough washing, was extracted with sodium acetate. The latter extract containing hyaluronic acid showed significant non-dialysable radioactivity, which was resistant to further dialysis after trypsin digestion and remained soluble after protein denaturation by organic solvents. Like hyaluronic acid, all the radioactivity was precipitable with acid haemoglobin, whereas, after incubation with hyaluronidase, only 24 per cent. was precipitable under the same conditions. When subjected to paper electrophoresis at pH 5, where chondroitin sulphate acid and hyaluronic acid are easily separated, the radioactivity exhibited the same mobility as hyaluronic acid.

Analysis of an acid hydrolysate revealed 52.6 per cent. glucuronic acid and 42.3 per cent. n-acetylglucosamine. These components when isolated showed relatively greater specific activities for hexosamine than for glucuronic acid.

It is hoped that further studies employing this technique will afford a better insight into the intermediary metabolism of mucopolysaccharides in rheumatoid arthritis.

Discussion.—Dr. Charles Ragan (New York, N.Y.): I should like to confirm what Dr. Yielding has inferred regarding tissue culture studies. Synovial tissues from patients with rheumatoid arthritis, after long periods of growth, produce and look exactly like synovial tissues from normal subjects and from rats. Again, we may be faced with the problem of de-differentiation.

Dr. Currier McEwen (New York, N.Y.): How long was it possible to keep up the incubation of slices?

Dr. Yielding: 3 hours was our maximum time. In one time study we took equal aliquots at 30 minutes,
1 hour, and 3 hours. It seemed that at 3 hours the rate of synthesis was beginning to level off, but the rate was apparently linear up to that time. We felt that a 3-hour period was convenient for our use, since we did not have to worry about the problem of bacterial contamination and we obtained a favourable yield of fluid.

**Dr. Karl Meyer (New York, N.Y.):** I think this is a very significant contribution to the problem of the biosynthesis of hyaluronic acid. I am rather astonished at your discrepancy between the quantity of glucuronic acid and the activity of the hexosamine, and I believe this ought to be studied further. Of course, one may have to consider the possibility that the product synthesized in vitro is not a normal polysaccharide formed by the polymerization of a repeating disaccharide unit. Perhaps this possibility could be tested by exhaustive hydrolysis of the product by testicular hyaluronidase followed by the isolation of the tetrasaccharide units and then degrading these further by stepwise degradation with β-glucuronidase and β-hexosaminidase. Another method might be the hydrolysis of the polysaccharide with bacterial hyaluronidase which with normal hyaluronic acid gives quantitatively a 4:5 unsaturated disaccharide. Such a comparison of your polysaccharide with known hyaluronic acid might be interesting.

**Dr. Yielding:** The difference in specific activities of the hexosamine and glucuronic acid has been of considerable interest to us, too. This may reflect a difference in the precursor pool size, since the incubations were brief in time. It would be of interest to observe the relative specific activities in relation to time.

**President Robinson:** I cannot introduce the next paper without calling the attention of the American Rheumatism Association to the recent recognition of Dr. Karl Meyer's pioneering work in connective tissue disease and connective tissue chemistry, as evidenced by the recent joint Lasker Award to him and to Dr. F. O. Schmitt. I know I am expressing the feelings of the entire membership when I congratulate Dr. Meyer.

**Dr. Karl Meyer:** Thank you very much, Dr. Robinson, for your kind words. I only want to say that, of course, the recognition which has come to me was in great measure due to the excellent collaborators which I have had in the past and still have at present with me in our laboratory.

**Chondroitin Sulphate B and Heparitin Sulphate. By Karl Meyer, Philip Hoffman, and Alfred Linker, New York, N.Y.**

Studies on the nature and distribution of acid mucopolysaccharides in connective tissue have been continued, especially on chondroitin sulphate B and on a polysaccharide which we have named "heparitin sulphate". Differences in optical rotation, in colour reactions, and in enzymatic hydrolysis clearly distinguish chondroitin sulphate B from other chondroitin sulphates. The predominant uronic acid of B has been recognized as L iduronic acid, the 5-smer of D-glucuronic acid. Chondroitin sulphate B is the predominant mucopoly- saccharide of pig and ox hide and of ligamentum nuchae. In split ox hide it occurs mainly in the middle and flesh layer. It also has been shown to be identical with the so-called B heparin of beef lung. Furthermore, it has been isolated from the urine of one case of gargoylism. Heparitin sulphate is a monosulphated and (partially) N-acetylated mucopolysaccharide related to heparin. It has been obtained from amyloid and from bovine and human aorta. Heparitin sulphate is hydrolysed by the heparin-adapted enzyme of Payza and Korn (1956).

**Discussion.—Dr. Albert Dorfman (Chicago, Ill.):** First, as far as the rate of sulphuric acid B, I should like to confirm for the most part what Dr. Meyer has said. We also have been able to isolate iduronic acid and to carry the characterization one step further, and that is the reduction of the acid to idose or a mixture of idose and idosan which would be obtained from this compound. Our work has been done with a preparation obtained from Winterstein. We so far have not found evidence that has convinced us that the pure polysaccharide contains glucuronic acid.

I should like to comment also on Dr. Meyer's brief mention of gargoylism. As he knows, we have been interested in this particular substance for the last few years and reported our work in a discussion last June at a Mucopolysaccharide Metabolism Conference (Cifonelli, Ludowieg, and Dorfman, 1957). We have isolated large amounts of polysaccharide in the urine of these patients. We have now been able to examine two different patients and have found mucopolysaccharide in the urine of both. The substance has been identified as chondroitin sulphuric acid B.

Finally, I should also like to comment briefly on the question of the anticoagulant properties of this compound, since I think these are of some considerable biological interest. Marbet and Winterstein (1951) reported originally that this substance has a small degree of heparin-like action. Dr. Grossman, in our laboratory, has studied this question extensively (his manuscript is now in press), and has found that the antithrombin activity is highly dependent on the conditions of the assay, particularly the concentration of thrombin. At low thrombin concentrations this compound is more active than heparin, and at high thrombin concentrations it loses almost all its activity. In addition, at high thrombin concentration, it is able not only to neutralize the antithrombin effect of normal plasma but also partially to neutralize the antithrombin effect of mixtures of heparin and plasma.

**Dr. Ward Pigman (Birmingham, Ala.):** The Masumune group in Japan has isolated another uronic acid, galacturonic acid, from a number of animal tissues (Masumune, 1949). I wonder whether you have ever run into this and whether you have looked for it.

**Dr. Meyer:** Yes, we have looked for it. Masumune for some time claimed that the uronic acid of hyaluronic acid was galacturonic acid on the basis of some colour reactions. Later he or his students claimed that the uronic acid of the acid polysaccharide of hog gastric mucosa was galacturonic acid. I do not believe that there is at present any evidence for the occurrence of galacturonic acid in any mammalian tissue. Dische reported some years ago that heparin gives a colour reaction for galacturonic acid. This, of course, does not mean that heparin contains a galacturonide, especially since the publication of Wolfson in which chemical data were presented indicating glucuronic acid as the uronide of heparin. It would be desirable, however, to obtain
Some Effects of "Viscosity" on the Electrophoretic Analysis of Human Synovial Fluid and Blood Serum.

By David Platt, Ward Pigman, and Francis Patton, Birmingham, Ala.

Human synovial fluid is highly "viscous", but, as shown previously and in the present work, does not behave as an ideally viscous fluid. Although electrophoretic analyses have been reported earlier for synovial fluid, very little is known of the effect of viscosity on the electrophoretic behaviour of large molecules, especially in the complex systems occurring in blood serum and synovial fluid.

The present study reports some of the effect of "viscosity" on the electrophoretic mobilities of the different components of blood serum and synovial fluid. For this work the following general procedures were followed:

1. The addition of a viscosity-increasing agent (methylcellulose);
2. The treatment of the fluids with hyaluronidase;
3. The analysis of fluids having a wide range of apparent "viscosity";
4. The analysis of fluids of different degrees of dilution.

The electrophoretic analyses of the untreated fluids, at pH 8.6 in veronal buffer, indicate that the absolute mobility of the hyaluronic acid component remained unchanged over a wide range of apparent "viscosity". In contrast, the absolute mobility of the protein components increased as the apparent "viscosity" increased. When methylcellulose was added, the absolute mobility of all the components moving in the viscous medium was markedly decreased.

When the synovial fluids were treated with hyaluronidase, the mobility of the hyaluronic acid component was similar to the value obtained for the untreated fluid, but the mobilities of the protein components changed to values similar to those for blood serum. The π component, previously reported, completely disappeared upon hyaluronidase treatment. Since its mobility was between that of hyaluronic acid and albumin, the π component appears to be a hyaluronic acid-protein complex, which is readily broken by hyaluronidase. Dilution of synovial fluid caused a decrease in the absolute mobility of the protein components.

Since an increase in "viscosity" would be expected to cause a lowering of the absolute mobilities, as was found by the addition of methylcellulose; hyaluronic acid apparently complexes in some manner with some of the proteins under the usual conditions of electrophoresis. The formation of "complexes" may increase the absolute mobility of protein components and appears to be responsible for the appearance of the π peak.

Discussion.—Dr. Marian W. Ropes (Boston, Mass.): I am fascinated by the appearance of a component between hyaluronic acid and albumin. I think this adds a little more weight to the likelihood that there is an association of protein and hyaluronic acid in synovial fluid. However, I think the interpretation of an interaction between the protein and hyaluronic on the basis of the changes in mobility seems unwise. Surely, until one has used highly viscous substances with various changes it would be unwise not to interpret the changes as being caused physically by the viscous substance rather than as due to any complex formation.

It would be interesting, I think, to use highly viscous substances of various changes; I judge that this has not been done, and I should like to ask whether it has.

Dr. Platt: As further evidence for our proposal of an interaction between hyaluronic acid and the proteins, I should like to briefly discuss the effect of hyaluronidase on the electrophoretic analyses. The variation in some of the points presented on the graph seems to be greater than would be expected. These points are the values of the protein components of the hyaluronidase treated synovial fluids. Regardless of the viscosity of the samples analysed, the hyaluronidase-treated fluid protein components had mobility values similar to those of serum. In the untreated fluids the mobility value of the proteins appeared to depend on the viscosity of the sample.

This would seem to indicate that the enzymatic treatment disrupted some type of interaction between hyaluronic acid and the protein, and increased the mobility of the protein.

We have not tried any viscous materials other than methylcellulose.

Dr. David Hamerman (Bronx, N.Y.): Did you try electrophoretic studies at pH values other than 8.6? It would be of interest to study the migration of hyaluronate and the π component at an acid pH, say 6 or 5.5, but not acid enough to make a mucin clot. Hyaluronate would retain its negative charge at this pH. It would also be of interest to see whether, at pH ranges of 9-10, the supposed protein-hyaluronate complex could be broken and what the electrophoretic pattern would be like then.

Secondly, when the π component was made to disappear upon addition of hyaluronidase, was there an increased increment of "free" hyaluronate in the electrophoretic pattern?

Dr. Platt: Veronal buffer, pH 8.6, ionic strength 0.1, was the only buffer used in this investigation. In our preliminary investigations, we tried phosphate buffers at pH 6 to 7.6 and a veronal-sodium veronal-sodium chloride buffer, pH 8, and concluded that the straight veronal-sodium veronal buffer, pH 8.6, gave us the best resolution of the protein components.

In answer to the second question, we did not attempt to do any quantitative recovery after hyaluronidase treatment. However, the hyaluronic acid peak, which is a sharp spike in the very viscous fluids, was not changed to any great extent by the enzyme.

Dr. J. H. Fessler (Boston, Mass.): I am interested in these interactions of hyaluronic acid and protein. It seems that such interactions can be classified into two groups: one consisting of hydrodynamic interactions and the other involving some type of firmer binding.

I wonder whether you studied the π component after isolation. Ogston and Stainer (1952) isolated a hyaluronic acid-protein complex by a process of filtration on

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* Pre-doctoral Research Fellow of the Arthritis and Rheumatism Foundation and also supported by a grant from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service, Bethesda, Md.
sintered glass filters, and I believe that in exploratory electrophoresis experiments this complex migrated as a single component. A more definite linkage than just hydrodynamic interaction would seem to be involved there. Have you any views on the nature of the interaction between hyaluronic acid and protein in the π component?

**DR. PLATT:** I do not know. It seems to be one which is easily broken down. Extremely mild treatment with hyaluronidase reduces the viscosity of the fluid to less than half its original value and the π peak can no longer be detected by electrophoresis.

**DR. KARL MEYER (New York, N.Y.):** I have two questions. First, did you do any dilution experiments? The other has to do with the condition of your enzymatic experiments. I presume your hyaluronidase units are in the order of magnitude which are customarily defined in the literature as viscosity reducing or turbidimetric units. With only a few such units, you can only expect a very limited breakdown of hyaluronic acid especially in a natural medium such as synovial fluid where the breakdown is inhibited. In such a fluid you will find the action limited to the highest molecular weight or size fractions of hyaluronic acid, that is, you would find an effect on the most viscous fractions and none or little on the less viscous fractions.

**DR. PLATT:** We used the standard turbidity reduction units. The conditions for the treatment were two: for extremely mild treatment the fluids were diluted with the veronal buffer and incubated for 2 hours at 30° C. with the enzyme; for a more drastic treatment, we added the hyaluronidase directly to the undiluted fluid and incubated it at 37° C. We were thus able to obtain the desired conditions.

We did study the effect of dilution on electrophoretic behaviour at the ascending boundary, and the results were similar to those already described. As the fluids were diluted the viscosity decreased and the mobility of the protein components decreased, whereas the mobility of the hyaluronic acid remained virtually unchanged.

We have not studied the effect of dilution on the π component. However, it is of interest that, in one fluid diluted with two volumes of buffer and analysed in the 2 ml. cell, the π component was not detectable, but when the fluid was diluted with four volumes of buffer and analysed in the 6 ml. cell the π component was present.

**DR. PIGMAN:** In the normal electrophoresis of synovial fluid, the viscous component is hyaluronic acid, and hyaluronic acid is the most rapidly moving component. Our results indicate that in its movement it is pulling the other components with it. In the experimental situation that we had with the blood sera, the viscous component was methylcellulose, which was virtually immobile. In this situation the blood serum components were moving, and as our experimental results showed they were being held back by the viscous component. However one wishes to interpret the cause of the viscous effect, its result is simply to pull the other molecules along when it is moving. When the viscous component is stationary, it holds the other molecules back.

The problem is how to interpret these results. Most ideas about viscosity are based on hydrodynamic concepts. There is a need for interpretations based on concepts of molecular interactions and the structure of solutions. Perhaps there are some underlying principles which would interrelate viscous, electrophoretic, and ultracentrifugal behaviour.

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*Biochem. J.*, 52, 149.

The Occurrence of Rheumatoid Arthritis and Diffuse Fibrinoid Diseases in Patients with Congenital and Acquired Agammaglobulinaemia. By ROBERT A. GOOD, JEROME ROTSTEIN, and WILLIAM F. MAZZITELLO, Minneapolis, Minn.

Although numerous theories concerning the aetiology and pathogenesis of rheumatoid arthritis and other forms of the so-called fibrinoid (collagen) diseases have been proposed, no clear delineation of their essential nature has been forthcoming. Prominent among the theories of aetiology and pathogenesis of these diseases have been those implicating hypersensitivity, the formation of antibody, and disturbances of gamma globulin metabolism. We have had three cases of rheumatoid arthritis among our patients with agammaglobulinaemia. In each the manifestations of the joint disease typical of rheumatoid arthritis. Each had complete agammaglobulinaemia as revealed by both zone and free electrophoretic study of the serum. In addition, complete immunological unresponsiveness was demonstrated in two and profound immunological inadequacy in a third after intensive antigenic stimulation with a wide variety of antigens. Detailed study of case reports of patients with agamma-globulinaemia showed arthritic manifestations to be a common feature. In at least five additional cases in the literature, manifestations are reported suggestive of rheumatoid arthritis.

Other forms of fibrinoid disease were also observed in agammaglobulinaemic patients. Hansen *et al.* studied a patient with agammaglobulinaemia who had dermatomyositis; Van Gelder studied a case of agammaglobulinaemia with scleroderma. Finally, we have recently studied tissues of a patient with presumed agam-maglobulinaemia who died of a disease morphologically definable as generalized collagen disease or disseminated lupus erythematosus.

These observations suggest the conclusion that ana-phylactic or classical immunological mechanisms are not basic to the occurrence of rheumatoid arthritis or other members of the fibrinoid disease group. They also indicate that excessive production of gamma globulin often observed in patients with fibrinoid diseases is a concomitant event and is not basic to the aetiology or pathogenesis of these diseases.

**Discussion.—DR. WILLIAM J. KUHNS (Pittsburgh, Pa):** Were any of the serological tests that are customarily employed in rheumatoid arthritis carried out on these patients? I refer to such tests as the sheep cell agglutination test or the latex-fixation test.

Was the possibility considered that toxic agents liberated during repeated infections had any bearing upon the episodes of arthritis without any particular relation to the absence of gamma globulin?

**DR. ROTSTEIN:** Through the courtesy of Dr. John Vaughan, of Richmond, Virginia, serological tests were
performed on these three patients. The first was that with the F2 fraction plus the rheumatoid serum; there was no sedimentation. The second was conducted with sheep erythrocytes sensitized with rabbit antibody; there was no precipitation. The third was carried out with Rh-positive red cells coated with Rh-positive antibody; there was no precipitation. There was no inhibition for either system.

We have considered Dr. Kuhns’ idea very carefully. The patients presented by us at this meeting met every criterion for rheumatoid arthritis that has been established by the American Rheumatism Association. In addition, no patient had a decrease in arthritic manifestations when treated with antibiotics. When fever occurred the joint symptoms and signs cleared. When jaundice occurred in the first patient, the arthritis abated, as Hench’s observations led us to believe that it would. A consultant rheumatologist saw the patients and concurred in the diagnosis of rheumatoid arthritis. Therefore we accept all these patients as cases of rheumatoid arthritis. These cases were not associated with overt infection and at this time we know of no evidence that rheumatoid arthritis is associated with the liberation of toxic elements after infection.

DR. JOHN H. VAUGHAN (Richmond, Va): Dr. Rotstein has already referred to a possibility of delayed hypersensitivity mechanisms. Did you report testing these patients with tuberculin or other bacterial antigens?

DR. ROTSTEIN: The first patient, who died, was not tested to tuberculin. The other two had a negative tuberculin test. Both had positive skin reactions to 2,4-dinitrofluorobenzene.

DR. MELVIN H. KAPLAN (Boston, Mass.): Would you elaborate on the extremeness of the fibrinoid degeneration seen in the synovial tissues of these patients?

Secondly, would you conclude, as a result of your findings in this type of arthritis, that a primary consideration be given to the role of tuberculin-type hypersensitivity in rheumatoid arthritis, in general, to the exclusion of anaphylactic or circulating antibody?

DR. ROTSTEIN: Many patients, who are labelled as agammaglobulinaemic do have, by immunochromical determination, a small amount of gamma globulin and are truly hypogammaglobulinaemic. However, two of our three patients were truly agammaglobulinaemic as shown by immunochromical methods. Therefore, we believe that we have shown that those antibodies which are contained in the gamma globulin fraction of the serum proteins are not basic to the aetiology of rheumatoid arthritis.

DR. GERALD P. RODNAN (Pittsburgh, Pa): Was a search made for L.E. cells and were the serum uric acid concentrations estimated in these patients?

DR. ROTSTEIN: All the patients had bone marrow and peripheral blood lupus cell tests; all were negative. Uric acid determinations were done in all instances; none were elevated.

DR. THEODORE B. BAYLES (Boston, Mass.): I have discussed with Dr. Craig of Dr. Janeway’s group the patients with arthritis associated with agammaglobulinaemia, and Dr. Peter Kulka has reviewed the biopsy material. It seems to me that, at this point, knowing little about agammaglobulinemia but a little more about rheumatoid arthritis, I should prefer to reserve a final decision whether this is rheumatoid arthritis or not. From what I have seen and can find out, I should be unwilling to accept the idea that this is a bona fide rheumatoid arthritis in children.

THOMAS McP. BROWN (Arlington, Va): I wonder if Dr. Rotstein has any way of determining whether there are any fixed tissue antibodies of any sort that could be measured. It seems unwise to me to conclude that circulating antibodies must be necessary to create a state of hypersensitivity. Certainly they aren’t in tuberculosis as far as we know. I know of no good method of excluding the point of the pH in the fixed tissue antibodies, even in the absence of circulating ones. I think it is dangerous to jump to the conclusion that hypersensitivity does not operate because circulating gammaglobulin is absent.

DR. ROTSTEIN: At the end of our paper we stated: “The recent demonstration that patients with agammaglobulinaemia possess the ability to develop a delayed type of allergy would make it seem unwise to discard, without further evidence, the hypothesis that all, or any, of these diseases may be due to some kind of allergic reaction, even though they do occur together with agammaglobulinaemia.”

Therefore we are agreed that hypersensitivity of some type may be basic to the aetiology of rheumatoid arthritis.

Lawrence and Pappenheimer (1956) is the most advanced study of the antibodies involved in the delayed type of allergy. With the most careful methodology they could not measure this type of antibody. In Dr. Good’s laboratories we have not yet pursued this type of work.

DR. SIDNEY COBB (Pittsburgh, Pa): Might there not be another interpretation of this information? Several years ago we called attention to the fact that patients with rheumatoid arthritis more commonly die with infections than people in the general population. Here we have a group of people who had an enormous number of infections, and it occurs to me this association seen in two different situations may play a role which is not obvious to us at the moment and is perhaps unrelated to the phenomenon of agammaglobulinaemia, except in so far as it permits large numbers of infections.

DR. ROTSTEIN: We have thought about this. One of our thoughts has been that the gamma globulin which becomes elevated in rheumatoid arthritis may not be an effective gamma globulin.

DR. JOSEPH J. BUNIM (Bethesda, Md.): Certainly the question uppermost in most people’s minds was whether this was indeed rheumatoid arthritis. I think we ought to point out that, although it is unquestionably true that patients with rheumatoid arthritis who develop jaundice, enjoy a remission in most instances, it does not necessarily follow that amelioration during the jaundice is a specific diagnostic point in favour of rheumatoid arthritis. I recall that in his original papers on this subject Hench stated that a few patients with rheumatic disease other than rheumatoid arthritis also enjoyed remissions or improvement in their clinical state when suffering from jaundice.

DR. PHILIP R. TROMMER (Philadelphia, Pa): Inasmuch as there has been an increasing evidence in the literature of a correlation of blood groups with disease states, I was wondering whether blood typing was done of these patients, so that it could be used for future reference.
DR. ROTSTEIN: Unfortunately, I do not have that data with me. It will be included in a paper now in the press (Good, Rotstein, and Mazziello, 1957).

DR. CURRIER McEWEN (New York, N. Y.): I have been looking forward to hearing this paper ever since I saw the abstract. I was present at the meeting last May at which Dr. Janeway reported some patients with agammaglobulinaemia and arthritis. I believe his designation of it as "undiagnosed joint disease" is preferable to calling it "rheumatoid arthritis". It would be most unwise of me to say it isn't rheumatoid arthritis; but like Dr. Bayles I wish to raise the question. Dr. Janeway's description of the cases he reported last year left some doubt whether, even clinically, they warranted the label of rheumatoid arthritis. I am particularly interested in the data just reported by Dr. Vaughan that the sheep cell erythrocyte agglutination tests were negative in these patients, even when done by the inhibition technique.

Dr. Ziff will point out a little later in this meeting that patients with Marie-Strümpell spondylitis, Reiter's syndrome, and arthritis accompanying mucous colitis and psoriasis also fail to show the sheep cell agglutination phenomena even though they do have gamma globulin. The sheep cell factor, like the L.E. factor, apparently appears in the gamma globulin fraction of the serum. If the patients reported by Dr. Rotstein truly have rheumatoid arthritis, it will be clear that the sheep erythrocyte agglutinating factor is not a necessary concomitant of that disease.

However, I would suggest that, until we really know the nature of the arthritis accompanying agammaglobulinaemia, we shall not contribute to clarification by labelling it rheumatoid arthritis. I believe it would be wiser to consider this form of joint disease as similar to rheumatoid arthritis but not necessarily the same thing.

DR. JOSEPH E. WARREN (Pittsburgh, Pa): I just want to know how great an increase there was in mucoproteins or alpha-globulins in these patients; was it very marked?

DR. ROTSTEIN: Each of our cases had an increase in the alpha-2 globulin.

DR. CHARLES RAGAN (New York, N. Y.): I have to disagree with Dr. McEwen and Dr. Bayles. If we plan to act on the basis of Dr. Rope's committee's recommendation, these patients do have rheumatoid arthritis. If we are going to say that they do not have rheumatoid arthritis, every other paper on the programme concerned with rheumatoid arthritis must be modified. By the definition of Dr. Rope's committee, these children have rheumatoid arthritis.

One reason why Dr. Janeway said he felt they might not have rheumatoid arthritis was that they did not complain of pain. In our experience, this is not uncommon in Still's disease. Far-advanced disease may exist without a history of pain.

I should like to point out that, even with complete absence of gamma globulin by paper electrophoresis, there may still be some present. Agammaglobulinaemia should be characterized by name—agammaglobulinaemia by paper electrophoresis or by immunochemical methods.

DR. ROTSTEIN: By immunochemical methods one patient had a minute amount of gamma globulin; two had none.

DR. PAUL J. BILKA (Minneapolis, Minn.): I think I should like to add a comment to Dr. Ragan's. As the person who examined the first patient in Dr. Rotstein's paper, I can say he had all the classic clinical signs of rheumatoid arthritis. If we are going to question that, I must agree with Dr. Ragan that we shall have to question every paper presented here, now, in the past, and in the future, concerning rheumatoid arthritis!

DR. HARRY BARTFELD (New York, N. Y.): The patients described may well have rheumatoid arthritis. It has been reported that 5 per cent. of patients having this disease in an active form have a normal sedimentation rate. It is possible that these patients may have agammaglobulinaemia or hypogammaglobulinaemia. Similarly, authentic cases of rheumatoid arthritis have a negative sensitized sheep cell agglutination or similar tests and these may well have a deficiency of gamma globulin or other blood proteins.

REFERENCES


Using the criteria for rheumatoid arthritis adopted last spring by this association we have been able to make the following estimates of the prevalence of this disease for the Arsenal Health District of Pittsburgh:

<table>
<thead>
<tr>
<th>Category</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definite</td>
<td>0.7 per cent</td>
</tr>
<tr>
<td>Probable + Definite</td>
<td>2.7 per cent</td>
</tr>
<tr>
<td>Possible + Probable + Definite</td>
<td>13.9 per cent</td>
</tr>
</tbody>
</table>

These estimates are based on a carefully studied sample of 798 individuals who were selected as a stratified random sample from the district, the total population of which is about 80,000.

Further estimates indicate that the preponderance of females suffering from this disease is more striking among sufferers from the severe forms than from the mild form, and similarly that the prevalence of the severe forms increases rapidly with age, though there is relatively little change with age for the mild form. The most interesting finding is the increase in prevalence with changing marital status. It seems that married persons are more often affected than single persons, and persons who are separated, divorced, or widowed are more affected than those who have remained married. This is true even when the rates are adjusted for the obvious age differences between the three groups. Another important finding is that the prevalence rates go up to approximately double when they are based on two examinations instead of only one. This is because the fluctuations of the disease frequently do not permit an adequate diagnosis on the basis of one examination.

Discussion.—DR. CHARLES L. SHORT (Boston, Mass.): I must thank Dr. Cobb and his associates for their most valuable work. Studies such as these should eventually give us information regarding the real rheumatoid arthritis, not just the rheumatoid arthritis that we see either in the office, the hospital, or the clinic. Up until now, as you all know, most of our information about the natural history of the disease is based upon such patients.
ANNALS OF THE RHEUMATIC DISEASES

I wonder if Dr. Cobb has any data in regard the age at onset of the disease in these patients. I ask this because, if the age at onset of rheumatoid arthritis is distributed through all age groups, we should expect to find a rise in prevalence with increasing age. Is this not so?

DR. COBB: I am sorry to say that so far we have made no satisfactory analyses of the ages at onset. One reason was that we found it difficult to determine what comprises the “onset”. Patients will give different points in time, depending on how carefully the history is taken. You are correct in assuming that the observed increase in the prevalence of the probable full rheumatoid arthritis could merely be based on a steady incidence rate, unvarying with age, assuming of course that there are no recoveries, which means that the cases accumulate with time.

DR. PHILIP R. TROMMER (Philadelphia, Pa): Had Dr. Cobb’s cases always lived in the Pittsburgh area, or were they recent emigres into the area who had come from various parts of the country, other than Pittsburgh?

DR. RUSSELL L. CECIL (New York, N. Y.): will Dr. Cobb elaborate a little on his interpretation of morning muscular stiffness. Isn’t that more likely to be just an age factor or possibly due to osteo-arthritis, rather than to rheumatoid arthritis?

DR. COBB: In answer to the question about migration, I am sorry that I do not have the figures on that in my hand and would hesitate to reply to it. A detailed analysis of this and many related factors is in preparation.

We have used morning stiffness as a criterion here because it is one of the definitions that were adopted by this Association last spring.

DR. JOSEPH J. BUM (Bethesda, Md): I am sure Dr. Cobb has given a great deal of thought to the question of the prevalence of rheumatoid arthritis in people below the age of fifteen. I wonder how we are to deal with this problem since it is not negligible. I believe that the prevalence of this condition is about one in every 100,000 of the population.

DR. COBB: For the study of a disease of very low prevalence rate such as Still’s disease, quite different methods are required. One possible pattern is that developed for the study of multiple sclerosis.

Results of the Sensitized Sheep Cell Agglutination Tests in Chronic Arthritis Diseases. By MORRIS ZIFF, GEORGES FALLET, PHILIP CARME, DOMINICK DITATA, JOSEPH LOSPALLUTO, and CURRIER MCEWEN, New York, N. Y.

Previous work in this laboratory utilizing the euglobulin fraction of serum demonstrated the sheep cell agglutinating factor in the serum of over 90 per cent. of patients with rheumatoid arthritis when the euglobulin was tested directly, and in all patients with clinically definite rheumatoid arthritis when the capacity of the euglobulin fraction of test sera to inhibit known positive-reacting rheumatoid serum was used as an index. The high incidence with which the sheep cell factor occurs in rheumatoid arthritis and the rarity of false positive tests in non-rheumatoid subjects indicates that its presence may be accepted as a specific characteristic of the disease.

To date, negative tests, both on the euglobulin fraction when tested directly, and by the inhibition procedure described, were given by all of seventeen patients with psoriasis and arthritis; 88 of 89 patients with ankylosing spondylitis, eleven of whom had peripheral joint involvement; sixteen of seventeen patients with chronic or recurrent venereal arthritis; and all of six with ulcerative colitis and arthritis. Five of eighteen patients with systemic lupus erythematosus, however, gave positive tests. The patients with positive tests in the last group had peripheral joint involvement resembling that seen in rheumatoid arthritis.

Twelve of twenty sera from patients with juvenile rheumatoid arthritis gave positive tests when tested directly on the euglobulin fraction, and all twenty gave positive tests when the inhibition procedure was used.

The results indicate that the arthritides studied, with the exception of systemic lupus erythematosus, are not associated with the presence of the sheep cell factor in the serum, even though they have some features in common with rheumatoid arthritis both clinically and pathologically. It would appear that the rheumatoid factor is not a by-product of chronic synovial inflammation but is significantly related to the rheumatoid process.

Discussion.—DR. WILLIAM J. KUHNS (Pittsburgh, Pa): I gather from Dr. Ziff’s talk that he may be slightly haunted by the last paper, but I would certainly agree that the sheep cell agglutination test is an extremely valuable test in rheumatoid arthritis, whether or not it is directly related to the aetiology of the diseases.

DR. CHARLEY J. SMYTH (Denver, Colo.): We have been interested in the test described by Dr. Ziff and associates and have now tested 1,300 sera from 222 patients with rheumatoid arthritis and various other rheumatic diseases and also many normal subjects. There were 93 patients with rheumatoid arthritis and the test was positive in 83-9 per cent.; there were 75 patients with degenerative joint disease and the test was negative in 71 and positive in four. In six patients with Marie-Strümpell arthritis, the test was negative in five and positive in one; in eight patients with lupus erythematosus disseminatus, seven were positive and one was negative; in two patients with Still’s disease, both were negative; in 28 patients with rheumatic fever the test was positive in two and negative in 26.

Of the patients with rheumatoid arthritis, we selected 42 whom we were able to follow with serial tests (minimum three, maximum seven) during a period ranging from 6 months to 2 years. At the initial examination the test was positive in 88 per cent., but at the end of the 6 months’ to 2 years’ follow-up, the number of positive tests gradually decreased to 71 per cent.

DR. CHARLES M. PLOTZ (Brooklyn, N. Y.): The test we did was positive in 10 per cent. compared with our series and yours with the sheep cell agglutination.

DR. ZIFF: The results obtained in disseminated lupus erythematosus depend a great deal on the type of patient tested. In patients with severe arthritis, the incidence of positive tests is high. If the degree of arthritis, past or present, is small, the incidence of positive tests is low.

DR. TIBOR BENEDEK (Chicago, Ill.): The speaker inferred from the result of a serological reaction that rheumatoid arthritis of the peripheral joints and rheumatoid spondylitis (rheumatoid arthritis of the spine) are...
two entirely different diseases. With growing methodological knowledge, he will realize that serological as well as immunological reactions (like skin-testing) are the weakest links in the chain of etiological evidence of the cause and pathogenesis of any disease. At best, they are equivocal. By their fallacy, though in reverse, one could infer that syphils and leprosy are etiologically and pathogenically identical diseases because the Bordet-Wassermann complement-fixation test is positive in both. The etiology and pathogenesis of rheumatoid arthritis, peripheral and spinal, are well established, and we now have a firm frame of reference for the proper evaluation of all the serological and immunobiological findings in this field. The constant and continued ignorance of this solid frame of reference is detrimental to further research and to the alleviation of the suffering of those attacked by rheumatoid arthritis, peripheral and spinal.

Dr. Ziff: I think the laboratory can help to reinforce the clinical impression.

Dr. Charles L. Short (Boston, Mass.): We should not discard the possibility that rheumatoid-like arthritis with psoriasis and so-called rheumatoid spondylitis may be variants of rheumatoid arthritis simply because of differences in serological reactions, when the clinical similarities are so marked. It is more important perhaps to find out why these serological differences exist than to conclude from these findings, which I agree are important, that we are dealing with completely independent entities. Conversely, I wonder how Dr. Ziff would explain the positive tests in lupus erythematosus. Do they mean that these patients have a coincidental rheumatoid arthritis?

Dr. Ziff: I don’t know the answer to that question. I think that this problem is one of the most interesting challenges we shall have to deal with in the next few years.

Dr. Philip R. Trommer (Philadelphia, Pa): Since you have had a great deal of experience with this test, and since the problem of rheumatoid arthritis is early diagnosis, how soon do you think the test becomes positive in the great percentage of cases? Can the test be used for the early diagnosis of rheumatoid arthritis?

Dr. Ziff: Our inhibition procedure is positive early, but it is a difficult test to do. The results with this method have convinced us that the rheumatoid factor is present early in the disease, though it may not be demonstrated easily by clinical screening procedures.

Dr. Trommer: What do you mean by early?

Dr. Ziff: In ten of eleven cases in our series of less than 6 months’ duration, the tests were positive. Dr. Wallace Epstein has told us of two cases of acute rheumatoid arthritis which gave positive tests on the day of onset. In surveying the literature and our own experience, the question seems to be one of how much disease the patient shows clinically. If there are early clear-cut changes, it is more likely that the test will be positive early. Often people showing very little anatomical evidence of disease for years will be negative for years, but I must emphasize that we have found the test helpful again and again even in early cases.

Dr. Wallace Graham (Toronto, Ont., Canada): Does the test remain positive during pregnancy?

Dr. Ziff: Yes.

REFERENCE


Studies on the Mechanism of the Binding of the Rheumatoid Factor to Sensitized Sheep Erythrocytes. By Glenn M. Clark, Charley J. Smyth, and Gabriel Haby (by invitation), Denver, Colo.

Cohn’s Fraction III was prepared from the serum of ten patients with rheumatoid arthritis and was shown in each case to have potent activity in agglutinating sensitized sheep erythrocytes. Repeated exposure of each sample at 4°C. to sensitized sheep-cell stroma removed all agglutinating activity and a portion of the protein-bound carbohydrate (Sheflet). Absorption in the same manner with non-sensitized stroma removed only a part of the agglutinating activity and much smaller amounts of carbohydrate. Fraction III from patients without rheumatoid arthritis showed agglutinating activity in low titre. This activity was completely removed by absorption with non-sensitized stroma. These findings indicate the presence of two sheep erythrocyte agglutinating factors in Fraction III, only one of which requires sensitization of the cells.

In an effort to determine whether the observed fall in carbohydrate was concerned in the binding of some component of Fraction III to haemolysin, sensitized stroma was washed with solutions of galactose and mannose (at 4°C.) before exposure to Fraction III of high agglutinating titre. Fraction III exposed to carbohydrate-treated sensitized stroma no longer lost its agglutinating activity.

These data are interpreted as evidence that the binding of a specific agglutinating factor in Fraction III of rheumatoid serum to sensitized stroma may be inhibited by blocking some active group in the haemolysin-coated stroma with galactose and mannose. This suggests that the binding of the “rheumatoid factor” to sensitized stroma occurs through a carbohydrate linkage.

Discussion.—Dr. Wallace Epstein, San Francisco, Calif.): Which other carbohydrates beside the two mentioned were tried in this blocking effect, and were oligosaccharides tried in terms of relative potency compared with the two monosaccharides.

Dr. Clark: We have been interested in trying to find out something about the specificity of the carbohydrates. We have only tried galactose, mannose, and glucose, as well as mixtures. We found that mannose is far more potent than any of the other sugars in preventing absorption with sensitized sheep cells, but all the sugars we tried had some effect.

Dr. William J. Kuhns (Pittsburgh, Pa.): Did you have the opportunity to carry out experiments with the fractions obtained by using other separation methods. It is known that electrophoretically separated fractions similar to those obtained by Cohn fractionation may behave differently immunologically and may also differ in their relative protein and polysaccharide content. Have you compared the behaviour of Cohn fractions with that of electrophoretically separated fractions?

Dr. Clark: No: we used no other method.

Dr. John H. Vaughan (Richmond, Va.): We are all very much interested in the possible role of the polysaccharides in these agglutination reactions. There is one thing that confuses me, however, and which I should like to know more about. In absorbing a specimen your
normal sheep cells reduce the polysaccharide content to a considerable extent, though not as much as do sensitized sheep cells. Does this not invalidate the probability that there is anything specific in this for the sensitized sheep cell reaction itself? Do you know whether there are any differences between the various sugars in their effectiveness in inhibiting absorption by normal cells or by sensitized cells? I think this information would help.

**Dr. Clark:** This question has caused us concern and sober reflection since our original excitement concerning this observation. It is obvious that large amounts of protein and polysaccharide (if this is polysaccharide) are removed by these absorptions. It would be difficult to imagine that three-quarters of Fraction III, which is the amount that can be absorbed by sensitized stroma, would enter into any specific antigen-antibody reaction. We have also attempted by electrophoresis and chemical methods to show some difference between material which is absorbed by the sensitized and nonsensitized stroma, and we have had no success whatsoever. We feel that the only indication of any specificity is concerned with the fact that some sugars seem to be more effective agents in blocking the absorption than others.

**Dr. Morris Ziff (New York, N.Y.):** Were these preparations made from inactivated and absorbed sera? Is the activity which was absorbable with unsensitized stroma possibly heterophil antibody?

**Dr. Clark:** Yes. We feel that this probably is a heterophil antibody. The reason that we did not try to remove it was that we had found changes in the protein and protein-bound carbohydrate content after absorption of sera with non-sensitized sheep cells.

**Dr. Ziff:** Would the unsensitized stroma take out the heterophil antibody and the sensitized stroma take out the rheumatoid factor?

**Dr. Clark:** That is right.

**Dr. Ziff:** What about the concentration of mannose solution? Was that high?

**Dr. Clark:** We used 1 per cent. mannose and 1 per cent. galactose, and they were allowed to stand in the ice box over night. This did not occur at room temperature.

**Dr. Ziff:** The important changes seemed to happen on the fourth absorption. That is, the figures all looked pretty much the same, until you reached the fourth tube, and then the values would drop down and give a significant difference. Do you have any explanation for that?

**Dr. Clark:** Yes. The explanation for that is that although we have examined five to ten sera in each type of experiment, all of the data from the representative experiments reported here were obtained from the serum of the same patient.


The C-reactive protein test for the detection of non-specific inflammatory conditions is a widely-used qualitative and semi-quantitative precipitation capillary method. By the use of latex fixation it has been possible to convert the method into a more quantitative agglutination reaction.

To a progressive serial saline dilution of serum is added an equal amount of a saline suspension of polystyrene latex particles (0.81 μ) and anti-C-reactive protein serum. The tubes are incubated for 2 hours in a water bath and centrifuged. The resultant strong agglutination occurs in positive serum in dilutions to 1:5, 120, and the end-point is reproducible.

By the use of this method it is possible to demonstrate the presence of C-reactive protein in low titre in sera where it was not detectable by the older method. A group of 120 rheumatic fever sera and 120 controls were studied.

**Discussion.**—Dr. Francis W. McCoy (Columbus, Ohio): We were very enthusiastic about this latex test when it was reported last summer by Plotz and Singer (1956) and we incorporated this latex phenomenon in a few serological examinations in our laboratory. Being essentially interested in time-saving devices and accuracy, we compared the so-called macro-method of Dr. Plotz, with a drop modification, developed in our laboratories. The technique is not essentially different. We found 100 per cent. agreement in testing sera which showed a positive reaction and negative reaction by both techniques.

We then proceeded to compare the reaction using this test and the standard and modified forms of the sheep cell agglutination test on a total of 125 sera. There were 58 sera in this group of unselected illnesses: 47 were positive by the latex fixation test, and 51 by the sheep cell test. Forty sera reacted by both tests, and five reacted with the latex fixation test but were negative by the sensitized sheep cell test. Eleven were positive by the sheep cell test and negative by the latex fixation test. This gave us a rough agreement with the sheep cell agglutination test of 69 per cent.

What interested us so much was that, of 105 patients with active rheumatoid arthritis, 92 gave a positive reaction by this method. Of twelve in whom we felt the disease was either quiescent or arrested because of therapy, eight gave a positive reaction.

From patients with rheumatic fever, diffuse collagen disease (such as lupus erythematosus), gout, fibrositis, and rheumatoid spondylitis, we got no positive reaction, but of five patients with peripheral rheumatoid arthritis associated with spondylitis, three were positives. In a group of miscellaneous diseases the one positive result was in a case of infectious mononucleosis; five were positive out of eighteen with tuberculosis, seven out of 24 with syphilis.

It is interesting that the last three groups parallel closely the sheep cell agglutination reaction.

Our technique is very easily accomplished, takes only half an hour, and seems to be very accurate in confirming the clinical impression of rheumatoid arthritis.

**Dr. William J. Kuhrns (Pittsburgh, Pa.):** It appears that, depending upon the pattern of agglutination found in dilutions of 1:20 to 1:160, a serological differentiation between rheumatic fever and rheumatoid arthritis is possible. Is this correct?

**Dr. Plotz (Brooklyn, N.Y.):** In answer to Dr. Kuhrs, the test as we developed it was not designed to differentiate rheumatic fever from rheumatoid arthritis. If you want to do so, and we really have not enough data to say this is an absolute test, but you have to run at least a five-tube test. We routinely run this test up to dilutions of 1:10,240 because we are interested in determining ultimate titre.
In answer to Dr. McCoy, your discussion will be gratifying to Dr. Singer who has worked so hard with the latex test, and will be pleased to know that another group has not only confirmed our findings but also developed a refinement upon our technique.

REFERENCE

Hexosamine Content of Serum Globulins. By **ALFRED JAY BOLLET** Detroit, Michigan.

In normal sera, the hexosamine content of the alpha-1 globulin averaged 17.5 mg. per cent., alpha-2 25 mg. per cent., beta 17.4 mg. per cent., and gamma 24.7 mg. per cent. The ratio of hexosamine to protein (×100) was found to average 4.3 in the alpha-1, 4.1 in the alpha-2, 2.9 in the beta, and 1.8 in the gamma globulin. In both rheumatic and some non-rheumatic diseases the hexosamine content of the alpha-1 and alpha-2 globulins increased significantly, but the ratio of hexosamine to protein increased significantly only in the alpha-1. Minimal changes occurred in the beta globulins. Sera with increased gamma globulin had an increased hexosamine content of that fraction with no change in the ratio of hexosamine to protein. Anti-inflammatory hormones caused a fall in the hexosamine content of the alpha-1 and alpha-2 globulins, but a corresponding fall in hexosamine to protein ratio occurred only in the alpha-1.

The sero-mucoid fraction contained 5.69 mg. per cent. hexosamine in normal subjects, with a ratio of hexosamine to protein (×100) of 7.2. The amount of hexosamine and the ratio of hexosamine to protein were increased in both rheumatic and non-rheumatic inflammatory diseases. Anti-inflammatory hormones caused a fall in the hexosamine, but a rise in the protein in the sero-mucoid in most instances, with a decrease in the ratio of hexosamine to protein.

These observations indicate that an increase in at least one component of the alpha-1 globulin and the sero-mucoid which is richer in hexosamine than the rest of the fraction occurs in a variety of illnesses, and that there is a fall in a hexosamine-rich component with anti-inflammatory hormone therapy.

Discussion.—**DR. M. R. SHETLAR** (Oklahoma City, Okla): We have made a similar study in which we did strip paper electrophoretic studies and applied the periodic acid–Schiff reaction directly to the paper strips. The total protein-bound carbohydrate was quantitated by determination of bound hexose by our tryptophan method. We studied 33 cases of rheumatoid arthritis by this technique, and are able to confirm Dr. Bollet’s data, with the addition that we found the a2 globulin-bound carbohydrate (when expressed as a percentage of the protein moiety of this fraction) to be significantly elevated in rheumatoid arthritis. I noticed that Dr. Bollet reports an elevation of protein-bound hexosamine in this fraction which was not significant. I imagine that a larger number of samples might have yielded data similar to ours.

We went further and attempted to correlate each of the estimations of carbohydrate bound to the various fractions with a clinical appraisal of rheumatic activity, as evolved by Dr. R. W. Payne of our group. We found that both the a1 and a2 globulin-bound hexose carbohydrate, expressed in mg./100 ml., of serum were significantly correlated with clinical activity. When expressed as a percentage of the corresponding protein moiety, only the a1 globulin-bound carbohydrate was found to have a significant correlation with activity. None of these correlations was as good as that obtained between total serum glycoprotein (PR) and clinical activity.

I was interested in the information concerning the sero-mucoid. The demonstration of a change in the carbohydrate to protein ratio of this fraction is indeed unusual. I should like to ask how the protein content was determined on the sero-mucoid fraction.

Dr. Bollet mentioned that the sero-mucoid travels with or has about the mobility of a2 globulin. Our studies with continuous paper electrophoresis and studies reported from England utilizing two-dimensional paper electrophoresis both indicate that fractions of the sero-mucoid complex move with both a1 and a2 globulin in veronal buffer at pH 8.6.

*Dr. Bollet*: We did note some change in the ratio of hexosamine to protein in the alpha-2 globulin but this did not seem significant, particularly in view of the number of cases we were studying. It certainly was minimal when compared to the change in the ratio of hexosamine to protein in the alpha-1 globulin, and in addition no change could be detected in the hexosamine to protein ratio in the alpha-2 globulin following hormone therapy.

The seromucoid protein determinations were done using a casein standard.

I think I said seromucoid is largely alpha-1 globulin. I realize there is some question about this, and that some is probably alpha-2 or migrates between alpha-1 and alpha-2.


The immediate anti-inflammatory activity, as manifested by the serum protein-bound polysaccharide-protein ratio (PR), of various glucocorticoids, glucocorticoid-salicylate combinations, and salicylates were compared in patients with rheumatoid arthritis.

Salicylates in tolerable doses were found to exert a significant though modest anti-rheumatic effect. The addition of small amounts of glucocorticoids to the salicylates produced no objective evidence of drug reinforcement. Prednisolone exhibited approximately five times the anti-inflammatory potency of cortisone insofar as the production of changes in PR and prednisone proved slightly less effective than prednisolone.

**Discussion.** **DR. EDWARD E. FISCHEL** (New York, N. Y.): Did you study the Westergren sedimentation rate simultaneously with other studies? I ask this because there are many acute-phase tests being studied which, while of great theoretical interest, rarely have practical advantage over a well-performed erythrocyte sedimentation rate estimation. Many of the claims concerning the
superiority of some of these tests over the sedimentation rate are based on the use of sedimentation rate methods other than the Westergren.

DR. WILLIAM R. MERCHANT (Pittsburgh, Pa): Had you any patients in the series who did not respond to the hormones, and what were the polysaccharide-protein ratios in these particular individuals?

DR. KARL MEYER (New York, N.Y.): I should like to make a comment which has nothing to do with the content of the paper but with the terminology used. I think it would be very appropriate, unless the authors have contrary information, not to use the term "polysaccharide" but only to talk about carbohydrates or hexuronic acids or whatever sugars and sugar derivatives are being tested for. It is possible that what increases in these diseases consists wholly or partly of polysaccharides, but this has not been proved to my knowledge. In fact, it seems to me that serum mucoid fractions, as well as other mucoids about which we know something, contain not polysaccharides but short multiple branches, which are substituents of polysaccharides. I believe we should keep the terminology simple and not imply more than we know.

DR. HOWARD F. POLLEY (Rochester, Minn.): Were the levels of plasma salicylates determined in these rheumatoid patients, and if so was there any correlation between the levels of salicylates in plasma and the antirheumatic effect, sedimentation rate, or "polysaccharide-protein ratio"?

DR. HERMAN H. TILLIS (Newark, N.J.): What do you mean by the tolerance levels of the salicylates? Was that a symptomatic or laboratory evaluation?

DR. PAYNE: In our experience the sedimentation rate, among acute phase reactant tests, has exhibited relatively poor correlation to the quantitative aspects of rheumatoid arthritis. We also suspect that glucocorticoids often decrease the sedimentation rate considerably in excess of their anti-inflammatory action. Though sedimentation rates are routinely obtained in our clinic we find this data of very little value in a study of the type presented here.

A treatment-resistant rheumatoid patient was recently treated with prednisone 250 mg. every 6 hrs. Though this patient exhibited only a slight clinical response to this medication, she developed a transitory but substantial fall in the protein ratio, which rose again when the prednisone was reduced to the doses ordinarily used.

Serum salicylate concentrations were not followed. I agree that this data would have been of interest in the present study.

By "tolerance" I mean both the number of these rather large pills that our patients will graciously accept, as well as an amount that is relatively free from untoward effect after prolonged administration. The daily doses of medications used in this study are, I believe, not unusual in the long-term treatment of rheumatoid arthritis.

Certainly, studies of this type could be enlarged in several directions. It would be of interest to determine the effect of larger doses of salicylates, particularly those that are better tolerated orally. Other glucocorticoid-salicylate ratios might also be explored.

DR. ALBERT DORFMAN (Chicago, Ill.): If one is to relate various laboratory tests to clinical activity, it is necessary to specifically define the criteria for clinical activity more exactly. Obviously the accuracy of any correlation will be a function of the accuracy of the two measurements that are being correlated. There is at present no definitive evidence that the decrease in sedimentation rate that accompanies hormone treatment accurately measures the effects of the drug on the disease process, but in rheumatic fever at least the correlation seems as good as any other measure of activity.

DR. PAYNE: I believe that the sedimentation rate is a somewhat better index of inflammatory activity in rheumatic fever than in rheumatoid arthritis. However, even here it is a relatively poor acute-phase reactant.

DR. WILLIAM R. MERCHANT (Pittsburgh, Pa): I phrased my original question very poorly. In the very interesting case you told us about, were there any changes in protein polysaccharide?

DR. PAYNE. I have not seen the run on that patient, but it will be of considerable interest.


In the search for a steroid having only anti-inflammatory activity it is important to evaluate not only anti-inflammatory action but potential side-actions as well. Now that whole series of new corticosteroid analogues are being synthesized it is pertinent to inquire whether one can predict the change in biological function which will result from the introduction of a given substitute into the steroid molecule. The present report summarizes the first metabolic studies in man and dogs of seven new synthetic corticosteroids, and extends our knowledge to the effects of:

(a) methyl-substitution;
(b) dehydrogenation;
(c) halogen-substitution within the corticosteroid nucleus.

On the basis of these and previously reported observations, the following generalizations now appear secure:

(i) Methyl substitution in Position 2 greatly potentiates the electrolyte-regulating activity of 11β-hydroxysteroids without appreciably altering ACTH-suppressing, nitrogen-wasting, or eosinopenic activities.

(ii) Dehydrogenation at Positions 1 and 2 potentiates the ACTH-suppressing, nitrogen-wasting, and eosinopenic activities of corticosteroids which possess an 11-oxygen group without increasing electrolyte-regulating activity.

(iii) Halogen substitution in the 9α position potentiates all corticosteroid activities, with electrolyte-regulating activity being increased proportionately more than other activities.

When two or more of the above modifications are combined in the same molecule the effects on biological potency are multiplied. Introduction of the above substituents in other positions may have quite different results biologically. For example, the introduction of a methyl group in Position 6 of prednisolone resulted in no significant potentiation of electrolyte-regulating activity, although methylation at carbon 2 greatly potentiated such activity in all the 11β-hydroxysteroids studied.

Discussion.—DR. JOSEF FRIED (New Brunswick, N.J.): I should like to comment with regard to the 21-fluorinated corticoids, which Dr. Liddle has mentioned briefly.
These compounds possess both glucocorticoid and anti-inflammatory activity, but lack salt retention. Introduction of fluorine into the 9-position and a double bond in the 1,2-position lead to the expected increase in glucocorticoid and anti-inflammatory activity without affecting an increase in salt-retaining activity. In fact, 9α,21-difluoro-21-desoxyhydrocortisone and 9α,21-difluoro-21-desoxyprednisolone are devoid of salt-retaining activity in the rat at 500 μg./animal.

DR. JEROME ROTSTEIN (Washington, D.C.): Slocumb was the first to describe peculiar, non-Cushing-like symptoms in patients who had received high doses of steroids. These observations were confirmed and extended by Rotstein and Good (1957) in their description of the steroid pseudo-rheumatoid state. Smith and Good (1956) were able to produce erythema nodosa-like lesions in a group of children who had received very high doses of prednisone. Bonner and Homburger (1957) recently described toxic dermatitis associated with prednisone therapy. In the treatment of periarteritis nodosa I found that steroids in high doses may have an inflammatory rather than an anti-phlogistic action. It can therefore be predicted that one of the results of these very potent steroids will be bizarre, and perhaps uncontrollable, diseases in the animals and humans to whom they are administered.

DR. LIDDLE: In response to the comments of the first speaker, I should like to say that we have restricted our own studies to dogs and man. It not infrequently happens that species differences occur in the biological effects of steroids, and it will be interesting to see how well the results of assays in rats carry over to man.

It is my feeling that, while it is interesting to perform these exercises of correlating structures and functions of steroids, and while such systematization of our knowledge may serve as guides to future work, the generalizations drawn from such studies should never be applied rigidly. The degree of unpredictability of action of new steroids is still so great that each new compound should be evaluated from several points of view in the hope that eventually we shall find steroids which are truly specific in their activities.

In reply to Dr. Rotstein, I would certainly agree that the bizarre reactions to extremely large doses of steroids which have been described by these various workers are indeed unpredictable. I believe, however, that the discussant is using the word "unpredictable" in quite a different sense from that which I was employing in my efforts to relate the structures of steroids to their more ordinary functions.

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Recent studies suggest that the introduction of additional unsaturation on the C-1 position of hydrocortisone, as well as introduction of other substituents (2-methyl and 9α-fluoro groups) on the steroid molecule, lead to increased biological activity. Prolonged plasma levels of these steroids have been observed after their oral and intravenous administration.

It has been suggested that the more prolonged plasma half-life of steroid analogues is due to a decrease in their rates of metabolism at the cellular level of tissue organization. It is of considerable practical and theoretical importance to be able to determine if steroid analogues exert their increased effects by a continuation of an already existing phenomenon or because of a different mechanism brought about by the introduction of various substituents on the molecule.

Studies were undertaken to determine if elevated plasma levels, as well as increased "glucocorticoid" activities of steroid analogues, could possibly be related to their rates of inactivation in rat liver microsome-supernatant fractions. Rat liver preparations which contained a TPN-2H generating system were used to follow 3α-ketone and C-20 ketone reduction rates. Reduction rates of biologically active steroid analogues were correlated with relative potency ratios in the liver glycerol deposition assay. A close inverse relationship was found to exist between rates of reduction of the 3α-ketone and C-20 ketone positions of highly potent hydrocortisone analogues and their enhanced "gluco-corticoid" activities. Among the hydrocortisone analogues studied were: 2-methylhydrocortisone, 2-methyl-9α-fluorohydrocortisone, 3α-hydrocortisone, 3α,9α-fluorohydrocortisone, 19-norhydrocortisone, 2-methylenehydrocortisone, 6β-fluorohydrocortisone, and 2-methylcortisone.

Discussion.—DR. JOSEF FRIED (New Brunswick, N.J.): How good was the quantitative correlation between the rate of metabolism and glucocorticoid activity? Also was there any correlation between metabolic rates and mineralocorticoid activity?

DR. GLENN: The quantitative relationship is that the faster a steroid hormone is metabolized (in this in vitro system at least) the less activity it has as a glucocorticoid. The less rapidly it is metabolized in the liver, the more active it becomes as a glucocorticoid. That is our general impression. We have by design steered away from attempts to correlate with electrolyte activity and have concentrated our attention primarily on the correlations with glucocorticoid activity, because we feel there is much less known about the manner in which the steroids exert their effect on the electrolyte pattern.

DR. FRIED: I should like to ask how your data can be reconciled with those of Tomkins (1956), who studied the action of the enzymes of a cell-free liver extract on a variety of natural and synthetic corticoids and found that the rate of reduction of ring A proved to be equal for all the steroids studied with the exception of prednisolone, which seemed to be the sole exception, since prednisone fell in line with all the other substrates.

DR. GLENN: We are aware of those studies. Liddle and Richard (1956), and Liddle, Richard, and Tomkins (1957) have also recently shown that 2-methyl-9α-fluorohydrocortisone is metabolized either not at all or extremely slowly in rat liver enzyme systems.
In our particular instance, we are using the microsome-supernatant fractions derived from rat liver. It is a relatively crude enzyme preparation. I do not know specifically just how our enzyme systems vary from those used by Dr. Tomkins. Any in vitro system of this nature is highly artificial. We are adding maximum quantities of reduced triphosphopyridine nucleotides, isocitrate, and glucose-6-phosphate dehydrogenase which contribute to the further reduction of steroids. There is undoubtedly a difference in the manner in which the experiments are conducted.

DR. LEON L. WIESEL (Brooklyn, N. Y.): In the course of investigations on the metabolism of the various synergistic agents to prolong their effects, as a by-product of this work, we studied the inactivation of these substituted steroids by the method of Schneider and Horstmann (1952) using liver slices. We were unable to show, at time-periods of 1, 2, or 3 hours, any difference in the rate of metabolism of the various steroids. I wonder if you would comment on this?

DR. GLENN: There are differences (mainly of a technical nature) in the manner in which slices and homogenates metabolize steroids. There are other problems here and these problems are ones of solubility. Many of these steroids cannot be put into solution with ethanol. However, in our laboratory we have a new steroid solubilizing agent and we know beyond a doubt that any differences in the metabolism which we obtain with these steroid analogues are due to differences in metabolism and not to differences in solubility in the in vitro preparation.

DR. WIESEL: We have been able to demonstrate in vitro that such substances as para-aminobenzoic acid, isonicotinic acid, hydrazide, and antabuse very strongly inhibit the destruction or inactivation of steroids by liver tissue. Does your solubilizing agent possibly work in that fashion?

DR. GLENN: No; that has been checked. It does inhibit to a very slight degree.

DR. RALPH L. DORMAN (Shrewsbury, Mass.): Dr. Glenn has discussed the relationship between the biological activity of a compound and the rate of inactivation. Unless one properly considers the possibility that compounds probably vary in their relative activities merely on the basis of their inherent structure, I believe the issue can become confused. In other words, we may say that in vivo biological activity of a compound is dependent, among other factors, on the activity of the compound per se and on the rate at which the compound is converted to an inactive form. Dr. Glenn has presented very interesting data on the latter point.

DR. GLENN: We have only attempted to correlate our findings with the parent hormone, hydrocortisone. I think we have to realize that, although this in part explains some of the increased biological activity of the steroid derivatives, it does not explain all of them, as was mentioned with regard to electrolyte regulating activity.

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bacterial  β-glucuronidase, although only about half this fraction was detectable with the phenylhydrazine reaction.

Discussion.—Dr. E. Myles Glenn (Kalamazoo, Mich.) I have a point that substantiates Dr. Peterson's result in another area. An analogue 2-methylcortisone in contrast with 2-methylhydrocortisone, is extremely low in biological activity. We find that in a rat liver enzyme system we can cause a conversion of cortisone to hydrocortisone. However, we cannot demonstrate a conversion of the 2-methylated derivative of cortisone to the corresponding 11-hydroxylated derivative. This might help to explain why the 2-methyl analogue of cortisone is inactive, whereas the 2-methyl analogue of hydrocortisone potentiates the activity of the parent hormone.

Dr. Hildegard Wilson (Washington, D.C.): We did some experiments several years ago on the transformation of cortisone and hydrocortisone (E and F) by synovial tissue after intra-articular injection in human subjects. In contrast to Dr. Peterson's findings, we did find a very substantial conversion of E to F by this tissue. These experiments were done by isolation and identification procedures, and have been published (Wilson, Fairbanks, Scialabba, McEwen, and Ziff, 1956). Two patients were studied and, of course, there may be individual differences.

The first interesting point is that in spite of the extensive conversion of E to F in the knee, E is essentially inactive when given intra-articularly. This remains a paradox. Another point is that in the liver E and F are metabolized in large part to the same or closely-related end-products, as judged by the dominant metabolites found in the urine. Synovial tissue, on the other hand, produces metabolites which are mainly entirely different from those formed by the liver, and moreover, the end-products of E and F are quite different from each other.

If one thinks of the liver as being an organ of degradation and excretion of steroid compounds, and of synovial tissue as being a target organ upon which the steroids are exerting their hormonal effect, then these metabolic differences suggest that some specific transformations are involved in the hormonal action.

Dr. John H. Vaughan (Richmond, Va.): In your studies of the rate of breakdown of cortisone, the metabolic products of cortisone, if that is what the phenylhydrazine reaction means, had a half-life of around 50 minutes, as I recall, yet your hydrocortisone, as I understood you to say, had a half-life of around 100 or in one case 120 minutes. Why doesn't this longer component show up in the earlier study of the cortisone breakdown?

The second question relates to the solubility of cortisone. Was the cortisone given as a particulate, or were some means made to solubilize it? Does precipitation in the serum and uptake by the reticulo-endothelial system of the liver play a part in the more rapid rate of disappearance of cortisone than of hydrocortisone?

Dr. Peterson: We are able to give a maximum of 400 mg. hydrocortisone or cortisone intravenously. It is easy to get solution because we first dissolve it in alcohol and make it up to volume with saline.

President Robinson: Was this the free alcohol?

Dr. Peterson: The free alcohol. We cannot give the acetates in solution this way.

When cortisone-4-C14 was infused, a plasma half-life of about 30 minutes was found for true cortisone, whereas the radioactivity had a half-life of about an hour. This we attribute to the fact that the radioactive metabolites convert for the most part of hydrocortisone plus other metabolites. Since hydrocortisone has a slower plasma disappearance rate than cortisone, it is not surprising that the half-life of the radioactive steroids was found to be longer than the half-life of the cortisone.

This same situation applies to the interpretation of the phenylhydrazine colorimetric assay of the plasma steroids after the infusion of cortisone. In normal subjects we have not followed the curve of disappearance of the phenylhydrazine-reacting material for more than 2 or 3 hours; however, if we had followed it for 5 or 6 hours, I would anticipate that it would eventually approach the curve of hydrocortisone. We did do such a study in a patient with cirrhosis of the liver in whom hydrocortisone was known to be slowly metabolized. In this individual, one could demonstrate an initial rapid decline in the concentration of the phenylhydrazine-reacting material in the plasma (half-life≈55 minutes), followed in about 2 hours by a much slower decline (half-life≈300 minutes).

Lawrence E. Shulman (Baltimore, Md.): Can you account for the anti-inflammatory action of cortisone when applied locally to the eye? Has any substance, such as an enzyme, been found in the secretions of the eye that will mediate the conversion of cortisone to hydrocortisone?

Dr. Peterson: I am aware of the fact that according to ophthalmologists, cortisone is effective when given locally in the eye. This is the subject of further study. Perhaps the eye can convert cortisone into hydrocortisone? I am not going on record as saying that cortisone per se is inactive biologically. We have some interesting data in vivo which might suggest that one can account for most, if not all, of the biological activity of cortisone on the basis of that fraction that is converted to hydrocortisone, presumably by the liver. Also, we are unable to demonstrate any conversion of cortisone to hydrocortisone by the synovia of the knee joint.

REFERENCE


A new class of synthetic steroids has been produced which possesses anti-inflammatory and metabolic properties equal to or somewhat better than other compounds currently available. These new compounds are related to highly potent salt-retaining steroids and despite this fact they do not cause sodium or fluid retention. The particular compound tested, 16x-hydroxy-Δ1-9z-fluoro-hydrocortisone, has at an average daily oral dose of

* American Cyanamid Company, CL 19823.
13.5 mg. produced comparable or superior clinical improvement in eighteen patients with rheumatoid arthritis, most of whom were previously maintained on other steroids. Metabolic balance data indicate that therapeutic doses of the new steroid do not cause sodium retention or potassium or magnesium loss. Some degree of suppression of endogenous adrenal function is produced by the new compound as evidenced by diminished urinary ketosteroids. The steroid has been demonstrated to possess life-maintenance properties in a totally adrenal-ectomized patient. With the exception of the development of frank diabetes in a latent diabetic subject, no undesirable effects of any sort have so far been observed in 3 to 21 weeks of continuously successful treatment of patients with rheumatoid arthritis with daily doses of 4-24 mg. of the new steroid.

Discussion.—Dr. Joseph J. Bunin (Bethesda, Md): Dr. Hellman, Dr. Gallagher, and others of the Sloan-Kettering group, and Dr. Freyberg and members of his arthritis clinic are certainly to be congratulated for the very careful and conservative conclusions which they have drawn in this well-planned and meticulously executed study. We have had an opportunity to evaluate this steroid for a very short time in very few patients. It is much too early to draw any conclusions on the basis of our work, but our observations so far have been consistent with those now reported by Dr. Hellman.

The principal question, of course, is whether or not major side-effects will result from the administration of this new steroid. That question will better be answered at the fourth interim scientific session of the American Rheumatism Association in 1957.

Dr. Ralph Jessar (Philadelphia, Pa): Dr. Hollander, who has been ill and is prevented from being here to-day, has asked me to read the following statement concerning our experience with this new synthetic steroid:

"During the past month we have treated six rheuma-
toid arthritis patients with the 2-mg. tablets supplied to us—and I emphasize the tablet because there has been some question whether the capsule form might have been more active than the tablets we received.

All the patients had previously been receiving prednisone or prednisolone, and we switched to the new tablets without comment except for dosage instructions. Two were observed in the hospital; the others, as out-patients, at least at weekly intervals.

"We can confirm, even on the basis of this short period of observation in this small number of patients, that the steroid has antirheumatic effect and no observable indi-
cating side-effects. In clinical potency, however, it appeared at best to be no more effective mg. for mg. than prednisone or prednisolone.

"All our patients lost weight on the steroid. In three, oedema was definitely lessened. All noted some anorexia. Three patients had weakness, exhaustive fatigue which they had not previously noted. The epigastric discomfort which had previously been present in two of our patients on the other steroids persisted on this agent.

"During the past week, three of our six patients have resumed their previous steroid because of some increase in arthritic symptoms and signs, despite a dose of the new steroid as much as one-third larger than the dose of prednisolone."

"We remain to be convinced that this steroid has any practical advantage over previously available compounds. If long-term observation of many patients on this steroid shows a significantly lessened incidence of undesirable side-effects, however, the advantages would be self-evident."

Dr. Charles Ragan (New York, N. Y.): Through the courtesy of Dr. Freyberg, we have received some of this new steroid and have administered it to five patients. In two, the dosage gave a result equivalent to prednisone. In two, prednisolone seemed to be more potent than the new steroid seemed more potent. It is far too early to predict our results with untoward effects. Diuresis of inflammatory oedema with loss of salt and water was observed with the first administration of prednisone.

Dr. John Lansbury (Philadelphia, Pa): This matter of weight loss seems interesting. If the patients were previously taking other steroids, it could be explained on the basis of a correction of a previous salt-and-water retention. However, if the patients were not previously taking other steroids, then the weight loss might represent an increased anti-inflammatory action which caused the liberation and excretion of the water bound by the inflammatory process. We must be certain that the weight loss does not indicate a specific sodium-losing action, since this might induce a serious sodium deficiency on long-continued use.

Dr. Harry Freeman (Worcester, Mass.): A summary of results obtained at the Memorial Hospital of Wor-
cester, Massachusetts, using the same compound, are presented in the Table.

<table>
<thead>
<tr>
<th>Prednisone (Tablets) to Prednisolone (Capsules)</th>
<th>Change</th>
<th>Dosage</th>
<th>Results</th>
<th>Total Cases</th>
<th>Percentage Good Fair</th>
</tr>
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<tr>
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<td>3</td>
<td>4</td>
<td>18</td>
<td>78</td>
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<tr>
<td>Prednisone Fair</td>
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<td>4</td>
<td>6</td>
<td>33</td>
</tr>
<tr>
<td>Prednisone Poor</td>
<td>6</td>
<td>3</td>
<td>4</td>
<td>13</td>
<td>69</td>
</tr>
</tbody>
</table>

All patients in Stage II, III, or IV: Class 2 or 3.

These patients show moderate degrees of pathology and dysfunction. They had been on prednisone for at least 6 months before the start of the experiment, and we knew the maintenance dose of prednisone quite well. We ran a double-blind test, with six patients on the compound and six on the placebo. All six patients on the placebo promptly relapsed within one week, whilst two of the six patients who received the new compound (CL19823) at a dosage level of 30 per cent. of their previous prednisone dose apparently had a satisfactory clinical result during a 2-week period. Thus our results were approximately successful in only one-third of the six cases.

We felt we would have to increase the dosage ratio in comparison with the prednisone dosage and we were also...
concerned about the anxiety of some patients when shifting to a new medication. We therefore set up the following experiment:

We had two batches of capsules which were identical in appearance, one containing 1 mg. prednisone and the other 0.5 mg. of the new steroid. Eighteen patients who had received prednisone steadily for several months were placed one on the prednisone capsule with no change in dosage. Four of them promptly relapsed and this continued until they went back to prednisone tablets; the other fourteen did pretty well—a success ratio, as it were, of 78 per cent. Of these fourteen, we then placed thirteen on Cl19823; they received the same number of capsules daily but the dosage was equal to one-half of that of prednisone, though the patients were unaware that they were being given a different compound. Four of these relapsed, but we demonstrated the clinical effectiveness of the new compound in 69 per cent. of the thirteen cases during a 2-week period.

This study was done with the collaboration of Dr. Ralph I. Dorfman, of the Worcester Foundation for Experimental Biology, Shrewsbury, Mass., and Drs. Samuel Bachrach and Harold H. McGilpin, Jr., at the Arthritis Clinic, Memorial Hospital, Worcester, Mass.

Dr. Charles M. Plotz (Brooklyn, N.Y.): Was there any change in the eosinophils in the patient who developed a rash while on this drug?

Dr. Woodrow Kessler (New Brunswick, N.J.): It is interesting that diabetes has been precipitated in certain patients. I wonder whether Dr. Hellman has any information on the glomerular infiltration rate of some of these patients or on the mechanism by which the insensible loss is seen in sodium and water.

Dr. Charley J. Smyth (Denver, Colo.): I am moved to cast the spell of extreme scepticism upon an apparently euphoric audience after hearing about yet another new steroid substance. We are continually hearing of a new one and then a few months later of another new one, and we never quite get back to the old one to learn what the old ones have done to the patients and to the disease.

Our friends, the British, are somewhat more sceptical about steroid therapy in rheumatoid arthritis than the Americans. We should not lose sight of the fact that they conducted a multi-centre, carefully controlled study of rheumatoid arthritis, comparing cortisone with aspirin for one year, and came up with statistical evidence there was no difference between aspirin and cortisone.

It is high time that those in a position to make long-term observations should report at meetings of this sort what has happened to patients treated with dear old cortisone and hydrocortisone. From all the clinical evidence that I am familiar with, we still do not know what is happening to the underlying disease process as a result of steroid therapy. Until such evidence as this is available, we are just grasping at new straws.

I feel strongly that those of us who are sincerely trying to evaluate therapy should exert a tremendous effort to try to answer the following question: What are the steroids doing to the fundamental disease process; not the symptoms, and not the short-term anti-inflammatory process? What happens to the patient a year or two years later? We might even go so far as our friends in the area of cancer research and ask what the results are even after 5 years of steroid therapy.

Dr. Hellman: We have not made any measurements of glomerular filtration rate or renal blood flow, and cannot give an explanation for the sodium-and-water loss observed during the first few days of administration of this compound. Our working hypothesis is that there may be at least two mechanisms for the retention of sodium; one perhaps depends on a steroid for its action and the other may not. During the first few days of administration, the new compound may serve as a biological antagonist to some endogenously produced substance. The administered steroid may cause suppression of a salt-containing compound which is normally produced by the endogenous adrenal-cortical secretion. During the first few days sodium loss may then be observed, but afterwards other homeostatic mechanisms come into play to abolish the sodium-losing properties.

The totally adrenalectomized patient is not solely dependent on steroid for the reabsorption of sodium. The administered steroid does not act as an antagonist and the patient's life is maintained by virtue of the other corticoid properties of the steroid.

These observations have been confirmed in adrenalectomized animals by the American Cyanamid group.

The weight loss is most marked in patients who have been on previous steroid therapy; but it also occurred in some patients who had never been treated with steroid therapy. I suspect that some portion of the weight loss may be related to some degree of fluid and electrolyte retention caused by the previous steroid therapy and that this is abolished by the use of the new steroid.

With respect to the remarks of other investigators who have used this compound for relatively short periods of time and us together, we have had no small numbers of patients, and it is likely that among them some will do very well, some will have average benefit, and some will do poorly. Only with the passage of time will the actual merits of the compound be evaluated.

I was wondering, Dr. Robinson, if you would ask Dr. Freyberg if he has any concluding remarks.

Dr. Richard H. Freyberg (New York, N.Y.): First, I wish to express the appreciation of Dr. Bernsten and myself for having been asked to collaborate with the investigators of the Sloan-Kettering Institute in the clinical investigation of this new class of steroids. The metabolism studies of the new steroid revealed properties that were considered to be quite favourable, and our drug was then developed to determine if its anti-inflammatory and antirheumatic effects it might possess. The early studies indicated that it possessed a definite antirheumatic effect, and attention was directed to its evaluation in prolonged use in patients with rheumatoid arthritis.

One important difficulty was that there was a very small amount of material with which to work. The method employed during the latter portion of these studies was abruptly to substitute the new steroid for that which the patient had been receiving after we had arrived at an accurate appreciation of the effect of the first drug. No effort was made to improve the patient, but we tried to determine how much of the new steroid would be needed to keep the patient at essentially the same stage of partial suppression of the rheumatoid process. The judgment was based solely upon clinical evaluation and one must appreciate that although this method may indicate whether a new agent has definite antirheumatic value, it is not a method that has great precision. This new steroid was found to have an antirheumatic effectiveness approximately equivalent to that of prednisone or hydrocortisone (in some of the earlier studies it seemed that a slightly greater effect was obtained with an equivalent dosage).
The amount of the steroid required is not in itself important. What is important, particularly in patients with rheumatoid arthritis, is the ratio of anti-rheumatic properties to the potential for undesirable side-effects. We are particularly concerned with this aspect of the problem and, stimulated by the results of these early studies, we believe it is important to determine whether some of the troubles that plague the physician and the patient during the prolonged administration of any steroid may be diminished.


Fluoridation of Public Water Supplies and its Relationship to Musculo-Skeletal Diseases. By Charles Leroy Steinberg, Dwight E. Gardner, Frank A. Smith, and Harold C. Hodge, Rochester, N.Y.


Clinical Significance of the Staining of Lipoprotein by Schiff’s Leucofuchsin-Sulphurous Acid, with particular reference to Myeloma. By William Q. Wolfson, Fort Riley, Kansas.