AMERICAN RHEUMATISM ASSOCIATION

PROCEEDINGS OF THE FIRST INTERIM SCIENTIFIC SESSION

Dr. Edward Boland, President of the American Rheumatism Association, took the chair at the opening meeting of the first interim scientific session, held on November 4, 1954, at the Clinical Center, National Institutes of Health, Bethesda, Maryland.


*Triturus viridescens viridescens*, a urodele amphibian possessing the ability to regenerate limbs, was used in this study. After amputation, daily intramuscular injections of cortisone acetate were administered. Three groups were tested: one receiving 0.005 mg., one 0.037 mg., and the control none.

In those animals receiving 0.005 mg., a definite delay in wound healing was noted. Mitotic counts of epidermal and connective tissue cells revealed that cortisone inhibits wound healing by decreasing the rate of mitosis of both tissues. After 6 days, the animals adjusted to this dosage. A compensatory increase in the mitotic activity of epidermis and connective tissue followed. After 12 days, the experimental animals had completely overcome the initial inhibitory effect and regenerated at the same rate as the controls.

The most striking results were observed in those animals receiving 0.037 mg. daily. Gross morphological observations revealed a gradual degeneration of epidermis, dermis, muscle, connective tissue, and nerves in a proximal direction beginning at the amputation surface. This degeneration began on the 6th day and continued for a period of 14 days. The radius and ulna were the only elements left in the lower arm of some specimens. These bones were attached to the protruding humerus head by tendon remnants.

A complete histological study of these specimens is now being undertaken. This extensive degeneration appears to be due to an uncontrolled phagocytosis by those elements which usually leave the wound site after the inflammation reaction subsides.

Discussion.—Dr. WILLIAM H. KAMMERER (New York, N.Y.): I am afraid I missed it if the Doctor stated the weight of the experimental animal. I wonder if he could correlate or compare the dosage of cortisone used in this experimental animal with that used in man.

Dr. MANNER: We had that in mind when we set up the dosage of cortisone for these experimental animals, which was done on a weight proportion basis. The 0.0005 mg. is comparable to a 25-mg. dosage daily in a man weighing 120 lb.

Dr. HANS WAINE (Boston, Mass.): I think it is particularly gratifying that Dr. Manner has again directed fundamental research to a group of animals with interesting articular structures. Lubosh (1910) called attention to the fact that certain of these amphibians throughout life, instead of the typical articulation that is found in the higher species, have synchondrotic joints. In other words, a rather primitive type of articular cartilage makes up the joint for the total adult life of the animal. For that reason, it appears that these animals would constitute a good model for studying the effects of various influences on differentiation of joint tissues in normal and abnormal conditions. Has Dr. Manner observed any effect on the articulations?

Dr. MANNER: In a recently published report on the normal histology of regeneration we took that fact into account. In the adult animals the entire epiphyseal end of long bones is cartilaginous in nature. The fibroblasts which accumulate in the blastoma end—I use the term “fibroblasts” to denote an embryonal mesenchymal cell as distinct from a fibrocyte; some histology books use the term synonymously—are capable of differentiating into chondroblasts, which then become the chondrocytes of the adult cartilage. This is approximately the sequence that occurs in forming the new articular ends.

Dr. CHARLES RAGAN (New York, N.Y.): What happens to regeneration in the salamanders when he “stresses” them, and is the whole process of regeneration dependent upon environmental temperature; do injections alone of an inoculant vehicle modify regeneration?

Dr. MANNER: As to the effect of the environmental temperature upon regeneration, we find in most metabolic effects a direct correlation between rate and temperature: if the temperature is increased (and we tried it one summer where the running water in which these animals are kept reached a temperature of approximately 70°), the rate but not the quality of regeneration differs.

When we first started on the animals we injected the control animals as well as the experimental animals. The control animals were injected with a salamander isotonic salt solution, a mixture of various salts closely approaching the isotonic concentration of salts in the animal’s tissue fluids. There appeared to be no effect upon the regeneration even though the animals were injected daily with this solution.

A “stress” situation arose where the animals were injected daily, as we used a size 18 needle and the tail is rather small.

Electrophoretic Analysis of Normal and Arthritic Synovial Fluid. By DAVID PLATT, WARD PIGMAN, K. LEMONE YIELDING, and HOWARD L. HOLLEY, Birmingham, Ala.

Electrophoretic analysis of normal synovial fluid has been carried out for the first time. The electrophoretic...
patterns of normal synovial fluid differ significantly from those reported for traumatic and arthritic synovial fluid. An important difference was the presence in five of eight normal fluids of two components whose relative mobilities were faster than that of the albumin fraction. Previously, two rapid-moving components had been detected occasionally in the electrophoretic patterns reported for pathological fluids. To distinguish the second component from the previously known hyaluronic acid, it is proposed that it be known as the $\pi$ component.

The relative mobilities of the components of normal synovial fluid differ from those of serum obtained from the same individuals and from arthritic fluid and serum. The relative mobilities of the arthritic fluids are similar to those reported by Perlmann, Ropes, Kaufman, and Bauer for traumatic fluid. The A/G ratio in the normal fluids was greater than that of the arthritic fluids.

Arthritic fluids from individuals at the acute and recovery stages were analysed and compared. Upon improvement of symptoms, the relative electrophoretic mobilities of the alpha-two and beta globulins increased. A decrease in the relative mobility of the hyaluronic acid also occurred upon clinical improvement. Other components showed a less marked effect. As might be expected, the relative mobilities approached those of the normal fluids as a result of the treatment. The electrophoretic analyses of synovial fluid were carried out using the Perkin-Elmer Model 38 Tiselius electrophoresis apparatus. The buffer used in this investigation was a veronal buffer, pH 8.6, ionic strength 0.1. The relative mobilities were determined by comparing the mobilities of the various components with that of the albumin fraction.

**Discussion.**—DR. MARIAN W. ROPES (Boston, Mass.): We have for years been hoping to find a component that moved with a mobility between that of hyaluronic acid and albumin. We have seen one such peak and one questionable peak in abnormal fluids. We hoped to find it because many of us think there may be, in vivo, a combination between hyaluronic acid and albumin, and we wonder whether this component will turn out to be such a combination.

In the matter of difference in mobilities in normal and abnormal fluids, we have been much disturbed; as Dr. Platt suggests, we should be interested in knowing whether the mobilities in normal fluid remain unchanged in fluid treated with hyaluronidase. We have found the mobilities in viscous fluids are not reliable, and that adding hyaluronidase to fluid or serum does not alter mobility or distribution of protein components; I think it is possible, therefore, that when hyaluronidase is added to normal fluid, the mobilities of normal fluids may approach those of all other fluids and of serum. We know that when we originally studied viscous fluid without hyaluronidase, we had variations in mobilities.

I think also it would be interesting to know the absolute mobilities rather than the relative ones, because of possible variations, although the results are so consistent that probably the relative mobilities are representative.

I find the so-called "$\pi$ component" of extreme interest.

DR. PLATT: One of the difficulties in determining absolute mobilities in normal fluid components is the rather limited amount of the sample available for analysis. Generally we are quite fortunate if we obtain enough fluid for one sample. If conductivity measurements on the sample were carried out, there might not be a sufficient amount remaining for the electrophoretic run.

One other point. Perhaps we can also ascertain the mobilities by running them on the paper electrophoresis apparatus and checking them with the known absolute mobilities of some of the fluid we have analysed with the Tiselius apparatus.

DR. MORRIS ZIFF (New York, N.Y.): In 1940 Dr. Chargoff and I added heparin to plasma and observed a little peak migrating ahead of the albumin complex. It was very much like this $\pi$ component, and I wonder whether this component is not a complex between albumin and hyaluronic acid.

DR. PLATT: It might be so. We do not know the chemical nature of these materials and are hoping to get some leads by use of the paper electrophoresis apparatus wherein we can use a fairly small sample for analysis.

DR. KARL MEYER (New York, N.Y.): I feel that this is an interaction of hyaluronic acid with one of the protein components. Dr. Ragan and I have investigated chemically quite a number of pathological synovial fluids and we never saw any chemical difference between the latter and normal fluids, in our case only, the synovial fluid. Cattle synovial fluid may not be quite normal, because of oedema in animals which have been transported.

What disturbs me more is the difference in mobilities between the hyaluronic acids. Theoretically, of course, a hyaluronic acid having only one carboxyl group in the repeating unit should have the same mobility regardless of its degree of polymerization, if the viscosity is taken into consideration; I wonder whether that has been done because in these highly viscous fluids, of course, the viscosity cannot be disregarded as it can in serum.

The formula used for calculating mobilities is of some significance here. There is, however, one possibility which occurs to me, and that is that in the normal fluids, parallel to the higher degree of polymerization of hyaluronic acid, the carboxyl groups are masked in contrast to the depolymerized fluids in which the carboxyl groups are unmasked and active. There is a possibility that might explain this really enormous difference between the relative mobilities which Dr. Platt has shown here.

DR. PLATT: Since a high viscosity is a property of normal synovial fluid, to destroy or greatly decrease the property by artificial means, such as hyaluronidase, would then make the normal fluids resemble the arthritic fluids. The viscosity is lowered to some extent by the ascending cell of the electrophoresis apparatus, since the components move through the buffer. The possibility exists that the $\pi$ component might be an instrumental anomaly, but since it appears in both the ascending and descending portions of the cell, the possibility of its being an anomaly seems remote.

**Effect of Hydrocortisone on Radioactive Sulphur Uptake in Cartilage.**—BY CHARLES W. DENKO, DELBERT M. BERGENSTAL, AND ALLAN T. KENYON, Chicago, Ill.

Previous studies on the fixation of radioactive sulphur-35, $^{35}S^\text{S}$, into tissues have demonstrated its organic binding into connective tissue, especially cartilage. It is incorporated largely into sulphate-bearing compounds.
i.e. chondroitin sulphate. Growth hormone stimulates this process markedly in hypophysectomized rats.

The influence of hydrocortisone on sulphur uptake was investigated similarly, using young female rats. The synthetic activity of tissues in binding S35 was studied in varied states influenced by hydrocortisone, growth hormone, and combinations of hydrocortisone and growth hormone. Hormones were injected intraperitoneally daily for 4 days as was radio-sulphur. After the animals were killed tissues were removed, and ashed, sulphur precipitated as barium sulphate, and radioactivity determined as a measure of S35 fixation.

Hydrocortisone administration resulted in a decrease in concentration of S35 in the cartilage, xiphoid, costal, and articular tibial (as the tibial cap) of hypophysectomized rat. Growth also usually decreased. Growth hormone administration counteracted depressing influence of hydrocortisone; conversely, hydrocortisone inhibited growth-hormone in stimulating S35 fixation.

Discussion.—DR. THEODORE B. BAYLES (Boston, Mass.): Some years ago there emanated from the Rockefeller Institute a report on the effect of thyroid function on the incorporation of radioactive sulphur into the costal cartilage of growing rats, showing that if thiouracil was given to these animals there was a marked diminution in radioactive sulphur uptake, which could be reversed or corrected by the administration of thyroxin.

I do not wish to discuss whether cortisone depresses thyroid function or not, but I wondered if this factor was considered in these experiments.

DR. DENKO: We felt that the thyroid function might be important in the sulphur fixation of these animals. Therefore, we carefully did our first series of investigations on the effect of thyrotropin on these tissues. We found that thyrotropin had no stimulatory or inhibitory effect that we could note in our experimental procedure.

We also ran a few animals using thyroxin, and found that we could demonstrate a noticeable effect of thyroxin, either, so we felt that thyro, thyrotropin, and thyroxin had an appreciable influence in sulphur fixation in our experimental animals.

Therefore, we felt that whatever effect we got was directly due to hydrocortisone. Furthermore, the previous studies demonstrated that in vitro or tissue culture work showed that cortisone would inhibit the sulphur uptake and I feel somehow that the thyroid function would not enter into such a procedure.

Mucoprotein of Cartilage. By MAXWELL SCHUBERT, and JENNIE SHATTON, New York, N. Y.

From cartilage a material has been isolated which behaves as a single substance, a compound of chondroitin sulphate and protein, that is, a mucoprotein. It is soluble in water and gives viscous solutions. Precipitated from solution as different salts it shows a constant composition. It can be distinguished from mixtures of protein and chondroitin sulphate in three different ways. First, the protein of the mucoprotein cannot be precipitated by any of the usual protein precipitants. Second, the polysaccharide and protein are simultaneously adsorbed to kaolin and so the polysaccharide of the mucoprotein can be completely removed from solution by repeated treatment with kaolin. This is not true of free chondroitin sulphate.

Third, the mucoprotein does not pass through a glass bacterial filter while free chondroitin sulphate passes through readily. The electrophoretic mobility of the mucoprotein appears to be identical with that of chondroitin sulphate. The mucoprotein in solution gives strongly metachromatic colours with appropriate dyes. The mucoprotein is susceptible to attack by two different enzymes, hyaluronidase and trypsin. Not more than half the chondroitin sulphate of cartilage could be extracted in the form of this mucoprotein.

How the other half is bound is not yet clear. The existence of such a mucoprotein in connective tissue raises other possible interpretations of what is meant by depolymerization of ground substance, such as are thought to occur in rheumatic disease.

Relation of Mucoprotein, Mucopolysaccharides, and Collagen Formation. By THOMAS G. KANTOR, and MAXWELL SCHUBERT, New York, N. Y.

Chronic inflammations ending in dense scar formation are characteristic in the pathology of many arthritic conditions. Controlled foreign body reactions in subcutaneous tissue afford an opportunity to study the genesis of dense scar tissue histologically and chemically. Quartz-induced granulomata were studied histologically in the subcutaneous tissue of rabbits and the time of reticulin and collagen formation determined. Histochemical tests were used to establish the presence of mucoproteins or mucopolysaccharides.

In addition, chemical studies of the same material have been performed at three stages during the development of these lesions. Collagenous tissue, hexosamine, and water content were all determined, and an attempt was made to isolate and characterize the polysaccharides.

Preliminary results indicate that non-hexosamine containing polysaccharides is present during the stage of reticulin formation. Hexosamine-containing polysaccharides are present at all stages, but particularly as the lesion matures and collagen is actively laid down.

Discussion.—DR. WARD PIGMAN (University of Alabama): We know that these mucopolysaccharides and mucoproteins may be quite soluble in water. It has been one of the difficulties in histological examinations of tissues that one must avoid extraction of such materials during the staining procedure. Dr. McManus and Dr. Mowry, at our school, have worked out a procedure which they applied particularly to protecting dextran in tissue, and this procedure involves using aqueous alcohol rather than aqueous pyruv stain.

I wonder if you have gone into that at all here, because as you have already indicated, many of these materials are somewhat soluble. Has there been an attempt to avoid solubilization during the staining procedure, with resultant loss of materials?

DR. KANTOR: My feeling is that the 10-minute procedure of periodate oxidation is probably not enough to get out too much in the way of soluble polysaccharides. We know from other staining methods (such as metachromicity and the Hale procedure) that they take extensive washings at times to diminish the staining intensity of these other stains. It may be true, however, that the Schiff stain depends on a more soluble polysaccharide.

DR. PIGMAN: In your fixing procedure is there any possible loss of these materials at those times?
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DR. KANTOR: The fixing procedure mainly is that of Carnoy, which is followed by strong alcohol dehydration. No water is used at all and we hope that Carnoy, which is a mucoprotein precipitant, will maintain the position of all the polysaccharides in the tissue.

DR. PIGMAN: These tissues contain water-soluble fractions, and generally most of these are not picked up in the usual procedures.

Further Studies on Mucopolysaccharides. By KARL MEYER, EUGENE A. DAVIDSON, ALFRED LINKER, and PHILIP HOFFMAN, New York, N. Y.

The mucopolysaccharides occur in mammalian connective tissues in complex patterns typical of the tissues of their origin. Since last year two new mucopolysaccharides have been isolated, keratosulphate and chondroitin. Keratosulphate is composed of equimolar concentrations of N-acetylglucosamine, galactose, and sulphate; its structure is still unknown. Chondroitin, composed of N-acetylgalactosamine and glucuronic acid, resembles hyaluronic acid in many of its physical and chemical properties. Both form highly viscous solutions and in both the hexosaminic bonds are hydrolysed by testicular and bacterial hyaluronidases at comparable rates. The bacterial hyaluronidases yield from both substrates 4-5 unsaturated hexuronides. The repeating deacetylated disaccharide of chondroitin was isolated in a yield of 75 per cent. and crystallized. Its infra red absorption spectrum was identical with that of the disaccharide chrosin obtained from chondroitin sulphate A of cartilage and C of umbilical cord.

Studies of chondroitin sulphate B (which occurs in skin, heart valves, tendon and large blood vessels) revealed further differences from the other chondroitin sulphates. Besides its higher optical rotation and resistance to testicular hyaluronidase, it showed a markedly faster rate of acid hydrolysis than either A or C. One mole of CO₂ per repeating unit was liberated by decarbxylation, whereas the carbazole reaction for uronic acids gave only half the expected value. On acid hydrolysis, it yielded a disaccharide with infra red spectrum different from that of chondrosin. It thus shows little relation to the chondroitin sulphates, apart from its analytical values. The biological implications are being studied.

Agglutination and Inhibition by Serum Globulin in the Sensitized Sheep Cell Agglutination Reaction in Rheumatoid Arthritis. By MORRIS ZIPF, PATRICIA BROWN, JACQUES BADIN, and CURRIER MCEWEN, New York, N. Y.

A naturally occurring inhibitor of the sensitized sheep cell agglutination reaction has been demonstrated in the globulin fraction of normal and pathological sera. Precipitation of the euglobulin fraction of rheumatoid serum effected partial removal of this inhibitor from the agglutinating factor, and increased the sensitivity of the agglutination reaction. The modified Rose test performed on the euglobulin fraction was compared with the modified Rose and Heller agglutination tests, using whole serum, and the modified Rose test on the cold globulin fraction in a group of patients with rheumatoid arthritis and in control individuals. The highest percentage of positive tests was obtained with the euglobulin fraction, and with a decrease in the percentage of false positive reactions.

The capacity of the euglobulin fraction of rheumatoid and non-rheumatoid sera to inhibit the agglutination of sensitized sheep cells by known positive test sera was also studied. It was found that non-rheumatoid euglobulin fractions inhibited the agglutination of sensitized cells by positive test sera, but that rheumatoid euglobulin did not. Specifically, 95 per cent. of non-rheumatoid euglobulin fractions thus far investigated inhibited agglutination in a significant titre, while rheumatoid euglobulin fractions failed to agglutinate in all cases. This provides the basis for a sensitive test.

The difference in solubility of the agglutinating factors in rheumatoid arthritis and the false positive reaction in lupus erythematosus was observed.

Discussion.—DR. JOSEPH J. BUNIM (Bethesda, Md.): Dr. Ziff and his associates are to be congratulated for an ingenious modification of the agglutination reaction which now enhances its diagnostic possibilities and usefulness. We have had the privilege of applying this modification during the past 3 months here at the Institute, and have found it most useful. There was a group of fourteen patients with rheumatoid arthritis who had a consistently negative sheep cell agglutination by the Heller technique. When retested by the modified technique of Ziff and his associates, eight of those fourteen were converted from negative to positive.

We also had one particularly impressive case of a child with Still's disease, whom we were able to observe in the 6th week of his illness, and who had a definitely positive test by the euglobulin modification.

We have also found the test to be negative in spiro-dylis, and have been able to convert a positive reaction in a rheumatoid patient to a negative one by the intramuscular injection of human gamma globulin.

DR. WILLIAM KAMMERER (New York, N. Y.): When I first started work with Dr. Cecil some years ago, the "old" streptococcus agglutination test was in favour and I learnt from him about its merits, demerits, and possible implications. I developed an interest in these biological phenomena, and was happy some 4 or 5 years ago when I was able to continue my interest in a small way in my association with Dr. George Heller at the Veterans Hospital, Bronx, N.Y., who has devoted himself to research in this field.

After Dr. Heller had made a modification of the original Rose test, which appeared to be an improvement, his chief interest focused on finding in what fraction of sera was the reactor responsible for this agglutination phenomenon. Naturally, his attention turned to the globulin fraction of serum and he demonstrated that the haemagglutination test for rheumatoid arthritis can be inhibited by including Plasma Fraction 2 as a reagent. Agglutination did occur with tannic acid erythrocytes sensitized with Fraction 2.

Nevertheless, the number of positive reactions with known cases of rheumatoid arthritis did not increase as he had hoped it would by using the tannic acid erythrocytes sensitized with Fraction 2 above the level that we obtained with whole serum, which was about 7% to 8% per cent.

He studied the other fractions of globulin that he had available and found that there was no inhibition when
Fraction I, IV-1, IV-4, and V, but, with Fraction III some degree of inhibition was obtained.

Regarding euglobulin, which is sort of a hodge-podge, which can be prepared by several different methods of salting, the interesting question is, what fraction of euglobulin is responsible for this phenomenon, particularly since the individual globulin fractions, as studied by Heller, did not enhance the sensitivity of the test.

I should like to know whether euglobulin prepared by other methods of salting have been used, and, if so, whether the results were any different?

Lastly, I should like to add my congratulations to those of Dr. Bunim, because I think that Dr. Ziff and his co-workers have furnished us with a diagnostic tool that may be very useful and helpful.

Dr. Ziff: I am not sure whether Heller, who has made excellent contributions in this field for many years, has actually tested directly with purified globulin fractions.

Dr. Kammerer: Yes, he has done it directly with purified globulin fractions as prepared by Cohn's cold ethanol method.

Dr. Ziff: Against tannic acid-treated cells, not against amboceptor-sensitized cells?

Dr. Kammerer: Against both.

Dr. Ziff: We have tested the euglobulin precipitate by the method of Dr. Nana Svartz of Sweden. We did not get as high a percentage of positives as she obtained in patients with rheumatoid arthritis, but no doubt this is because we are sensitizing differently and using different criteria for a positive test. I think the results depend upon the separation at least in part of the agglutinator from the inhibitor substance. No doubt we do not remove all the inhibitor, but sufficient appears to be removed, so that the percentage of positives goes up, and false positives down.

The test is somewhat more complicated. The dialysis procedure takes several days.

Dr. RALPH H. BOOTS (New York, N.Y.): It seems to me that, despite the numerous agglutination tests which have been devised, the most useful diagnostic and laboratory procedure for differentiation of rheumatoid arthritis is the degree of depression of the sedimentation rate. As you will recall, this was first described in this country over 20 years ago by Dr. Dawson and myself; the method which we used was the Westergren modification of the Fahraeus technique. Since that time, there have been many modifications, and all of them seem to complicate the diagnostic procedure rather than to help it. It may be a different story with agglutination phenomena. Dr. Ziff's method of the sensitized sheep cell agglutination seems to be a distinct contribution and renders this type of test of more value.

The first agglutination phenomenon, the haemolytic streptococcus agglutination, was first introduced by Cecil, Nichols, and Stamsby to show the relationship between haemolytic streptococci and rheumatoid arthritis and not as a diagnostic procedure. It was only later that it was used for diagnosis.

Dr. Dawson and I found that its great disadvantage was that very few patients gave a positive test in the first year of the disease. Later, after 4 or 5 years, the percentage of positives would run as high as 80 per cent. In the late stages of the disease the diagnosis is so easy to make from clinical signs that the test, as we used it, was of relatively little value.

Dr. Ziff stated the number of positive agglutinations in relation to the duration of the disease; I should like to know whether he gets more positive agglutinations in patients with early rheumatoid arthritis than we obtained with the streptococcus agglutination test.

Dr. Ziff: The inhibition procedure is based on the ability of the euglobulin fraction to inhibit known positive serum. We have not found a patient with rheumatoid arthritis, even early, whose euglobulin fraction was able to inhibit a known positive serum. We assume that this means that there is sufficient agglutination in this fraction from rheumatoid serum to neutralize the inhibitor. It proves to be a more sensitive way of demonstrating the agglutination.

Dr. CHARLES RAGAN (New York, N.Y.): Do your controls include any patients with rheumatic fever?

Dr. Ziff: There were fourteen with rheumatic fever.

Dr. HOWARD C. COGGESHALL (Washington, D.C.): We found quite a number of patients with sensitive reactions to penicillin and sulphur and various serum had positive tests. I wonder if you have studied this aspect?

Dr. Ziff: The one case of serum sickness was negative.

Dr. CURRIER McEwen: I have one comment on one of Dr. Boot's remarks. No one would want to depreciate the usefulness of the erythrocyte sedimentation rate or a test as that for C-reactive protein, but it remains a non-specific test and in no way selective, and do not distinguish between rheumatic fever, rheumatoid arthritis, lupus, etc. Therefore, it would be clinically very useful to have a test which is more selective for rheumatoid arthritis.

We do not believe that this modification of the sheep erythrocyte agglutination test has yet been brought to a point where it can be used with complete confidence diagnostically. When one is testing the diagnostic value of a new procedure, one uses it first on clinically clear-cut cases. We hope that it will prove useful in early cases not yet clinically diagnosable, but we do not want to be very emphatic about that yet. It will take much more observation and many more patients—especially earlier cases and those giving false positive results—before one can be sure of the reliability of the test.

Dr. RUSSELL L. CECIL (New York, N.Y.): I think all these agglutination reactions have suffered from being a little too complicated for practical use in routine diagnosis. I wonder if Dr. Ziff could tell us just how practical his test would be for use in ordinary clinical laboratories. If it were not too complicated and there were not too many pitfalls, it would be a valuable addition to our diagnostic armamentarium.

Dr. Ziff: The inhibition test, I think, is difficult to do as a routine, because it is in a sense two tests. The direct test for agglutination, in which we found 92 per cent. positives, is more sensitive than the whole serum test and would be well worth doing. As it requires dialysis for serum of 2 days, it takes 2 days longer to get the answer.

Uricosuric Effect of Phenylbutazone. By E. R. Huffman, C. J. Smyth, George M. Wilson, Jr. (by invitation), and Robert Hill. (by invitation), Denver, Colo.

The investigation of the effect of oral phenylbutazone at various blood levels upon the serum and urinary uric acid in ten gouty and eight non-gouty arthritics. A step-wise fall in serum uric acid to normal occurred in all but two of the gouty patients. After a
butazone as a complementary or antagonistic effect in uric acid metabolism.

In both studies, the cumulative amounts of extra uric acid appearing in urine during drug ingestion and the decrease in urinary urate excretion are quite limited. However, the greater amount of uric acid catabolized or disposed of by non-renal pathways, and the increase in uric acid secretion, as compared to the control period, is of considerable importance. The increase in the rate of uric acid synthesis is not limited to the renal pathway, but is also apparent in the extra-renal metabolism of uric acid.

Dr. Wilson: I agree that the uricosuric effect of this drug probably bears no relation to its remarkably dramatic effect in the acute gouty patient. We studied two patients during an acute attack of gout, who received 100 or 200 mg. of phenylbutazone every 2 hrs; we did not observe any increase in urate excretion, and the level of uric acid did not decrease.

Dr. Wilson: This is the most important aspect of the problem. In our experience, it requires 600 mg. phenylbutazone daily to attain a consistent blood level above 10 mg. per cent., the level at which significant uricosuria occurs. This is a rather large dose; in a small series of cases that we have analysed we were unable to maintain patients for a long period on this dosage without incurring toxic side-effects requiring discontinuance of the drug. We believe, therefore, that Benemid is the drug of choice for uricosuria therapy.

Discussion.—Dr. John Steinbrocker (New York, N.Y.): I enjoyed hearing this careful and significant discussion of phenylbutazone excretion. We are not yet able to perform such experiments, but the results are clear and in line with our own studies. We have not been able to demonstrate an increase of urate excretion during the administration of phenylbutazone. One of four patients showed a questionable increase; this patient was found to have increased urate excretion and three showed none.

We think that the careful work just described must indicate that there is something about the technique of looking for these phenomena that evaded us. As many workers have found increased urate excretion with phenylbutazone as have found none.

Dr. Wilson: I think it is significant that the drug causes salt and water retention, and that with large doses it is difficult to demonstrate any uricosuria.

Dr. John Norrie Swanson (Toronto, Ont., Canada): I should like to support Dr. Wilson's contention that phenylbutazone is uricosuric. In two patients that we studied in our investigation unit on a standard diet, we, too, found that the output of uric acid in the urine was increased while taking this drug. Our study was very short, being only 4 days on each occasion, and I should like to ask whether Dr. Wilson found that this effect is maintained. In other words, is phenylbutazone an alternative to Benemid? Or is it better than Benemid or aspirin? Has he any data to show that if it is combined with either of these two drugs, its effect is complementary or antagonistic?

Dr. Wilson: This is the most important aspect of the problem. In our experience, it requires 600 mg. phenylbutazone daily to attain a consistent blood level above 10 mg. per cent., the level at which significant uricosuria occurs. This is a rather large dose; in a small series of cases that we have analysed we were unable to maintain patients for a long period on this dosage without incurring toxic side-effects requiring discontinuance of the drug. We believe, therefore, that Benemid is the drug of choice for uricosuria therapy.

Dr. James Wyngaarden (Bethesda, Md.): We have been interested in the effect of phenylbutazone on uric acid metabolism, since the early reports that it caused a decrease in serum urate concentration without a corresponding increase in urinary urate. We studied this problem in two normal subjects on a low purine, low protein diet. Analyses for uric acid were made by a specific enzymatic method (differential spectrophotometric method of Kalckar modified by Praetorius).

Tests were conducted with N\(^5\)-uric acid during control periods and again after several days of phenylbutazone, 800 mg. per day. The variations of the miscible urate pool incident to drug ingestion were compared with rises in urine excretion. The rates of turnover of urate were compared before and during drug ingestion. Catabolism and extrarenal disposal of urates were estimated from the fraction of the turnover failing to appear in urine, and also from the fraction of the test dose of N\(^5\)-uric acid failing to appear in urine. Finally, uric acid spaces were calculated for all experiments.

In both subjects the cumulative amounts of extra uric acid appearing in urine during drug ingestion agreed within 10 or 15 per cent. with the degrees of contraction of the miscible pool, so that it appears that the renal action of phenylbutazone explained the observed changes in serum urate levels.

There were no appreciable changes in the rates of uric acid synthesis. There were slight decreases in the amount of uric acid catabolized or disposed of by non-renal pathways, and 20 to 35 per cent. increases in uric acid space between the control and later experiments, attributable I believe to fluid retention.

On the basis of these studies in two normal subjects, it would appear that phenylbutazone has only one important effect on uric acid metabolism—namely, the enhancement of urinary urate excretion.

Dr. William C. Kuzell (San Francisco, Calif.): Our results correspond more or less with those of Dr. Steinbrocker and his associates. We have been impressed by the fact that the anti-inflammatory effect of phenylbutazone does not depend on such large doses as are necessary to produce uricosuria. Furthermore, the use of quite small maintenance doses of phenylbutazone decreases the frequency of acute exacerbations without decreasing the serum uric acid. In studying patients for equally long periods before the administration of any drug, we frequently observed that they put out large amounts of urate in a cyclical fashion.

Dr. Wilson: I agree that the uricosuric effect of this drug probably bears no relation to its remarkably dramatic effect in the acute gouty patient. We studied two patients during an acute attack of gout, who received 100 or 200 mg. of phenylbutazone every 2 hrs; we did not observe any increase in urate excretion, and the level of uric acid did not decrease.

Uricolysis in the Normal and Gouty Human. By E. Bick, and M. Zucker, Long Island, N.Y.

The lack of uricase in humans has been used as evidence that uric acid is not degraded. Wyngaarden and Stetten have recently reported a demonstration of uricolytic activity in human serum by the method of Kuzell and Grodsky.
demonstrated that the nitrogen of uric acid labelled with \( ^{15}N \), when injected into a normal human is in part excreted in the urinary urea and ammonia. Marked alteration in the intestinal flora by sulphanamide therapy did not diminish the extent of degradation. Additional evidence for uricosis is the uric acid "deficit" observed by Benedict and her co-workers in their studies on the uric acid turn-over and pool. Tuttle and Cohen demonstrated oxidation or uric acid by a peroxidase, and Margoles and Griffiths found similar results with a cytochrome—cytochrome oxidase system.

Whole blood contains both of the above enzyme systems. The rate of uricosis in whole blood was determined. Since leucocytes contain large amounts of peroxidase, and erythrocytes have both peroxidative and cytochrome activity, the formed elements were separated and incubated with plasma. Addition of urate, such that the plasma of normal individuals was at hyperuricaemic levels, did not change the rate of uricosis. Repeat determinations at weekly intervals showed only minor variations. The rate of uricosis was determined in hospitalized individuals without hyperuricaemia or joint symptoms. In the normal individuals, there was no difference in the rate of uricosis between males and females. Eight determinations on seven gouty individuals showed that the addition of colchicine had no effect on the rate of uricosis. A diminished rate of uricosis was observed in whole blood and with both white and red cells in patients with gout. There was no correlation between the degree of hyperuricaemia and the rate of uricosis. The results were as follows:

| Subjects | Number of Determinations | Per cent. Plasma Mean Fall (per cent.) in Plasma Urate Incubated with Whole Blood White Blood Cells Red Blood Cells |
|----------|--------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Normal Gout | 25                      | 2-3                             | 34-4                            | 39-0                            | 29-8                            |

**Discussion.**—DR. L. MAXWELL LOCKIE (Buffalo, N. Y.): Was freezing used in any of these studies?

**DR. BIEN:** There was no freezing. The blood specimens were obtained and the experiment was started usually within an hour.

**Comparative Pathology of Experimental Arthritis due to Formalin, Blood, Immediate and Delayed-Type Hypersensitivity Reactions.** By D. BOKING and F. S. BRENN, London, Ontario, Canada.

In these experiments, the intra-articular lesions of immediate-type (anaphylactic) and delayed-type (bacterial) hypersensitivity in rabbits were compared. The immediate-type reaction was studied using egg albumen in rabbits actively sensitized. The delayed-type reactions were produced with old tuberculin (O.T.) injected intra-articularly in B.C.G.-sensitized rabbits. In addition, lesions produced by intra-articular injection of homologous blood and formalin were studied. In each group, the injections ranged from one to ten at 48-hr intervals.

The immediate-type hypersensitivity reaction resulting from a single injection of egg albumen consisted mainly of a polymorphonuclear exudate, maximal at 6 hrs. The delayed-type hypersensitivity reaction, with a single injection of O.T., consisted primarily of a synovioctic and subintimal fixed-cell proliferation, with lymphocytic and polymorphonuclear infiltration, and superficial "fibrinoid" deposition—maximal at 48 hrs or later. Repeated intra-articular injections of egg albumen or O.T. in normal and sensitized rabbits produced a proliferative synovitis characterized by fibrinous exudate and infiltrate on the surface, synovioctic and subintimal fixed-cell proliferation, and lymphocytic infiltration. In addition to these changes, repeated intra-articular injections of O.T. in sensitized rabbits caused an early pannus formation, subchondral bone invasion, and lymphoid collections.

Lesions resulting from formalin were characterized by extensive necrosis of cartilage, and subsequently, synovial membrane and adjacent bone, with relatively slight inflammatory reaction. It did not resemble the proliferative synovitis of rheumatoid arthritis.

Repeated intra-articular injections of homologous blood resulted in a mild proliferative synovitis.

Delayed-type hypersensitivity reactions simulated the histological appearances of rheumatoid arthritis.

**Sternoclavicular Articulation in Rheumatic Diseases.** By LEON SOKOLOFF and I. O. GLEASON, Bethesda, Md.

The sternoclavicular articulation is affected by various types of rheumatic disorder with greater frequency than is recognized clinically. This is illustrated by several examples of rheumatoid arthritis, gout, and infectious synovitis. The normal joint and the degenerative changes occurring with age were shown to distinguish them from the specific lesions of rheumatoid arthritis. Examination of this structure is recommended when other polyarthritis joints are dissected at necropsy.

**Review of the Published Data on a Syndrome resembling Rheumatoid Arthritis, Disseminated Lupus, and possibly other Collagen Diseases, induced by Hydralazine and other Hypotensive Agents.** By JOHN Lansbury and Fred B. Rogers, Philadelphia, Pa.

The occurrence during hydralazine administration of syndromes closely resembling those of rheumatoid arthritis and disseminated lupus erythematosus is an event of major importance. It seems that for the first time we have been able to induce a clinical picture closely resembling two or more connective-tissue diseases. Thus the mechanism of production of the same symptoms in spontaneous rheumatic disease may be discovered.

These artificially produced syndromes are not identical with the spontaneously occurring diseases. The drug-induced syndrome may not apply to both rheumatoid arthritis and disseminated lupus, but may represent gradations of lupoid disease alone. The high incidence (7.5 per cent.) of the drug-induced syndrome makes it unlikely that we are seeing here simply the precipitation of a latent rheumatoid or lupoid stage. We cannot dismiss these findings with a statement that they are
simply a “drug reaction”. The important thing is that parallel mechanisms seem to be involved in both the drug-induced and spontaneously occurring syndromes. Allergy, disturbed liver function, and blockade of the nervous pathways and blood-pressure-regulating mechanism may play a part in this process.

The pulmonary fibrosis occurring in hexamethonium administration, which histologically resembles sclerosis of the lungs, may also have some relevance.

Discussion.—DR. VIRGINIA P. BEELAR (Washington, D.C.): I reported a case in November, 1953, in which symptoms very typical of rheumatoid arthritis developed in a patient receiving doses of less than 100 mg. per day. Subsequently her sister also developed a similar syndrome on approximately 200 mg. a day. I do not feel therefore that excessive dosage is entirely the explanation.

DR. LANSBURY: I am very glad to have this information. One other patient in whom the syndrome did occur on rather small dosage turned out to have lupus when the records were examined.

DR. BEELAR: I followed this patient carefully during the last year, and her sedimentation rate has continued elevated without apparent explanation. She has had no further rheumatic symptoms of any sort.

DR. HILARY H. HOLMES (New York, N.Y.): Is there any chemical method for determining hydralazine in the urine or blood?

DR. LANSBURY: Hydralazine can be determined in the urine, but it is most unlikely that any substance resembling hydralazine will be found to occur naturally in the body.

DR. CHARLES L. SHORT (Boston, Mass.): This is an important subject, in that it represents the first experimental production of a syndrome resembling rheumatoid arthritis or disseminated lupus.

I should like to confirm the apparent antagonism between severe hypertension and rheumatoid arthritis. We are not yet sure that rheumatoids are apt to show hypotension or to have, in a large series, lower blood pressures than controls. But thus far, in a series numbering 600-800 rheumatoid patients, we have not encountered anyone who has died from severe hypertension at an early age. We have found this situation in two patients with rheumatoid spondylitis, but not in any with peripheral rheumatoid arthritis. I think this suggests that there is an antagonism between the two conditions.

I should also like to ask whether the rauwolfia compounds have produced any symptoms of a syndrome resembling this one. Just before I left Boston I had been seeing a spondylitic who had a moderate hypertension. He was being treated with rauwolfia and just when his diastolic pressure came down to a satisfactory level, a severe iritis developed.

DR. LANSBURY: I don’t know whether it is in the literature, but it is quite common knowledge that the administration of rauwolfia and its preparations to hypertensives may be associated with muscle pains. Whether this has any meaning in the rheumatological sense or not I don’t know, but it is an interesting point.

DR. WILLIAM C. KUZELL (San Francisco, Calif.): We approached this problem in a somewhat different way: in studying the ratio of the reduced to the oxidized glutathione in the blood of a great many subjects with a variety of rheumatic diseases, we found that the group comprising the most severe rheumatoid patients had the lowest ratios. The total blood glutathione was normal but reduced glutathione in the blood was decreased.

These rheumatoid people were generally also hypertensive. When they were treated in various ways and improved clinically the glutathione ratio rose. In trying to correlate the rise in the glutathione ratio with all other measurements, the only one which paralleled the rise in the glutathione ratio was the rise in the blood pressure.

The therapeutic agents which seemed to cause the change most regularly were cortisone and corticotropic.

Use of Combined Therapy and Rebound Suppression of the Treatment of Rheumatic Fever. By EDWARD FISCHER and CHARLES W. FRANK, New York, N.Y.

In most instances of rheumatic fever, salicylate and adrenocortical hormones appear to be equally effective in suppressing signs of activity. In severe cases, the combined use of both agents may offer some advantage, perhaps of a synergistic nature, in effecting rapid defervescence of inflammation. Toxicity of the hormones may be partially avoided by early withdrawal. The severity of the anticipated rebound can be substantially diminished by continued administration of salicylate.

Day-to-day changes in the severity of rheumatic carditis are difficult to evaluate. The presence of other suggestive or local signs may be without significance, reflects the possibility of an associated rheumatic carditis. Studies of the clinical evidences of rheumatic activity in conjunction with determinations of the E.S.R., serum complement, and C-reactive protein were done to aid the detection of inflammatory reaction.

In general it appears advisable to institute therapy which effects early, rapid, and prolonged suppression of rheumatic inflammation. Drug toxicity and the hazards of discontinuing therapy should be minimal.

Discussion.—DR. CURRIER MCEWEN (New York, N.Y.): Dr. Fischel has done us all a service in recalling attention to the possible usefulness of salicylates in the treatment of rheumatic fever beyond the antisympathetic and antipyretic effect.

Probably no one has been more insistent than I in the past that salicylates are useless so far as any benefit in carditis is concerned. The evidence to-day does not prove that the impression which most of us have had for many years is wrong. Dr. Fischel has been a lone voice in the wilderness for a number of years, and, largely through his work, we now have some reason to believe that the salicylates may have some beneficial effect in carditis. Certainly, the evidence is now overwhelming that the salicylate effect is not mediated through the adrenocortical mechanism, and if that is the case, it seems to me there is justification for using salicylates as well as cortisone or ACTH in rheumatic carditis.

It is difficult to judge, to-day, whether any of these agents plays any very pronounced role in controlling carditis; but in the present unfortunate state of uncertainty, I believe it is reasonable to suggest that a patient with carditis deserves the benefit of both hormone and aspirin therapy if one wants to do the best one can on the basis of the meagre information available.

Enzymatic Metabolism of Corticosteroids. By KURT ISSELBACHER and GORDON TOMKINS, Bethesda, Md. (Introduced by Joseph J. Bumim.)

The major pathway of adrenal steroid inactivation by...
results in the formation of the corresponding tetrahydro derivatives. This involves the addition of four hydrogens to the ketone and double bond of ring A. These reactions occur primarily in the liver, as previously demonstrated by perfusion and slice experiments. However, in such preparations, where cellular structure is maintained, it is not possible to make a detailed study of the enzymes and coenzymes involved.

By means of extracts free of intact cells, we have demonstrated that an enzyme system capable of converting cortisone to tetrahydrocortisone resides in the granule-free cytoplasm of the rat liver. The cofactors for these reactions are the reduced forms of the diand triphosphopyridine nucleotides. In addition, it has been observed that the enzyme involved in the reduction of cortisone is different from that concerned in the inactivation of hydrocortisone. These enzymes have been separated.

Preliminary Clinical Trials with 9-Alpha-Fluoro Hydrocortisone Acetate in Rheumatoid Arthritis. By Edward W. Boland and Nathan E. Headley, Los Angeles, Calif.

A halogenated derivative of hydrocortisone—9-alpha-fluoro hydrocortisone acetate—was administered as investigative therapy to thirteen patients with active rheumatoid arthritis. Seven patients received the preparation as initial medication; three of these were transferred later to hydrocortisone (free alcohol) for comparisons of dosage requirements. Six patients, being maintained on established daily amounts of hydrocortisone (free alcohol), were transferred directly to the fluoro compound and comparisons of the doses needed for similar degrees of rheumatic control were made.

The following conclusions were drawn:

1. Weight for weight, the antirheumatic potency of 9-alpha-fluoro hydrocortisone acetate was found to be much greater than that of the parent compound, hydrocortisone. This was indicated by the following:
   a. Initial suppressive doses of the fluoro derivative ranging from 3 to 8 mg. a day were sufficient, in five of seven patients, to promote rapid improvement in the rheumatic manifestations.
   b. Comparisons of maintenance dosage requirements for approximately equivalent degrees of clinical control revealed that, in eight of nine patients, the antirheumatic power of 9-alpha-fluoro hydrocortisone acetate, milligram for milligram, was roughly ten times that of hydrocortisone (free alcohol).

2. With the small total daily amounts of the fluoro compound employed, signs of fluid retention developed in twelve of the thirteen patients, being pronounced in some. This suggested that the substitution of a fluorine atom at the ninth carbon position increased the electrolyte activity of hydrocortisone to an extent proportionately greater than it enhanced its antiphlogistic property. The excessive salt-and-water retaining effect of the fluoro derivative would seem to preclude its practical application in systemic therapy.

3. The observations with 9-alpha-fluoro hydrocortisone acetate are of interest chiefly because they demonstrate that the anti-inflammatory potency of hydrocortisone may be enhanced, and other of its properties modified, by altering its formula. This raises hope that, through changes in structure or other chemical substitutions, a more successful therapeutic agent for rheumatoid arthritis may be produced in the future.


Aldosterone was administered intramuscularly for 6 days to two patients who had rheumatoid arthritis. Total daily doses were 200, 250, 400, 800, and 280 μg. in one patient; 800, 800, 800, 600, 1,000, and 640 μg. in the second. No antirheumatic effect could be detected clinically after the use of these doses in these patients.

Retention of fluid and sodium chloride occurred. Detailed studies, including studies of metabolic balance, were made in one patient. The doses used in this preliminary study were considerably larger than those reported to be effective in studies on patients with Addison's disease. However, the range of doses necessary for production of antirheumatic effects, if any, may not have been reached by the doses used in this study. When supplies of aldosterone permit, larger doses will be employed for further study of antirheumatic, anti-inflammatory, and other effects. The compound, 9-alpha-fluoro hydrocortisone acetate, administered orally to a group of rheumatoid patients in total doses of 4 to 8 mg. daily produced cortisone-like antirheumatic effects. These same doses also produced potent metabolic activities, particularly in regard to retention of sodium chloride and fluid, and excretion of potassium.

Discussion.—Dr. Clark (Denver, Colo.): We have treated nine patients who had previously been regulated on cortisone dosage varying from 50 to 67 mg. per day with doses of 9-alpha-fluoro hydrocortisone acetate ranging between 3 and 4 mg. per day. All except one of our patients developed signs of toxicity which necessitated discontinuing the drug. Eight gained weight up to 10 lb. and seven of them developed moderate to severe oedema. Five developed hypertension. One showed a systolic blood pressure rising to 210 and developed albuminuria; neither symptom disappeared until 2 weeks after discontinuing the drug.

Three patients spontaneously reported a distressingly severe nasty taste in the mouth, with scum forming on the teeth, and were very belligerent about taking the drug.

In four patients who developed insomnia and five who had severe headaches the drug was discontinued.

The one patient who showed no large weight gain and no toxic symptoms received potassium supplements in his diet, 0·6 g. three times a day; he has been on the drug since the middle of July and is still taking it with no apparent ill effects. He was taking 67 mg. cortisone daily and is now well regulated on 3 mg. 9-alpha-fluoro hydrocortisone acetate.

We did balance studies on two of our patients, and, as far as their sodium and potassium were concerned, both showed a marked fall in sodium excretion on a controlled intake of sodium and potassium, the excretion...
fell from 120 to 130 milli-equivalents per day to 6 milli-
equivalents per day in one, and 9 in the other.

Our observations are almost exactly the same as
Dr. Boland has reported, and agree with Dr. Ward's
finding regarding the electrolyte balance.

DR. THEODORE B. BAYLES (Boston, Mass.): I do not
feel that 9-alpha-fluoro hydrocortisone, or perhaps any
other type of cortisone, is the answer to rheumatoid
arthritis, but in our experience, running as long as
30 days on individual patients, we saw very little evidence
of hypertension or weight gain.

We studied five hospitalized patients on low sodium
diets of 1 g. or less and were receiving 0-6 g. potassium
chloride three times daily. The dosage ranged from
4-16 mg. daily for 10 days. The 9-alpha-fluoro hydro-
cortisone was given orally every 6 hours. This does not
mean that I feel this rigid programme is desirable, but
it does seem that with this programme one can carry
patients for a fairly long time with a fairly good dosage.

I agree that its antiphlogistic effect is about seven
times that of hydrocortisone.

DR. BOLAND: I did not wish to infer that it is impossible
to continue 9-alpha-fluoro hydrocortisone acetate
administration in some patients. We have treated three
patients for uninterrupted periods of 4 to 5 months,
but they showed moderate rigidity in blood pressure and
one showed varying degrees of oedema. All the patients
in the reported series, save one, were treated on an
ambulatory basis, and adhered to qualitative, not
quantitative, restriction of sodium in their diets.

Considering the frequency of unwanted side-reactions,
particularly fluid retention and blood pressure elevation,
no patient in the reported series was better controlled
with 9-alpha-fluoro hydrocortisone acetate than with
hydrocortisone (free alcohol), even though much larger
doses of the latter were required. For this reason
9-alpha-fluoro hydrocortisone acetate is decidedly
inferior to hydrocortisone as a therapeutic agent and in
our opinion it would not be practical for general use.

We have also administered 9-alpha-fluoro hydro-
cortisone (free alcohol) orally, and the results in patients
with rheumatoid arthritis are roughly the same as with
the acetate ester.

The importance of these findings with 9-alpha-fluoro
hydrocortisone is the demonstration that the anti-
inflammatory property of hydrocortisone may be
enhanced by a change in its formula. It was obvious
from various studies, including our own investigations
(which compared the effects of cortisone, hydrocortisone,
and their several esters) that some of the physiological
actions of hydrocortisone could be modified through
alterations in its chemical structure. It was surprising
to us, however, that the anti-inflammatory activity could
actually be made greater than that of the natural hor-
mone. This observation is significant because it implies
that through further alterations in the steroid nucleus
a compound may be contrived which possesses wide
dissociation between wanted anti-inflammatory action
and certain other physiological effects which we now look
upon as unwanted endocrine or metabolic complications.

DR. JOSEPH L. HOLLANDER (Philadelphia, Pa.): Our
group has had an opportunity to study 9-alpha-fluoro
hydrocortisone acetate injected intra-articularly in 35
cases of rheumatoid arthritis. We noted a local an-
tiphlogistic effect clinically from as little as 3 mg. by intra-
articular injection, but in most cases 5 mg. was necessary.

The effect of this dose in no case exceeded that of
37.5 mg. hydrocortisone acetate in anti-inflammatory
potency or in duration of effect.

Frequently, when the dose was increased to 7.5 mg.
into one knee, or 5 mg. into each knee, marked oedema
of the leg or legs developed and persisted for several
days. This is probably our most interesting observation:
that we could produce local oedema in the leg injected
without giving systemic cortisone.

Our findings thus confirm that the markedly increased
potency of this analogue, but the increased salt-retention
effect appears to nullify this advantage so far as intra-
articular injection is concerned.

DR. JOHN W. SIGLER (Detroit, Mich.): We have used
9-alpha-fluoro hydrocortisone intra-articularly in two
patients. In one of these, recurrent peptic ulcer pain
was noted after a 5-mg. injection into the knee joint.

Preliminary Observations on the Anti-rheumatic Potential
and Metabolic Effects, and Hormonal Properties
of Metacortandralone and Metacortadracin.

By JOSEPH J. BUNIM, MAURICE M. PECHET, and ALFRED
J. BOLLET, Bethesda, Md.

Metacortandralone is a crystalline synthetic steroid
possessing the physiological activity of an adrenocortical
hormone. A decrease of more than 50 per cent. in the
circulating eosinophils and a reduction in the urinary ketosteroids occurs 1 or 2 days after its oral administration.

Metacortandralone is an effective anti-rheumatic agent.
Each of the indices of objective joint changes (swelling,
tenderness, warmth, pain on motion, and range of motion) was significantly, rapidly, and consistently improved. Subjective improvement, both articular and in general, was striking and was greater than objective improvement. Metacortandralone is an anti-inflamma-
tory agent. Histological examination of synovial biopsies taken before and during its administration clearly demonstrated a marked subsidence of inflammation. The erythrocyte sedimentation rate and C-reactive protein were restored to normal (or near normal) in every case studied. The sensitized sheep cell reaction of the serum was not significantly altered.

Metacortandralone is approximately three to four
times more potent than cortisone and two to three times
more potent than hydrocortisone. Preliminary observa-
tions indicate that this enhanced potency is not accompa-
nied by a proportionate increase in the frequency or severity of undesirable side-effects. This new sterol
therefore possesses an augmented therapeutic ratio.

The maintenance dose varies with the severity of the
arthritis and has ranged from 5 to 25 mg. daily.

Haemopoietic stimulation resulted from the adminis-
tration of metacortadracin. In several patients, signifi-
cant increases occurred in the haemoglobin, haematocrit,
haemoglobin, red and white blood cells, and neutrophils.

The total cholesterol but not the cholesterol-ester
increased as the steroid was administered.

Balance studies on two patients with rheumatoid ar-thritis—one male aged 16 and one female aged 32—
demonstrated that the daily oral administration of
30 mg. metacortadracin for twelve successive days
causd no sodium retention and no loss of potassium or
nitrogen. When the daily dose was increased from
30 to 50 mg. (in the male patient) for 24 successive days, a negative nitrogen balance, consisting of a loss of 1·8 g. nitrogen daily, developed on and after the tenth day. The serum albumin fraction increased by an average of 0·7 g. per cent., and the serum globulin fraction decreased by an average of 0·9 g. per cent. The fasting blood sugar revealed no significant increase during metacortandralone administration. No patient developed glycosuria.

The undesirable effects thus far observed have been minor in nature and degree and often disappeared as the dose was decreased. These consisted of hirsutism, faint facial rounding, acne-form eruption, increased perspiration, increased appetite, transitory epigastric discomfort, fatigue, weakness, sleeplessness, and transitory mental depression, but not hypertension.

When the steroid was discontinued or the dose reduced below the minimal level, signs and symptoms of arthritis returned within a few days.

Metacortandracin, a member of the same family of synthetic crystalline steroids, appears to possess hormonal properties, antirheumatic effectiveness and potency, and a therapeutic ratio like those of metacortandralone.

This is a preliminary report based on short-term observation of only seven cases of rheumatoid arthritis, and we do not yet know what effects, favourable or unfavourable, will result from prolonged administration.

CHAIRMAN KAMMERER: I think it would certainly be no exaggeration to say that Dr. Bunim's work may well be the most exciting therapeutic event that we have heard of since May of 1950. His work is so new that it probably isn't appropriate to ask for discussion, but we will entertain any questions that may come from the floor.

DR. CHARLES RAGAN (New York, N.Y.): We are becoming aware of the changes in effects which may be brought about by minor changes in the chemical structure of these various steroids. Can Dr. Bunim tell us anything about the structure of metacortandracin?

DR. BUNIM: I do not know the chemical structure of this steroid, and I am told by the manufacturers (Schering) that they do not yet know it either.

DR. RAGAN: How was it found to be anti-inflammatory? Was it found in screening a bunch of steroids on a laboratory shelf?

DR. BUNIM: The research team of Schering Corporation have done a number of experiments on animals, and have found that these steroids possess the capacity to reduce the eosinophil count and to cause thymic involution. It was, therefore, their interest or curiosity which led them to inquire whether this substance might not also possess anti-inflammatory, anti-rheumatic capacities, and we were very glad to try to determine that for them by tests with human subjects.

DR. CHARLEY J. SMYTH (Denver, Colo.): I think we have witnessed a paper not unlike that of Hench, Kendall, Slocumb, and Polley in May, 1949; the date August 3, 1954, may also become an epoch in medical history. But since 1949, those of us who are interested in rheumatoid arthritis have been particularly concerned about the long-term results. Dr. Bunim has stressed, I think very properly, the fact that these are short-term studies. We have now followed in our clinic four patients for over 3½ years. We have seen patients with relatively early rheumatoid arthritis maintained continuously, and have seen the progression of rheumatoid arthritis manifested by involvement of additional joints, development of subcutaneous nodules, and progressive joint destruction. It is to be emphasized that these changes have occurred in spite of intensive, carefully controlled long-term hormone administration.

Our current feeling (and I am sure it is shared by many in this room) is that so far hormonal therapy in rheumatoid arthritis has not altered the ultimate progress of the pathological process of the disease, although the patients may have symptomatic relief and be much more comfortable, and able to lead a more productive life.

We should keep the long-term view in mind, because rheumatoid arthritis is often extremely protracted.

DR. BUNIM: At the Heart Institute in this Clinical Center, the effects of this drug on nephrosis was studied in two patients, and one of them developed a complication which I think ought to be discussed. A 29-year-old male had had nephrosis for 9 years and was becoming progressively worse. In January, 1954, he was treated with cortisone and then with hydrocortisone. Unusually large doses were used and he was receiving 300 mg. daily. When no favourable effect, or only slight improvement, was observed, the drug was discontinued after having been administered for 4 months. There was a hiatus of 3 months, in which he received no steroids. He was then put on metacortandralone, first in doses of 40 mg. a day for a period of 16 days and then of 70 mg., which is higher than the dosage we have used. He remained on this dosage for 10 days, and it was tapered during the next 4 days, and finally discontinued.

Five days after the last dose was given, the patient passed a tarry stool. He continued to bleed from the gastro-intestinal tract; finally he was operated upon and an ulcer was found in the posterior wall of the stomach, close to the pyloric sphincter. In the floor of this ulcer was a branch of the gastro-epiploic artery. A gastric resection was done and the patient recovered.

How much the antecedent steroid therapy of cortisone and hydrocortisone and the metacortandralone contributed to this ulcer, we do not know. The patient had no symptoms, and an x ray of the stomach and duodenum was negative 2 weeks before the tarry stool was noted.

PAPERS PRESENTED BY TITLE ONLY

Hormonal Control of Amino Acid Metabolism. By VICTOR H. AUERBACH, Boston, Mass.

Effect of Salt on the Biophysical Characteristics of Sodium Hyaluronate. By BARUCH BLUMBERG and KARL MEYER, New York, N.Y.

Clinical Experience with Phenylbutazone. By CHARLES W. DENKO, DAVID RUML, and DELBERT M. BERGENSTAL, Chicago, Ill.

Chemical Fractionation of a Type-6 Strain of Group A Haemolytic Streptococcus. By J. WILFRED HAHN and BETTY HARGIS, Chicago, Ill.

Adenosine Mono- and Di-nucleotide in the Pathogenesis of Rheumatoid Arthritis. By GREGORY HAYDU, New York, N.Y.

Flocculation Tests in Rheumatoid Arthritis. By JACQUES HOUII, Rio de Janeiro, Brazil.

Human Skin Collagen from Different Age Groups before and after Collagenase Digestion. An Electron Microscopic Study.* By MADELINE K. KEECH, New Haven, Conn.

* See this issue, p. 19.
Psychic and Physical Findings in the Fibromyalgias. By J. H. Irvine, New York, N.Y.


Rheumatoid Heart Disease associated with Rheumatoid Spondylitis. By Charles LeRoy Steinberg, Rochester, N.Y.

FUTURE ARRANGEMENTS

The annual general meeting, 1955, will be held on June 3 and 4, at Atlantic City, immediately before the meeting of the American Medical Association.*

In 1957 the annual meeting will be supplanted by the IX International Congress of the Ligue Internationale contre le Rhumatisme, which will take place on June 23-28 at Toronto, Canada.

It has been decided to replace the Annual Directory of the American Rheumatism Association for 1955 by the forthcoming edition of the Year-Book of the Ligue Internationale (Secretary: Dr. Richard T. Smith). This is the official directory of the international organization and will include the names and addresses of members and officers, and particulars of the committees, etc., of the American Rheumatism Association, as well as those of all other affiliated rheumatological societies.

An American Journal of Rheumatology.—A committee of the A.R.A. to explore the desirability and feasibility of establishing a new journal of rheumatology, with Dr. Joseph Hollander as Chairman, was set up by approval of the Executive Committee in 1953. This Committee reported to the Executive Committee in June, 1954, and the latter decided that the time was not yet ripe for such an undertaking, that further study and consideration would be forthcoming, and that a subsequent report should be made to the members concerning this proposed journal. Dr. Hollander’s committee prepared such a report on the pros and cons of the matter with the co-operation of Dr. Philip Hench. At the Executive Committee meeting in November, 1954, it was decided to poll the membership to obtain the reaction to the creation of such a journal.

LIGUE EUROPÉENNE CONTRE LE RHUMATISME

The Third European Rheumatology Congress will be held in Scheveningen, The Hague, from June 13-17, 1955. To enable definite arrangements to be made, those intending to attend the Congress are particularly requested to apply for membership and hotel accommodation as soon as possible.

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The Congress fees should be paid through the intermediary of a Bank to the Amsterdamsche Bank N.V., The Hague, in favour of the Treasurer of the Third European Rheumatology Congress, before May 1, 1955. After this date the fees will be raised.

Scientific Programme

Section 1. Rheumatic Fever

Prof. Dr. F. Coste (France): Le traitement de la maladie de Bouillad.
Dr. P. Van der Mer (Netherlands): Frequency of rheumatic heart disease in school children and its consequences, a study of the Rotterdam primary school population.
Dr. Gene H. Stollerman (U.S.A.): Prophylaxis of rheumatic fever.

Section 2. Connective Tissue