HEBERDEN SOCIETY

At the Annual General Meeting held on November 12 and 13, 1964, at the Wellcome Foundation, London, the following papers were presented:

Diagnosis and Treatment of the Costoclavicular Syndrome. By A. A. Wouda, G. G. Berging, L. Penning, and F. Homann-v.d.-Heide (Groningen): Certain positions and occupations may help to cause compression of the neurovascular bundle in the shoulder region. This can be demonstrated by clinical manoeuvres (Adson-, costoclavicular-, alternate costoclavicular-, and hyper-abduction tests). Murmurs may be heard in suprav- and infra-clavicular regions and stenosis curves registered. The blood flow may be registered in various positions by putting Light Dependent Resistance elements on both thumbs (functional plethysmogram). Angiography in normal and obstruction positions localised compression.

If exercises fail to widen narrow costoclavicular space, exploration of the shoulder girdle region with scalenotomy is necessary. If no improvement is obtained, the first rib should be removed from behind (posto-lateral incision); this approach yields no risks of lesions to the plexus, etc. Post-operatively patients are free of complaints, and tests and functional plethysmogram are normal.

Discussion.—Prof. J. H. Kellgren (Manchester): I am glad to draw attention to this rather rare but important syndrome, because many years ago in 1930 Lewis described some patients who had this nipping of the artery. At a later stage they get emboli filling up the distal vessels. I have done a few of these excisions of the first rib and would agree the results are good.

Prof. E. G. L. Bywaters (Taplow): This may sometimes cause gangrene of the digits.

Dr. W. H. D. de Haas (Amsterdam): As forced bracing of the shoulders is a well-known manoeuvre in first-aid to stop arterial bleeding, I should like to ask whether you did any angiographic investigations in normal controls?

Dr. A. Wouda: No.

Dr. J. T. Scott (London): We faced this problem when doing brachial arteriograms in patients with rheumatoid disease to study their peripheral circulation. It is difficult to justify these procedures in normal subjects. In our own studies we have gone on to use autopsy material, but I doubt if this would be possible here.

Dr. J. Cosh (Bath): The recordings from the finger pulse varied in different positions of the arm. I take it you accepted it as abnormal only if the pulse disappeared entirely, though in some of your recordings the pulse excursion was reduced. Did you regard this as normal?

Dr. Wouda: Then we call the test negative. It is only positive if it is flattened.

Dr. J. Cosh: You studied the form of the curve as well as its excursion?

Dr. Wouda: Yes. Actually the up-stroke goes up very quickly. If the form changes, the up-stroke goes slowly before it disappears. This would suggest of course that there is a little obstruction, but I think the patients had no difficulties.

Prof. E. G. L. Bywaters: Prof. Kellgren has said that this is comparatively rare. It probably is in this advanced form, but I think that minor costo-clavicular obstruction must be really quite common in middle-aged people. It might be possible to get some information on angiographic appearances in people who are studied for cardiac purposes.

The Temporomandibular Joint in Adult Rheumatoid Arthritis. By Mr. A. S. T. Franks (Eastman Dental Hospital, London) and Dr. B. G. Watkins (Arthur Stanley Institute, Middlesex Hospital): A clinical and radiographical study has been carried out on the temporomandibular joint and dental health of 100 patients with rheumatoid arthritis and the results compared with control groups. 80 per cent. of the patients had either clinical or radiographical evidence of temporomandibular joint disorder, but only 53 per cent. gave a history of such a complaint. Of the patients with natural teeth (51 per cent. of the total), 75 per cent. had unreplaced missing teeth and 65 per cent. unbalanced loss. These figures did not differ significantly from the control series. However, 56 per cent. of the patients had radiological signs of temporomandibular change, compared with a negative result in the control group. 69 per cent. of the patients with radiological evidence of temporomandibular joint arthritis were unilateral chewers. This figure was significant compared with the control series. 91 per cent. of the patients with radiological changes had more than eight other small joints of the body affected by arthritis and in none was only the temporomandibular joint involved. There was a significant tendency for radiological signs to be present in those patients with more than three large joints of the body affected and with a Grade III or worse functional incapacity. There was a significant increase in incidence of radiological signs of affected temporomandibular joints as the general functional incapacity became worse. The results of the study suggest that the appearance of arthritis in the temporomandibular joint may be related to an uneven distribution of function between right and left joints and that this joint is affected later than other small joints.

Discussion.—Prof. E. G. L. Bywaters (Taplow): Is there any congruence between unilateral chewing and unilateral temporomandibular joint involvement?
Mr. Franks: No. The actual incidence of unilateral chewing in those patients with disease was 79 per cent. However, of the total, 43 per cent. had unilateral disease. An interesting feature was that the side affected did not seem to be related to the side used in chewing. The joint involvement might be ipsilateral or contralateral to the side on which the patient chewed. Some experimental work has been carried out in the U.S.A. on rats which had one side of the occlusal table mutilated. It was found that the pathological response of the condyle was the same in both joints even though there was only unilateral interference with the occlusion. In the present study there did not appear to be any relationship between the side of joint disorder and the side used for chewing.

Observer Variance and Prevalence of Rheumatoid Arthritis (RA) and Osteo-arthritis (OA) in a Longitudinal Population Study in The Netherlands. By Hans A. Valkenburg: During a period of 1 year 200 people, selected at random from the population of three villages in the south of the Netherlands, were seen at monthly intervals and examined for the presence of RA and OA. All were aged 40 years and over; seven physicians were involved, and all had no information on previous findings.

The completion rate of the follow-up study was virtually 100 per cent. as only people who were admitted to the hospital or were on holiday were not seen at the moment they were requested to come; 2 per cent. died during the survey.

Observer variance was small where the diagnosis of RA was concerned, but increased to values of 35 per cent. in “cases” of OA or spondylarthrosis (SA). Hence the conclusion was reached that for reliable clinical evaluation of (mild) OA and SA X rays are indispensable.

During the year of follow-up fluctuations in incidence of RA were more often due to hospital admission and differences in grading between observers (specially between Grade 1 = doubtful, and Grade 2 = certain but mild) than to real variations in incidence. No definite increase in prevalence was observed as a result of prolonged observation. In a single instance RA became apparent during the period of observation, but had disappeared clinically at the end of the survey.

The study was the result of a joint project between the Department of Rheumatology, The Institute for Rheumatism Research, and the Department of Microbial Diseases, University Hospital, Leiden. The observers involved were: Drs. A. Cats, H. Colenbrander, M. J. Haverkorn van Rijsewijk, H. Hazevoet, K. A. E. Meijers, J. Pietersen, and H. A. Valkenburg. At the end of the survey, diagnostic cases with observer variations of 2 grades or more were re-evaluated by Prof. J. Goslings, again without information on previous findings.)

Discussion.—Prof. J. H. Kellogg (Manchester): I should like to congratulate Dr. Valkenburg on his really excellent paper. I have one question. Was there any qualitative difference between the people who had episodes of joint pain and swelling who appeared in your follow-up, serologically or radiologically?

Dr. Valkenburg: I only have a few data because they have not yet been analysed. We had three male cases, two of whom were present for one month only and the other for a longer period. Two of these were sero-positive; one was ill for 1 month only. In the total group of 100 males only three were sero-positive, so that we had one false sero-positive male. In the women the serological results left us completely at a loss, because only one woman, who had rheumatoid arthritis throughout the year, was sero-positive, and all the twelve others were sero-negative. The X rays have not yet been evaluated.

Prof. E. G. L. Bywaters (Taplow): If a random survey was made at any one point in the year, you would not pick up some of these, so that the total incidence of three to eight over a period would be reduced to something like five in a normal population survey.

Dr. Valkenburg: Yes, but depending upon the time of the year, the incidence might vary between 2 and 7 per cent.

The Value of the Tangential X-Ray View of the Metacarpal Heads in Rheumatoid Arthritis. By D. A. Brewer-Ton (Westminster Hospital, London): A standard antero-posterior X ray view of the hand gives an outline of the metacarpal-phalangeal joints in extension, but not in flexion where they are most commonly used in functional activities. Therefore X rays have been taken of these joints when flexed at a right angle, using a tangential view almost along the line of the shafts of the metacarpals. In this view the grooves each side of the metacarpal heads are seen in silhouette and erosions may be demonstrated more clearly than in antero-posterior views of the same joints.

Tangential views and standard views have been used in the assessment of patients with early rheumatoid disease to assess the clinical value of the new method.

Discussion.—Dr. J. J. R. Duthie (Edinburgh): Do you feel that this view could reveal an erosion in this area which is loosening the attachment of the collateral ligament and giving rise to ulnar deviation?

Dr. Beverton: This is going beyond what I was talking about. I do not feel that it is loosening the attachment but there is no doubt that the collateral ligaments frequently become lengthened. Some of these views have shown fairly clearly what the surgeons know—that these erosions and synovial proliferation often begin on the lateral aspects of the metacarpal heads under the collateral ligaments. The ligaments are two-thirds surrounded by synovial tissue.

Dr. J. J. R. Duthie: We have seen this sometimes at operation where X rays showed nothing. Surgeons are coming to the conclusion that this may well be an important factor in causing ulnar deviation.

Dr. Beverton: We have not yet done a correlation with synovectomy.

Prof. E. G. L. Bywaters (Taplow): You have said that the apparently narrowed joint space was a false appearance due to flexion of the metacarpal-phalangeal joint. A more probable explanation, in my opinion, is that there is differing thickness of the cartilage in this affected metacarpal head at differing points in the head. It is very difficult to get apparent joint narrowing with flexion of the normal metacarpal-phalangeal joint.

Dr. Beverton: This is something that I would like to study more. In looking at an antero-posterior view, there is a tendency to assume that the joints have been...
placed in neutral positions, but if there is 30 degree fixed flexion of a joint you cannot get a clear idea of the joint space. To do this one should take x rays at right angles to the proximal phalanx.

DR. F. J. ANDREWS (Stoke Mandeville): Have your surgical colleagues ever altered their proposed technique as a result of this examination of the angle of the x ray?

DR. BREWERTON: No. They know far more about what goes on inside a joint than we do.

DR. F. J. ANDREWS: Do you think that prospective information given by this view might have an influence on the surgeons?

DR. BREWERTON: This is fairly well in advance of our knowledge, but one would like to follow this and see how relevant these views are.

Diagnosis of Chondrocarninosis Articularis. By J. A. G. van IJZERLOO and W. B. H. MEINERS (Rhoon): In eleven patients suffering from joint complaints, calcification of the articular cartilage was discovered in ten. In nine a knee joint could be aspirated. The synovial fluid was examined by Giemsa staining of the sediment. In all, intra- and extra-cellular crystals could be demonstrated. These combined findings of calcified cartilage and crystals strongly suggested the diagnosis chondrocarninosis.

Discussion.—DR. A. ST. J. DIXON (London): I wonder if I might have permission to show two slides briefly? These show acute podagra—it couldn't be more typical—in a young woman with a ruptured calcific deposit in the capsule of the toe joint. I show these because we need to define or avoid the term "pseudo-gout", which is also used for chondrocarninosis articularis, which is a different condition, and could also be used for the topfaceous deposits of calcific material sometimes seen in the fingers in calcinosis.

DR. W. A. BOURNE (Hove): What were the serum calcium phosphorus and phosphatase levels?

DR. van IJZERLOO: These were always normal.

DR. H. L. F. CURREY (London): I wonder if you have examined the synovial fluid using polarized light? Our experience of examining several fluids is that, during the acute attack, one finds large numbers of crystals fairly easily, but if one looks carefully by polarized light one sees small numbers of crystals—not only in fluids from this sort of patient but also in those from patients with other arthropathies. Of course the number present is very small and it is not possible to confirm their composition by x ray diffraction studies and very difficult to know what the significance of material present in very small quantities is.

DR. van IJZERLOO: We have only studied some of the synovial fluids with polarized light.

DR. H. L. F. CURREY: There is a great difference in the ease with which we find crystals. They have to be fairly numerous to be found by ordinary light microscopy, but looking under crossed nicols they are fairly easily picked up.

DR. F. Dudley Hart (London): How many of these patients had acute pain?

DR. van IJZERLOO: Six had episodic attacks.

PROF. E. G. L. BYWATERS (Taplow): I should like to amplify a little what Dr. Dixon said about two types of calcification. I described some patients, in a communi-
cation to the Heberden Society some years ago, who have multiple calcinotic tendinitis which may sometimes give local pain. This is not associated in our experience with the deposition of calcium in the articular cartilage; there seem to be two rather different entities. In your cases I understand that the calcification was always in the articular cartilage or fibrocartilage. You saw none in the tendon space. Is this correct?

DR. van IJZERLOO: We have seen calcification peripheral to the joint in some cases.

PROF. E. G. L. BYWATERS: Were you able to rule out the presence of hypervitaminosis D?

DR. van IJZERLOO: We have no proof of this.

DR. J. J. R. DUTHIE (Edinburgh): I agree with Professor Bywaters about the two quite distinct entities. Dr. Dixon's case of a young woman is a classical case of pseudo-gout. We have seen several physiotherapists who get this acute pain in the feet, and we usually see deposits of calcium in relation to the tendons. There are two kinds of calcification: intra-articular and extra-articular.

DR. van IJZERLOO: Perhaps it would be possible to investigate these cases and obtain fluid and see if there are crystals there.

DR. J. Ball (Manchester): In metastatic calcification the mineral is amorphous and different from that present in pseudo-gout.

DR. G. D. KERSLEY (Bath): Were these areas easily visible on random films, or did they require careful examination of a number of films? How many deposits were there?

DR. van IJZERLOO: They were obvious and easily visible. Sometimes there were many and sometimes only a few.

PROF. E. G. L. BYWATERS: It is important to point out that crystal gazing is very much simpler and easier in polarized light.

Pseudo-gout following Renal Transplantation. By Lavinia W. LOUGHRIDGE and R. Y. CALNE (Westminster Hospital, London): A 33-year-old housewife received a renal transplant from a cadaver donor in December, 1962. Moderately satisfactory function was established, her blood urea fell to 90 mg. per 100 ml. and she was able to return home. 11 months after operation, she first developed pains in fingers and right shoulder without clinical abnormality of the joints. X rays showed widespread calcification in peri-articular tissues and in the blood vessels of the kidney graft. At this time blood urea was 142 mg. per 100 ml. and uric acid 4.8 mg. per 100 ml. 2 months later she began to have acute attacks of pain, swelling, and redness affecting multiple small joints of the hands, associated with local tenderness and the development of large firm nodules overlying the joints. X rays showed increased peri-articular calcification but no destruction of joint surfaces. Biopsy of a nodule revealed a soft white material containing a few amorphous crystals and giving strong chemical reactions for calcium and phosphate. A weak reaction for uric acid and urates was obtained. Blood urea had risen to 200 mg. per 100 ml. and uric acid was 7.8 mg. per 100 ml.

The patient died 2½ years after operation and throughout this time she suffered repeated similar attacks of acute arthritis with further nodule formation.
Discussion.—Dr. V. Wright (Leeds): We have had one or two cases with this complication, usually during the period of renal dialysis, and have attributed it to a rather high calcium content in the dialysing fluid. I understand that Scribner's group in Seattle have investigated this problem and they deliberately maintained the uric acid in the bath fluid and so were able to prolong the attacks of gout. It would seem possible therefore that one may in fact get both pseudo-gout and true gout with chronic renal failure.

Dr. Loughridge: They have such a similar mode of clinical presentation that it would be difficult to distinguish which the patients were having.

Dr. J. T. Scott (London): Decker (1965) has also recently mentioned his experience that acute attacks in this situation tend to coincide to some extent with the serum uric acid levels, and there is the point already mentioned that acute attacks seem to continue if the uric acid content of the dialysate fluid is kept high. But he found that the deposits undoubtedly contained calcium pyrophosphate, and put forward the hypothesis that both substances contributed to the gouty episodes.

Dr. J. Dudley Hart (London): Did this patient have first acute pain and was the calcification seen only later on x ray? Did a clinical painful episode precede the radiological change?

Dr. Loughridge: Yes.

Dr. J. Dudley Hart: The x rays were then normal and she later developed calcification?

Prof. E. G. L. Bywaters (Taplow): Where were the para-articular changes? In the joint itself, or within the capsule? Were they in the actual tendon sheaths?

Dr. Loughridge: This autopsy was performed by Dr. McGillivray, who found no calcification in the tendons: it was mostly in the joint capsules.

Prof. E. G. L. Bywaters: Was there any metastatic calcification in the arteries or in the lungs?

Dr. Loughridge: Yes, it could be seen in the arteries but not in the lungs.

Prof. E. G. L. Bywaters: Are you sure this was calcium pyrophosphate?

Dr. Loughridge: No.

Prof. E. G. L. Bywaters: Presumably this calcium phosphorus ratio was not sufficient to produce metastatic calcification?

Dr. Loughridge: She had a calcium level of 9·8 which fell later on with the deterioration of renal function and uremia.

Dr. W. A. Bourne (Hove): I have seen one case of chronic renal failure with metastatic calcification in the subcutaneous tissues, in the blood vessels and ultimately, I regret, in the bed sores, but there were no joint lesions.

Dr. J. H. Glyn (London): When this patient came in, what treatment did she receive?

Dr. Loughridge: She just had the ordinary analgesic treatment. She preferred codeine. She was not tried on colchicine. Codeine did seem to have quite a good effect.

Dr. H. L. F. Currey (London): Dr. Williams has told me about autopsies on two patients dying in chronic renal failure. There had been no record of articcular complaints during life, but at autopsy, there was an icing-sugar type of powdering over the joint cartilage looking like gout. Histological and crystallographic studies indicated that the material was oxalate.

Prof. E. G. L. Bywaters: Were these cases of primary oxalosis where the clinical picture is quite different?

Dr. H. L. F. Currey: Oxalate forms in general during life. The patients had died from what was thought to be chronic pyelonephritis.

Dr. Loughridge: Did they die at a very early age?

Dr. H. L. F. Currey: No. They were adults.

Prof. E. G. L. Bywaters: I have come across one case of primary oxalosis where there is intra-articular calcium deposition.

REFERENCE


Skeletal Aspects of Myositis Ossificans Progressiva

By A. W. T. Eade and E. B. D. Hamilton (King's College Hospital, London): Three patients with established myositis ossificans progressiva have been studied. The clinical features and the recognized hereditary skeletal abnormalities are reviewed, with special attention to the changes which take place in the spinal column. In one of the cases serial x rays showed the gradual fusion of the apophyseal joints. These changes superficially resemble the late effects seen in some cases of Still's disease and anklyosing spondylitis with which they may be confused.

Discussion.—Dr. J. Sharp (Buxton): Is anything known of the range of cervical movement before the development of radiological changes?

Dr. Eade: No, nothing. The only one of our patients on whom we have details is the one who went to have torticollis.

Dr. J. Dudley Hart (London): This appears to be the case in the Hunterian Museum and in one in Dublin. I think perhaps it is more usual for the dorso-lumbar spine to be fixed by ossification of the muscles of the spine. Did they have marked limitation of the thoracic spine?

Dr. Eade: Yes.

Prof. E. G. L. Bywaters (Taplow): Would you say something about the early symptoms?

Dr. Eade: The most usual thing, I understand, is for the patient to develop local swelling, pain, and sometimes tenderness. This usually subsides in a matter of a few days to a week or so, and at that time there is already radiological evidence of ossification.

Prof. E. G. L. Bywaters: What is thought about the mechanism? It looked possible that these bony sheets were all ultimately due to outgrowth from the periosseum that occurred with growth. It occurred to me that the fusion of spinal bones which was shown was not actually due to maldevelopment but to the same sort of fusing process that is found in periosteal outgrowths from the vertebrae; possibly the same interpretation would apply to what you call maldevelopments of the toes; this would then be an acquired lesion of bones due to genetic maldevelopment.

Dr. Eade: As far as abnormalities of the digits are concerned, these did sometimes occur in other members of the patient's family, although they did not develop changes in the muscles or spine. I think there is a choice whether one can regard the digital abnormalities as a separate thing, inherited in the same people.

Prof. E. G. L. Bywaters: Are those digital abnormalities present at birth?

Dr. Eade: Yes.
Dr. V. Wright (Leeds): Have you any evidence from a family survey that there is a dominant mode of inheritance?

Dr. Eade: No, but others have put forward evidence that this is so.

Dr. J. Cosh (Bath): Have you any information from your own patients or the literature about possible destructive or necrotic processes in muscle leading later to calcification? Did you study transaminases or stain for phosphatase in biopsy material?

Dr. Eade: There were no studies on these patients in their early stages. In the few patients who have been studied in recent times, since most tests became available, everyone says the same, that no abnormality was found. I may have a possible clue. In two cases, biopsy material from early lesions has shown phosphatase activity 1,000 times greater than that of normal muscle, and most of this increased phosphatase activity was associated with connective tissue and not muscle cells. This is the only tissue enzyme study I know of.

Multicentre Controlled Trial of the Effects of Gold Therapy with Different Dosages, followed by Maintenance Dosage. By Prof. J. Goslings (Leiden). The effect of gold in rheumatoid arthritis was studied by a double-blind trial. Patients were divided into two equal groups, one being given 2,500 mg. Aurothioglucose over 21 weeks, the other 1,000 mg. These two groups were then divided at random into equal subgroups, one receiving placebo injections, the other a maintenance dose of 50 and 25 mg. respectively weekly for an intended period of 3 years. The patients, aged 20 to 65 years, all suffered from active definite rheumatoid arthritis of 6 to 24 months' duration. The effect of treatment was assessed by the ESR and the number of joints affected. There were 202 patients comprised of four comparable subgroups of approximately fifty patients each.

During the first 6 months, all four subgroups improved similarly. During the second 6 months, gold was more effective than the placebo, the differences being statistically significant (P < 0.017 for ESR and P > 0.05 for number of affected joints). After 12 months there was no significant difference between high and low dosage groups, though evidence from some patients suggested that a high dose was preferable. Toxic effects were more common in the first 6 months and amongst those receiving the larger doses of gold. No definite conclusions concerning the most suitable dosage were reached and results after 2 and 3 years will be assessed later.

Discussion.—Dr. J. T. Scott (London): Would Prof. Goslings tell us if there were any cases of marrow aplasia or other severe or fatal complications?

Prof. Goslings: There were no fatal complications, and no serious complications from blood dyscrasias. There were some cases of leucopenia but no serious ones.

Dr. G. D. Kersley (Bath): Were there any renal complications?

Prof. Goslings: No serious ones. Only some temporary albuminuria.

Dr. J. Dudley Hart (London): How much actual gold was given?

Prof. Goslings: 50 per cent. of the aurothioglucose is actual gold.

Dr. J. Dudley Hart: The first group had 50 per cent. at 200 mg. a dose?

Prof. Goslings: Yes, the high dose group had 50 per cent. of 200 mg. solganol B ol. (= 100 mg. actual gold) a week, except the first 2 weeks when they had half the dose, making 2 g. solganol B ol. (= 1 g. actual gold) in 11 weeks.

Dr. R. M. Mason (London): In reporting a comparison of these groups you selected the sedimentation rate and the number of affected joints. I wonder why you selected these two things; in our experience they are two of the most reliable variables.

Prof. Goslings: We selected these two things to start with because we had the same experience as you about the reliability of these two indices.

Dr. W. A. Bourne (Hove): All the patients were having gold at some time. When I did a small control trial I found that some patients receiving the placebo which contained the same suspension medium as the one containing gold developed skin lesions which seemed to be due to the suspension medium, and I wondered whether some skin lesions in your patients might be placebo reactions, but of course with this type of trial it is difficult to say.

Prof. Goslings: I agree that some lesions could be caused by the suspension medium. We did not use placebos because the English trial had proven the favourable effect of gold on the course of the disease.

Dr. J. H. Glyn (London): Do I understand you are giving 50 mg. a week for 3 years? I would have thought this meant an increasing risk of complications because of accumulation of gold in the tissues.

Prof. Goslings: It is difficult to get proof about the quantity of gold you should give in order to keep it at the same level in the body and prevent accumulation. We have given in the high dose group 50 mg. solganol B ol. (= 25 mg. actual gold) a week, and 25 mg. solganol B ol. (= 12.5 mg. actual gold) in the other group. With these dosages we had only a few side-effects in the second half of the year and there were no differences between the two dosages and the placebo groups. As far as is already known, after 2 years in the trial in about 150 patients, there have been even fewer side-effects in the second year with these maintenance doses.

Microchemical Analysis of Connective Tissues. By J. A. Szirmai (Leiden): Although histochemical methods are indispensable for the localization of various chemical constituents at the tissue level, their lack of specificity or of quantitative measurement frequently restricts their application and prevents definitive conclusions being reached. This is true especially for some constituents of connective tissues, such as mucopolysaccharides, since no absolute criteria are available for their differentiation and quantitation by histochemical means. Therefore attempts have been made (in collaboration with Dr. S. Gardell of Lund, Sweden) to develop microchemical procedures for the fractionation and quantitative analysis of the various types of mucopolysaccharides, and also to adapt micromethods for the analysis of collagen and other constituents.

A scaled-down version of the fractionation of mucopolysaccharides by elution of their cetylpyridinium complexes has been worked out, which allows determination of polysaccharide fractions down to a few micro-
programs. A quantitative recovery is obtained after liberation of the polysaccharides from the tissue by proteolytic digestion, and the amount in each fraction is determined by a modified Elson and Morgan procedure. In addition to the possibility of separating various types of mucopolysaccharides, a marked heterogeneity within a given type could be detected and visualised by the construction of a so-called solubility profile.

Application of these methods to the study of various connective tissues, such as nasal septum cartilage, intervertebral disk, etc., has shown marked differences in the solubility profiles of the cetylpyridinium-complexes of a given type of polysaccharide. Similarly, considerable differences in the concentration of other constituents, like total protein and collagen, have been observed. The results indicate a greater heterogeneity in the composition of the connective tissues so far investigated than what could have been expected from either histochemical or macrochemical investigations.

Discussion.—Dr. G. Loewi (Taplow): Does the lack of difference in quantity refer only to costal cartilage or does it refer also to others?

Dr. Szirmai: This observation was carried out in one area, and I have to confess that more than one section had to be used to obtain analysis of a good sample from a reasonable area of the cartilage. We do not understand anything yet about the composition of these areas because the results are expressed in terms of dry weight and that is a non-conclusive term. We know that the water content must be very much different in the immediate surroundings of cartilage, so that many of our results have to be checked again.

Dr. G. Loewi: The only comment I had was concerning the Alcian blue technique of staining.

Dr. Szirmai: Our difficulty is that we have morphologists entering the chemical field and chemists entering the morphological field, and they shout across at one another. We have reason to doubt some of the outcome of these tests.

Dr. J. Ball (Manchester): I should like to congratulate Dr. Szirmai, and should like to ask if you found any relationship between the distribution of the various polysaccharides and the calcifiability of the cartilage?

Dr. Szirmai: We have no personal experience of this. Dr. Hjertquist from Sweden used this procedure in a study of normal and rachitic epiphyseal cartilage and found to his surprise and disappointment that there was no difference in the concentration or the profiles of the chondroitin sulphate in various histological regions and there was no basic difference between normal and abnormal.

Dr. J. Ball: In costal cartilage the central parts tend to calcify and I had understood you to say that keratosulphate was concentrated in the central parts of your specimens.

Dr. Szirmai: It could be, but I think that it is hard to say from the evidence available so far.

Dr. Stockwell (London): I think that many of the differences between the results turn on the fact that the chemists use nasal cartilage and we have used costal cartilage. Certainly there are differences in the keratosulphate and protein content of the polysaccharides of bovine nasal and human costal cartilage, as stated by Schubert, so this may account for part of the disagreement.

As far as the results of the alcian blue technique are concerned, we have found on using hyaluronidase extraction that staining rendered negative by hyaluronidase corresponds with the localisation of chondroitin sulphate demonstrated by the Scott method, confirming its specificity.

Dr. Szirmai: I should not say that this mattered. We know that every piece of connective tissue is different. This is why we want to have strict chemical analysis.

Mucopolysaccharides in Amyloidosis of Human Spleens.

By Helen Muir and T. Bitter (Medical Unit, St. Mary’s Hospital, London): The diagnosis of amyloidosis is based on histological criteria which are not altogether specific, and it is uncertain whether all forms of “primary” and “secondary” amyloidosis are in fact the same disease. The lesions show similar histological features including metachromasia, which suggests that mucopolysaccharides are present, but it has never been established what were the quantitative or qualitative changes nor whether they were the same in all forms of amyloidosis.

The mucopolysaccharide composition of spleens from twenty cases of generalised amyloidosis representing most varieties of the disease were therefore investigated and compared with eighteen controls from healthy individuals of similar age.

The control spleens showed an eight-fold decrease in total mucopolysaccharides between the ages of 14 and 98 years, heparitin sulphate consistently accounting for about a quarter of the total. In contrast, all but one of the spleens with amyloid infiltration showed an increase in total mucopolysaccharides and no effect of age was apparent. This was mainly due to increased amounts of heparitin sulphate the relative proportion of which rose markedly in all the diseased spleens, including those where there was little or no increase in total mucopolysaccharides. There were no consistent changes in other mucopolysaccharides and while traces of hyaluronic acid were found, no heparin or keratosulphate could be demonstrated.

The mucopolysaccharides from all the control spleens moved as single bands on cellulose acetate electrophoresis, whereas those from diseased spleens had a minor component of lower mobility. This was isolated in one case by preparative electrophoresis and consisted of a heparitin of relatively low sulphate content. Heparitin sulphate was the only mucopolysaccharide present in the insoluble residue from part of a diseased spleen which had been extracted with concentrated calcium chloride and urea. The possibility that heparitin sulphate is involved in the deposition of amyloid material will be discussed.

Discussion.—Prof. E. G. L. Bywaters (Taplow): Have you examined the senile amyloidosis which normally occurs in some instances in animals and also in man?

Dr. Muir: I am afraid not. We studied only those twenty cases; one was an 83-year-old man, but whether his amyloid was senile or due to his disease I am not prepared to say.

Prof. E. G. L. Bywaters: With what structures is heparitin sulphate normally associated?
Dr. Muir: It is found in blood vessel walls. It also occurs in the capsule of the spleen, and the lung, and in hog gastric mucosa. It may be more widely distributed than has been supposed because it has not been studied very extensively.

Prof. E. G. L. Bywaters: This is certainly a very important discovery about a substance which has puzzled people for many years, which will ultimately lead to a greater understanding of amyloid and the processes which lead to it.

Cytological, Electron-microscopical, and Preliminary Immunological Observations on Amyloid.* By L. Ruinen, J. H. Scholten, H. Boeré, and E. Mandema (Groningen): For more than a century, it has been known that amyloid is deposited in the spleen and liver of patients suffering from chronic inflammatory diseases, among which is rheumatoid arthritis. In experimental amyloidosis, it appears to be developed first in the marginal zone cells of the splenic follicle. These cells are very susceptible to irradiation and agents like endotoxin and imuran, and their role in the pathogenesis of amyloidosis will be discussed.

In extracts obtained by density gradient centrifugation from splenectomies of patients with primary and secondary amyloidosis, the histological properties characteristic of amyloid (congo-red positive, crystal violet metachromasia, and birefringence in polarization microscopy) were still present. These extracts were investigated by electron microscopy, using various fixation, embedding, and staining techniques. A fibrillar component was observed in both kinds of amyloid. Details of the structure were best seen by means of the negative staining technique (Boeré, Ruinen, and Scholten, 1965).

Rabbits were immunized with the purified amyloid extracts. The protein components of amyloid were investigated by immuno-diffusion and immuno-electrophoretic techniques.

Discussion.—Dr. G. Loewi (Taplow): Have you any other comment on the intracellular material?

Dr. Ruinen: We tried to identify some proteins produced by the marginal zone cells. We were unable to complete the experiments, but we hope that the material produced in these cells can be shown to be a component of amyloid.

Prof. J. R. Duthie (Edinburgh): I find it surprising that trypsin did not change the electron microscopic appearance of amyloid. If this is so, could you not use it to remove protein contamination and thereby produce a cleaner antigen for your immunization studies? Could you not clean the trypsin?

Dr. Ruinen: From the literature it is known that the histological properties of amyloid are almost unchanged after trypsin digestion. Following a useful hint of Guest, we have been working with trypsin. The trypsin-treated extract, which is freed from proteic impurities, can probably be used for obtaining a more specific anti-amyloid-serum, provided that this material is still antigenic.

Prof. E. Mandema (Groningen): I think we can only state that we probably know a little more about the origin of the glycoprotein of amyloid. We hope to be able to show that these cells produce a protein of great importance for the deposition of amyloid in the spleen, and we also hope that immunological studies will prove whether this is a normal or an abnormal protein.

REFERENCE


Significance of the L.E.-Cell Phenomenon for the Symptomatology and Prognosis of Rheumatoid Arthritis. A Follow-up Study of 9 Years. By H. N. Hazevot, W. Humans, and J. H. Kieffts (Leiden): A group of 111 patients suffering from RA with L.E.-cells was compared to a control group of 98 RA patients without L.E.-cells. The symptomatology of RA with L.E.-cells was different from that without L.E.-cells. After a 9-year period, the differences originally present were less pronounced and less statistically significant, a tendency which also persisted in the L.E.-cell phenomenon itself. After 9 years, the original L.E.-positive group showed a more severe affection of the small joints of hands and feet by the rheumatoid process, more subcutaneous nodules, higher

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sedimentation rates, and lower haemoglobin values, as compared with the L.E.-negative group. So far the presence of the L.E. factor does not seem to have influenced the mortality rate.

Discussion.—Dr. D. Felix-Davies (Birmingham): In view of the fact that the L.E.-cell phenomenon is so constant, I wonder whether you can obtain further information by dividing your group into those who always have a strongly positive result and those who occasionally have them in a minor degree? Are there any differences when you divide the group further?

Dr. Hazewoet: There are now nine positives. There is no difference between those nine and the other patients of the originally L.E.-positive group.

Dr. J. R. Duthie (Edinburgh): Are you testing for the presence or absence of the antinuclear factor?

Dr. Hazewoet: No.

Prof. E. G. L. Bywaters (Taplow): Your proportion of rheumatoid patients showing L.E.-cell positivity has always been higher than that of other workers; is your 17 per cent. positive of 1956 still 17 per cent. in the sixty new patients or has there been an alteration?

Dr. W. Hims (Leiden): I am afraid that we cannot give an exact answer. We know we are at variance with a number of other workers, but think it is a question of technique. It is not everyone who is prepared to spend half a morning examining the L.E.-cell preparations of one patient and then to discuss the slides with others in the laboratory. It is the presence of the L.E.-cell which was the decisive factor. The question is therefore open for investigation.

Dr. W. A. Bourne (Hove): Did you vary the treatment when you found L.E.-cells?

Dr. Hazewoet: Our patients in 1956 came from doctors all over the country and we did not treat them all ourselves. There was a little difference in treatment between the two groups; the L.E.-positive cases received rather more corticosteroid and antimalarial drugs.

The Antiperinuclear Factor. A New Diagnostic Tool in the Diagnosis of Rheumatoid Arthritis. By E. Mandema and R. L. F. Niemhuis (Groningen): An investigation was made of the nature and the incidence of a new serum factor in rheumatoid arthritis, called the antiperinuclear factor (APF), because of the brilliant perinuclear granular fluorescence using Coons’ immunofluorescent technique, seen in human buccal mucosal epithelial cells. The factor is found in nearly 50 per cent. of rheumatoid arthritis patients and is specific for this disease.

The factor is active in the different immunoglobulin fractions of the serum. The nature of the fluorescing granules, present only in human buccal mucosal epithelial cells, whether they are kerato-hyaline granules, PPO, or lysosomal structures, is not yet established.

Discussion.—Dr. J. Ball (Manchester): I should like to ask whether the granules in the positive cells are auto-fluorescent?

Dr. Niemhuis: No. We used buccal mucosa and many controls for comparison, and in every one they fluoresced only with rheumatoid sera.

Dr. E. J. Holborow (Taplow): From reading a paper which appeared last year, I think perhaps the exchange of positive sera between interested groups might be of great benefit. Secondly, why do you call it “perinuclear staining”? Really it is cytoplasmic staining.

Dr. Niemhuis: You are right. You might perhaps even better call it “granular cytoplasmic fluorescence”.

Prof. E. G. L. Bywaters (Taplow): It might be considered worthwhile to exchange exact details of methods as well as sera!

Dr. W. A. Bourne (Hove): There was no question that your buccal mucosa cells came from patients who had had at any time active stomatitis?

Dr. Niemhuis: No. Everyone shows these granules and we cannot expect that everyone used has had active stomatitis. Cells from rheumatoid patients give the same results with their own sera.

Dr. J. R. Duthie (Edinburgh): Have you considered investigating the possibility that they might be mycoplasma?

Dr. Niemhuis: This is a difficult question, because we do not know what these granules are. They could be residual bodies. The form of the granules and staining properties gives rise to the possibility that they might be a micro-organism, and what could it be?

Dr. J. R. Duthie: There is the possibility that mycoplasma-like organisms are commonly present in buccal mucosa, but only in patients with rheumatoid arthritis can antibodies to them be demonstrated.

NEW YORK RHEUMATISM ASSOCIATION

At the Annual Meeting held on April 19, 1966, the following officers were elected:

President: Dr. David J. Harmer
President Elect: Dr. Thomas G. Kantor
Vice-President: Dr. Robert H. Mannheimer
Secretary-Treasurer: Dr. Arthur I. Snyder