substrates and products have no effect. Fractionation of normal erythrocytes by hypotonic lysis reveals a decline in the specific activity of A-PRT as the red cells age. In patients with the Lesch-Nyhan syndrome, the decline in A-PRT activity with ageing of cells is much less marked. Furthermore, the concentrations of free PRPP are as much as 10-fold elevated in the erythrocytes of these children and of patients with partial H-PRT deficiency. A good correlation exists between intracellular PRPP levels and A-PRT activity. We therefore propose that the absence or deficiency of H-PRT from the erythrocyte produces an accumulation of substrate PRPP which stabilizes the A-PRT enzyme against the loss of activity which occurs during ageing of normal erythrocytes.

Discussion

DR. M. K. JASANI (*Horsham*) Is the role of PRPP in the heat-stabilization of H-PRT or A-PRT that of a substrate as you have surmised, or is it that of a co-factor? The data presented today could well support its role as a co-factor, adenine and hypoxanthine being the respective substrates.

DR. BOYLE We know that PRPP is a substrate, as it is consumed during the reaction. We have gone into the kinetics at some length and all the kinetic data that we, and others have, support the concept that PRPP is a substrate.

PROF. C. A. KEELE (*London*) Is there any evidence that these enzymes that are found in the lymph cells are also found in other types of body cells?

DR. BOYLE Yes, there is. We studied a foetus diagnosed *in utero* as having the Lesch-Nyhan syndrome, the mother having elected to have a termination of pregnancy. We found that the enzyme was not detectable in the twenty different tissues that we assayed, whereas in foetuses aborted for other reasons activity could be easily detected. Moreover, the elevation of adenine phosphoribosyl-transferase enzyme was noted in all the tissues of the Lesch-Nyhan foetus. PRPP levels are known to be increased in fibroblasts of children with the syndrome. Thus we have evidence that both enzymes exhibit the reciprocal relationship in many body tissues and that PRPP levels are elevated in the two tissues (blood and fibroblasts) in which they have been measured.

Uric Acid Clearance in Normal and Gouty Subjects. By M. L. SNAITH, H. JABLONSKI, and J. T. SCOTT (*Kennedy Institute, London*). This paper with the discussion thereon appears in this issue of the *Annals* (see p.285).

Synovial Effusion induced by Adrenocortico:rophic Hormone. By N. WILLIAMSON, R. COCKEL, A. G. JOHNSON, and C. F. HAWKINS (*Rheumatism Research Wing, Department of Experimental Pathology, University of Birmingham*).

In a group of twenty patients with ulcerative colitis, it was observed that synovial effusions occurred 2 to 5

days after starting injections of ACTH in doses of 30 to 60 i.u. daily. The first abnormal sign noted was an effusion into the knee joint, but oedema of the legs and weight gain were frequently seen later. The size of the effusion in some patients produced slight limitation of movement, though there was no other evidence of an arthritis as shown by pain or warmth. In all, complete remission followed the cessation of ACTH therapy.

Examination of synovial fluid from nine of these patients showed certain characteristics. The fluids were viscid, almost colourless, and contained numerous fibrils, few cells, and no crystals. The presence of this large number of fibrils prompted further study into the incidence and nature of fibrils seen in synovial fluids from other patients with effusions due to anasarca, trauma, degeneration, or inflammation. Two types of fibrils have been distinguished using histochemical and immunofluorescent staining. An electron microscope study has confirmed the presence of these two types of fibril, one having the characteristic pattern of collagen, and the other larger fibril being suggestive of degenerating fibrin.

Discussion

DR. A. G. S. HILL (*Stoke Mandeville*) If you are supposing that production of fibrils is normal in the joint and that there is a continual dynamic process of production and removal, presumably removal in the normal joint occurs by way of synovial cells rather than by cells free in the fluid. Have you examined synovial cells for fibrin in the same way as you have looked at the free cells?

DR. WILLIAMSON I have stained synovial membrane with an antifibrinogen, and some fibrinogen is occasionally present, which would fit in with this hypothesis. This is not very marked, certainly not as marked as one finds in inflammatory polyarthritis where there is much fibrin staining, but in these conditions I think the main factor is a breakdown of material which is being removed by a more active phagocytic process.

PROF. C. A. KEELE (*London*) Since there was absence of fibrils in the inflammatory state, this might indicate activation of hydrolytic enzymes such as occur in lysosomes. Have you tried adding various proteolytic enzymes to these fluids to see whether you can remove the fibrils?

DR. WILLIAMSON Yes, I have used collagenase hyaluronidase and streptokinase to see whether these would break down the fibrils. One does get some breakdown, using any of these three enzymes, though my results were not as good as I expected. The streptokinase removed the fibrils from a large proportion of these fluids, but never completely. It may have been that the incubation was not correct or that I did not add enough activator or other essential factors. The collagenase was rather disappointing. No fibrils seemed to disappear even though I obtained good van Gieson staining. I do not know whether this was due to the enzyme we were using. This work must be followed up in detail to find out how one can break these down. Staining smears after enzyme treatment was not very successful. It may be that

preparing the smear does not allow the enzyme to work well.

DR. A. J. PALFREY (*London*) It seems to me that Dr. Williamson has shown very convincingly that two types of fibre are present in these fluids, and that one of them looks like collagen. A logical conclusion is that there is a turnover in the fibres of the cartilage. These fibres may therefore be present either as newly formed or as damaged collagen fibres: both these possibilities have to be considered. Their unsatisfactory enzymatic behaviour fits in very well with these possibilities. The fibrinoid fibres may be associated with the presence of damaged collagen fibres, but I think this is more likely to be a result of the ACTH treatment. Fibrinoid fibres in synovial tissues commonly stick to the surfaces of all types of cells.

DR. WILLIAMSON I would not argue that the pictures of a cell eating material are not very convincing. I merely thought that this was a possibility, but obviously it could be surface adherence.

PROF. E. G. L. BYWATERS (*Hammersmith*) Have you measured the protein content or the fibrinogen of these fluids, especially of the transudates described?

DR. WILLIAMSON I have not performed quantitative estimations. We have detected fibrinogen qualitatively by gel diffusion in all these fluids with the exception of two of those induced by ACTH in cases of ulcerative colitis. The two normal subjects were not tested since very little fluid was obtained, but it is said that fibrinogen is not present in normal joint fluid. It seems that fibrin is present in fluids from patients with ulcerative colitis receiving ACTH; whether this represents fibrils which have been broken down and are detected as free fibrinogen, or free fibrin, I am not sure. I treated some of these again with streptokinase to see if one could find breakdown products. There was so little present that the gel diffusion estimations were not convincing. We do not have any figures on the total protein content of the fluids.

DR. H. L. F. CURREY (*London*) Have you had an opportunity of examining these fibrils by polarized light microscopy? I examine fluids without anticoagulant and I feel that I can differentiate between collagen and fibrin fibres.

DR. WILLIAMSON All our fluids are examined by polarizing microscopy to exclude crystals, and I was not convinced that one could make a distinction. I considered that they did not show birefringence, but this may be a matter of degree.

DR. H. L. F. CURREY (*London*) I think collagen fibres are clearly birefringent and the fibrin as well if you have a source of light sufficient to show them.

DR. WILLIAMSON I think the problem is that these are very fine fibrils and are probably coated with other proteins. This is something we could investigate further.

DR. D. J. WARD (*Oswestry*) We had a patient with ankylosing spondylitis who was started on ACTH and within 2 days developed a simple knee effusion—the knee was certainly not hot. There were less than 1,000 white cells per cu. mm. and the protein content was 5 g./100 ml. Perhaps these effusions are inflammatory after all.

DR. WILLIAMSON We did estimate the hyaluronate in these fluids and, except that they gave the impression of a diluted fluid, they were normal.

The Cracking of Joints—A Bioengineering Study. By V. WRIGHT, A. UNSWORTH, and D. DOWSON (*Leeds*). This paper with the discussion thereon is to appear in the July, 1971, issue of the *Annals*, vol. 30.

Synovial Fluid Waaler-Rose and Latex Tests. By E. C. HUSKISSON, F. DUDLEY HART, and B. W. LACEY (*Westminster Hospital*). This paper was printed in full in the January issue of the *Annals* (1971, 30, 67).

Discussion

DR. A. G. S. HILL (*Stoke Mandeville*) I think your false positives are a difficult group, because many of these conditions could conceal rheumatoid arthritis. Any polyarticular osteoarthritis may conceal a polyarthritis. What is the present concept at the Westminster Hospital of palindromic rheumatism and its relationship to rheumatoid arthritis?

DR. HUSKISSON We believe that many patients will ultimately develop classical rheumatoid arthritis. We were therefore interested to find a positive Waaler-Rose test in the synovial fluid of a patient with palindromic rheumatism, but four patients subsequently tested gave negative results. A titre of $\geq 1:16$ was taken as positive throughout the study.

DR. J. A. BOYLE (*Glasgow*). Could you give us some statistical confidence in the index of discrimination? For example, if you repeated the study, do you think you would still find that a Waaler-Rose titre of 1:8 was a better discriminant between rheumatoid arthritis and non-rheumatoid arthritis than a conventional titre?

DR. HUSKISSON This is what we were trying to find out. This was a retrospective study designed to examine the usefulness of synovial fluid tests and to get some idea of the best titres to take. We chose our index of discrimination because it was simple, the value representing the percentage of patients in our series who were correctly classified by any criterion. Our patients were to some extent selected and the optimum levels might not be reproducible. The results will, however, form the basis for a prospective study.

DR. J. A. MATHEWS (*London*) Some years ago I tried to break up white cells in joint effusions by two techniques, either freeze-thawing or ultra-sound, to see whether rheumatoid factor was released, so increasing the number of positive tests, and I failed. I wonder whether you have tried this?