Heberden Society

Annual General Meeting, November, 1970

The following papers were presented at the annual general meeting on November 20 and 21, 1970.

Crystal Deposition in Hyperparathyroidism. By R. GRAHAME, M. B. MITCHINER, and D. JUNE SUTOR (*Guy's Hospital and University College Hospital, London*). Articular calcification is well known to occur in hyperparathyroidism and an association has been reported between hyperparathyroidism and hyperuricaemia and gout. Yet the literature concerning the pathology of the joints with special reference to articular crystal deposition in this condition is scanty.

This clinicopathological case report concerns a 64-year-old man who in life suffered from attacks of both urate and calcium pyrophosphate synovitis. Hyperparathyroidism was diagnosed but he succumbed to the effects of a calcific mitral valvulitis before operative treatment could be undertaken. At autopsy a large parathyroid adenoma was found measuring $3 \times 1.5 \times 1$ cm. All joints examined showed a chalky encrustation of the cartilage which was demonstrated by x-ray diffraction to contain both sodium acid urate and a mixture of the triclinic and monoclinic forms of calcium pyrophosphate dihydrate. A tophaceous deposit adjacent to one big toe joint showed calcium pyrophosphate dihydrate in addition to sodium acid urate. The calcific material in the heart valve was identified as carbonated apatite.

Discussion

DR. J. T. SCOTT (*London*) The relationship between calcification and hyperuricaemia requires further investigation. When we looked at the records of twelve patients with hyperparathyroidism some years ago we found that eleven had hyperuricaemia but there were three unsatisfactory features to this study:

- (1) The exact nature of the acute synovitis which occurred in five patients was never clarified; these patients were either studied in retrospect or were seen before we knew the significance of crystals in joint fluid.
- (2) Some of these patients were in severe renal failure.
- (3) Even those patients without obvious azotaemia were not studied in detail with regard to glomerular filtration and urate clearance.

This was largely a retrospective study and sometimes the situations were relatively acute.

It is surprising that there has been no further definitive study of this matter. I have seen only two or three patients with hyperparathyroidism since then and these have had normal uric acid levels. The time is ripe for those studying patients with hyperparathyroidism or hypercalcaemia from other causes to clear up this problem.

PROF. E. G. L. BYWATERS (*Hammersmith*) First, since I understand there was no difference in distribution of the urate and the pyrophosphate within articular cartilage, did they appear in the same sites? Secondly, the distribution shown by x-raying articular cartilage appeared to be a series of 'nets', whereas that in the meniscus seems to be a series of dots. I should very much like to know what this corresponded to histologically. Thirdly, it appears that the deposition of pyrophosphate depends very largely on a synovial milieu. We have recently seen two cases of seropositive rheumatoid arthritis, when reviewing routine biopsy sections at Taplow, which showed positively birefringent crystals in the rheumatoid synovial membrane, in one case surrounded by giant cells and in the other in association with ischaemic villi and a good deal of hydroxyapatite. These have been studied only under the polarizing microscope; we have not carried out any x-ray diffraction studies. I suppose they might be something else, but I should not have thought so because histologically they look exactly like our sections from hyperparathyroid cases at Hammersmith showing pyrophosphate in the discs and in the articular cartilage.

DR. GRAHAME We always found the two crystals together wherever we looked in cartilage or in ligament. In some areas one type predominated over the other, but we never found pockets of either one or the other in isolation. I am afraid we cannot answer your second question.

Substrate Stabilization: Genetically-controlled Reciprocal Relationship of Two Enzymes. By J. A. BOYLE, M. L. GREEN, and J. E. SEEGMILLER (*University of California and The Centre for Rheumatic Diseases and University Department of Medicine, Glasgow*).

An increased activity of adenine phosphoribosyltransferase (A-PRT) of erythrocytes has been one of the puzzling accompaniments of the deficiency of hypoxanthineguanine phosphoribosyltransferase (H-PRT) found in the Lesch-Nyhan syndrome (X-linked uric aciduria). The present study investigates the mechanism responsible for the elevated A-PRT activity in these patients. Purification of A-PRT from normal human erythrocytes yielded a protein, 6,700-fold purified, homogeneous on gel electrophoresis and in the ultracentrifuge. 5-phosphoribosyl-1-pyrophosphate (PRPP), a shared substrate for both H-PRT and A-PRT, protects purified A-PRT against heat inactivation, whereas other

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