and the administration of drugs such as antituberculosis therapy [8]. Interestingly, a growing number of cases are being recognized in the USA associated with dietary supplements containing L-tryptophan in which intense eosinophilia is associated with features such as musculoskeletal pain, peripheral oedema and fever. Some cases resemble eosinophilic fasciitis. These observations raise many questions about the causation of this rare and intriguing disorder.

P. J. Maddison
Royal National Hospital for Rheumatic Diseases, Upper Borough Walls, Bath BA1 1RL

REFERENCES

CLINICAL SIGNIFICANCE OF CALCIUM SALTS IN SYNOVIAL FLUID

Besides calcium pyrophosphate dihydrate crystals, different calcium containing particles such as hydroxyapatites, other basic calcium phosphates (BCP), brushite, and oxalate crystals have been identified in pathological synovial fluid [1]. Several studies [1-4] like the one published in this issue by Hamilton et al. [5] have investigated the role of some of these crystals in inducing inflammation, their common association with osteoarthritis and the inter-relationship between such crystals. Thirty-six years after the initial identification of CPPD crystals in synovial fluid by McCarty, these and monosodium urate (MSU) are the most commonly identified crystals during routine synovial fluid analysis [6-8]. Natural and compensated light microscopy both remain helpful in identifying such crystals, although some expertise and prolonged observation is sometimes required for CPPD [8]. The role of the CPPD crystal in inducing articular inflammation and progressive joint destruction independent of other calcium phosphate crystals has been well documented in familial forms of the disease. The role of apatite or BCP crystal as primary agent in inducing acute or chronic arthritis is still a controversial issue: both 'Milwaukee shoulder' and 'destructive apatite arthropathy' reflect the overemphasis given to the presence of apatite crystals in such conditions [6].

The association of CPPD, apatite crystals and osteoarthritis in the absence of chondrocalsinosis that Hamilton et al. [5] defined as 'chronic pyrophosphate arthropathy' is still an entity which is not clearly understood. Other investigators using more sophisticated and precise techniques have demonstrated the coexistence of apatite and pyrophosphate crystals in up to 70% of the patients with osteoarthritis [2-4]. In the study by Hamilton et al. [5], the presence of particles which stained with alizarin red S as evidence of BCP crystals must be interpreted with caution. Alizarin red S also stains small pyrophosphate crystals, other calcium phosphates, calcium oxalate, and other cations. The staining properties of alizarin red S solution may vary from batch to batch [8]. Furthermore, in several studies only heavily stained samples were shown to contain BCP crystal while particles with lesser degrees of staining rarely showed BCP crystals when examined under transmission electron microscopy [3, 4]. Furthermore, precipitation of the stain demands the constant use of positive and negative controls [3, 4]. A recent study exploring the sensitivity and reliability of synovial fluid crystal analysis has shown many discrepancies in the reading of alizarin red S stained fluids prepared by suspending synthetic BCP in synovial fluid [7]. For these reasons correlation between the different parameters studied by Hamilton et al. in relationship with the alizarin red positive scores must be taken with reservations.

Better quantitative techniques for clinical apatite or BCP crystal identification are required in order to investigate the relationship of CPPD and apatite crystal in osteoarthritis [8]. Binding with 14C disodium etidronate has been used but this is an indirect method and it is not readily available in most research or clinical laboratories [2]. Tetracycline is a good marker for apatite crystals in new bone recently formed at the calcification front or in other more hydrated and less mineralized calcified matrices [9], but in our hands has shown minimal binding to synovial fluid apatite containing particles. The specificity and sensitivity of alizarin red S stain can be improved by performing the staining on dry and absolute alcohol fixed synovial fluid preparations [10].

The origin of the apatite crystals in patients with osteoarthritis and 'chronic pyrophosphate arthro-
pathy' is not clear. Apatite crystals can be formed inside matrix vesicles of osteoarthritic cartilage, but in advanced osteoarthritis and severe rheumatoid arthritis could also come from subchondral bone [11, 12]. It is likely that pyrophosphate and apatite crystals are formed in different areas of degenerating articular cartilage [12]. Pyrophosphate crystals have been observed in close contact with collagen fibres and glycosaminoglycans outside of matrix vesicles [6]. Pyrophosphate in vitro and probably also in bone acts as an inhibitor of apatite crystallization, but this effect has not been so far demonstrated in articular cartilage or synovial fluid [11]. Although BCPs are commonly observed in osteoarthritic joints they usually are not associated with increased leucocyte count within the arbitrary non-inflammatory range between 0 and 2.0×10^9/l. It seems possible that these crystals might have the ability to activate synovial cells which may not be expressed in joint fluid cytology [1].

Hamilton et al. [5] provided a novel observation about intermittent CPPD crystal identification in patients with 'chronic pyrophosphate arthropathy'. This might suggest the presence of intermittent crystal shedding, active crystal 'traffic', transformation in other calcium phosphate phases or active crystal dissolution by pyrophosphatases. This drop in the crystal count could also be due to the presence of small and/or few crystals or sampling error. Quantification of crystals in synovial fluid as well as crystal size measurement are not easy parameters to measure in routine wet synovial fluid preparations.

In summary, better techniques for the clinical identification of apatite and BCP crystals are required in order to understand the significance of such crystals in patients with osteoarthritis and other forms of destructive arthropathies. Precise BCP crystal identification, quantification of individual crystals, their sizes, exact location in synovial fluid cells, synovium, and cartilage might be helpful to investigate the exact role of apatite or other BCP crystal in this setting. We hope that in the near future, techniques such as flow cytometry, now in use for characterization and quantification of cells, might become available for identification and quantification of different synovial fluid crystals [8].

A. J. Reginato
Cooper Hospital, University Medical Center, One Cooper Plaza, Camden, New Jersey 08103, USA

REFERENCES