studying the effect of nicotinic acid on uric acid metabolism. We have been treating patients with nicotinic acid who had hypercholesterolæmia. We noted that as the serum cholesterol was reduced the serum uric acid was elevated. This took place fairly consistently. However, the renal output of uric acid was not diminished, and we wondered if perhaps the intestinal cycle of uricolysis could be affected. We carried out some *in vitro* tests and we showed that nicotinic acid incorporated into solutions of uric acid prevented the breakdown of uric acid by intestinal organisms. We have also encountered patients who have been on antibiotics who suddenly developed acute gout in the absence of diminished renal excretion of uric acid. Further *in vitro* tests showed that nicotinic acid could act as a bacteriostatic agent. I am wondering if Dr Seegmiller has tested the effect of nicotinic acid with ETDA?

Dr Seegmiller: We have not used nicotinic acid in patients treated with this drug. We are rather limited in the amount of nicotinic acid that can be administered, and I have not checked to see whether or not we could get a comparable dose of nicotinic acid into the patient to whom we gave nicotinamide, because of the pharmacological effects of flushing and so forth that nicotinic acid produces. We have administered large doses of nicotinamide to some of our gouty overproducers, based on the rather naïve concept that perhaps if they had a defect analogous to that induced by the 2 ethylamino-1,3,4-thiadiazole they might also be helped by nicotinamide, and we found no evidence of any effect in a balance study. The precise way in which nicotinamide exerts its effect, as I said, is not known. It is very interesting to note, however, that there has been some suggestive evidence that this compound might be incorporated in nicotinamide adenine dinucleotide, and there is only one place in purine biosynthesis where such a dinucleotide has been involved – in the conversion of inosinic acid to xanthylic acid which is a puridine nucleotide-requiring enzyme. We have wondered whether or not this drug could be inhibiting this particular step of purine synthesis, but this is still in the realms of speculation.

Dr R W E Watts (*London*)*:* Has Dr Callaghan considered the possibility that the antibiotics have in fact acted on the kidney in the same way that the thiazide diuretics will sometimes produce an attack of gout?

Dr Cruess-Callaghan: Urinary uric acid was not reduced in the cases we have encountered. I wondered if the uric acid which normally enters the bowel could contribute to the urate pool and therefore elevate the serum uric acid level?

Dr Seegmiller: I am afraid we have no direct information on the transport of uric acid within the gut. The fact that Sorensen recovered uric acid in fæcal contents after intestinal bacteriostasis suggests that it is not reabsorbed appreciably by the bowel.

Dr V Eisen
(*Department of Pharmacology, Middlesex Hospital Medical School, London*)

Urates and Kinin Formation in Synovial Fluid

Kinins are polypeptides comparable in molecular size and pharmacological potency to the peptide hormones of the posterior pituitary. We are particularly concerned with kinins derived from plasma proteins. The chemical structure of three of these is known and is shown in Table 1, together with their principal pharmacological actions. The pain-producing effect of kinins has been extensively studied in man on the exposed nerve endings of an uncovered cantharidin skin blister, a method developed by Keele (Keele & Armstrong 1964).

When one considers the effects of kinins on the microcirculation, on leucocytes, and on the nerve endings of pain-conducting fibres, it becomes evident that these readily formed polypeptides may well act as mediators in the general and local reactions of the body to damage.

Enzymes which form plasma kinins are found in plasma itself and outside plasma (Fig 1). We may therefore talk about intrinsic and extrinsic plasma kinin formation. As first described by Margolis (1958), intrinsic plasma kinin formation is initiated by activation of the clotting Factor XII (Hageman factor). Activation of Factor XII initiates blood clotting and dissolution of blood clots, and also activates a specific kinin-forming enzyme called plasma kallikreinogen. Its active form, plasma kallikrein, then releases kinin from the kinin-yielding globulin kininogen. A plasma carboxypeptidase described as kininase digests kinin to inactive split products.

The fibrinolytic system contributes to plasma kinin formation by activating kallikreinogen and by attacking directly the substrate kininogen.

Outside plasma, kallikreins are found in the pancreas, salivary glands and urine. Some of the enzymes contained in lysosomes may be capable of forming plasma kinins.

Kinin-forming enzymes are restrained by inactivating agents.

The association of acute gouty arthritis with kinin formation started with studies by Seegmiller (Seegmiller *et al.* 1962) and McCarty (Faires & McCarty 1962), who provided dramatic support for the hypothesis proposed by Alfred Garrod in 1876, that the urate crystals present in most gouty joint effusions contribute to the symptoms of the acute attack. Injections of microcrystalline suspensions of monosodium urate into the knee-joints of healthy or gouty volunteers produced intense acute arthritis within one to three hours. The attacks closely resembled spontaneous gouty

Table 1
Plasma kinins of known structure

H – Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg – OH
Bradykinin

H – Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg – OH
Kallidin (lysyl-bradykinin)

H – Met-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg – OH
Methionyl-lysyl-bradykinin

Pharmacological effects of plasma kinins (doses for bradykinin):
(1) Vasodilatation (100–500 ng/kg; 1–100 ng/ml)
(2) Increase of vascular permeability (1–10 ng/ml)
(3) Actions on smooth muscle: contraction of rat uterus (0·1–1·0 ng/ml) and guinea-pig ileum (1–10 ng/ml); relaxation of rat duodenum (1–20 ng/ml)
(4) Production of pain (100–1,000 ng/ml)
(5) Bronchoconstriction (200–500 ng/kg)
(6) Migration of leucocytes (1,000–5,000 ng/ml)

arthritis, with severe local pain, warmth, tenderness, swelling and effusion.

Both groups of workers reported that the severity of the inflammation was roughly correlated with the percentage of crystals ingested by white blood cells present in synovial effusions.

On the basis of these findings, Seegmiller *et al.* (1963) suggested that acute gouty arthritis may develop in the following way:
(1) Crystals of monosodium urate deposited from body fluids with raised uric acid levels, must be present in joint tissue.
(2) An inflammatory reaction to the crystals develops.
(3) The inflammatory reaction is propagated by the addition of more urate crystals to the area of inflammation. This is attributed to the increased amounts of lactate produced by leucocytes engaged in inflammatory reactions and phagocytosis. The resulting lowering of the pH promotes precipitation of urates. A vicious circle is thus established.

More recent work suggests that kinins occur in the course of the induced and the spontaneous urate arthritis. Goldfinger *et al.* (1964) found that in gout and other types of arthritis, the synovial kinin concentrations were well correlated with the degree of tenderness. In some gouty effusions more than 50 ng/ml were found. Similar levels were induced by injections of microcrystalline suspensions into the human knee-joint. This concentration is amply sufficient to produce vasodilatation and increased capillary permeability, and would just be detected by the free nerve endings of a bare blister base. The pain receptors in the joint cavity may be more sensitive to bradykinin. It is also possible that 5-hydroxytryptamine is released from disintegrating platelets. This 5-hydroxytryptamine could potentiate the pain-producing effect of bradykinin, as some recent work by Sicuteri *et al.* (1965) suggests. Colchicine suppressed the crystal-induced arthritis and reduced the synovial kinin level. In dogs, acute arthritis could be produced by intra-articular injections of 0·5 μg–0·5 mg of bradykinin.

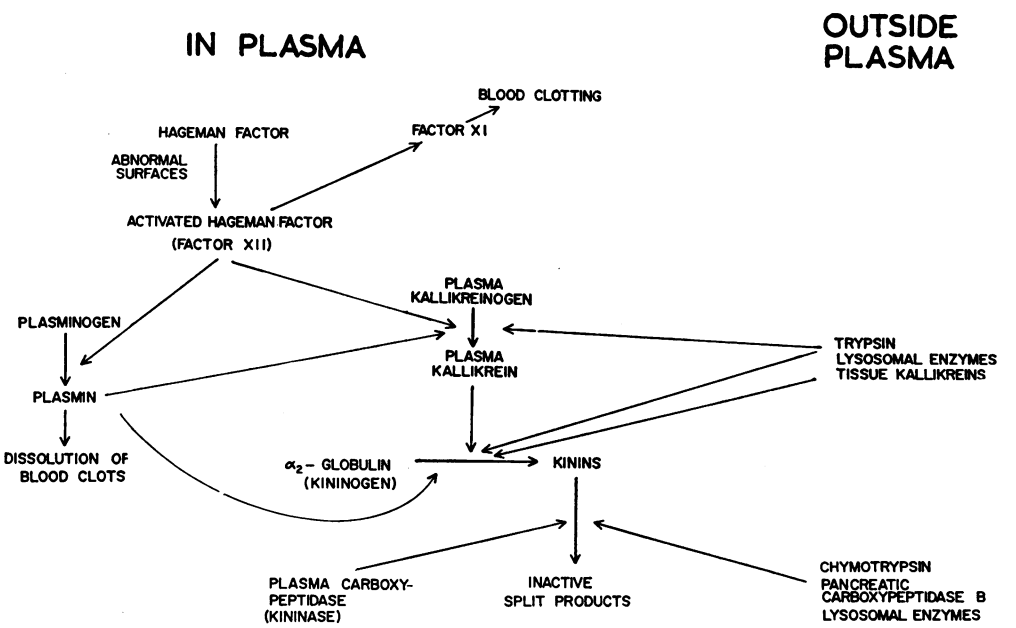

Fig 1 *Formation and destruction of plasma kinins*

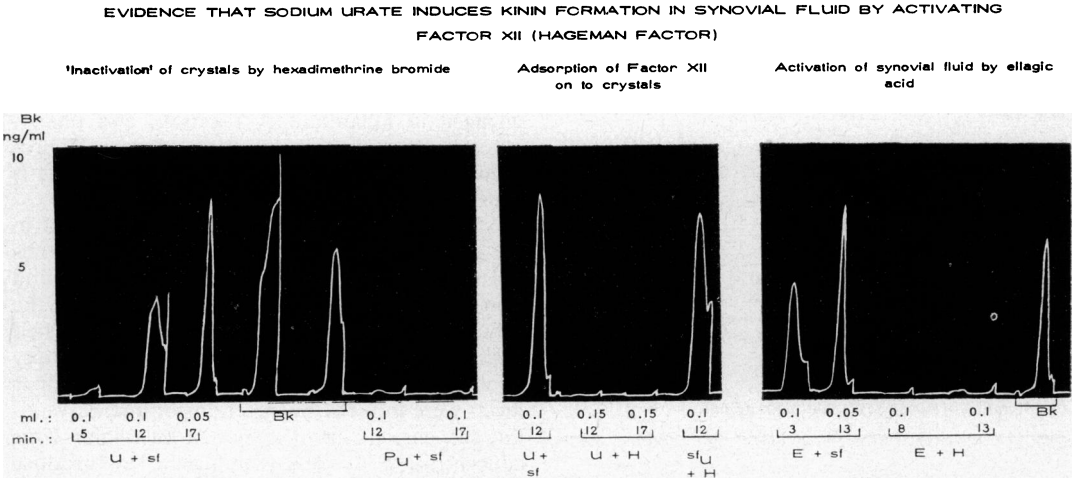

Fig 2A Fig 2B Fig 2C

Fig 2 *Evidence that sodium urate induces kinin formation in synovial fluid by activating Factor XII (Hageman factor). Rat uterus;* Bk=*responses to bradykinin 5 and 10 ng/ml. Volumes and times of tests as specified.* A, *kinin formation was induced in synovial fluid* (sf) *by 5 mg/ml of clean monosodium urate crystals* (U), *but not by crystals which had been immersed in hexadimethrine bromide (0·2 mg/ml) for twenty minutes, and then washed* (PU). B, *clean crystals activated a synovial fluid (test* U+sf). *After washing, these crystals activated Hageman trait plasma (test* sfU+H) *which does not respond to clean crystals because it lacks Factor XII (tests* U+H). C, *10^{-5}M ellagic acid induced kinin formation in synovial fluid* (E+sf), *but not in Hageman trait plasma* (E+H)

How are these kinins formed? Professor C A Keele and I are investigating this question and we have evidence for two possible pathways in gouty arthritis: (1) By a direct action of urate particles on the proteins in synovial effusions. (2) As a consequence of the phagocytic activity of the leucocytes in these effusions.

Action on Synovial Proteins

Kellermeyer & Breckenridge (1965) have recently reported that urate crystals promote blood clotting by activating the Factor XII in plasma. We examined whether Factor XII is present in synovial effusions, and whether it is capable of initiating synovial kinin formation. We used effusions from patients with rheumatoid arthritis. Their protein concentrations and composition are not unlike those in acute gouty arthritis. The fluids were rendered cell-poor by centrifuging soon after aspiration, then mixed with buffer and with 1–5 mg/ml of urate crystals. The mixtures were tested on isolated organs either directly, or

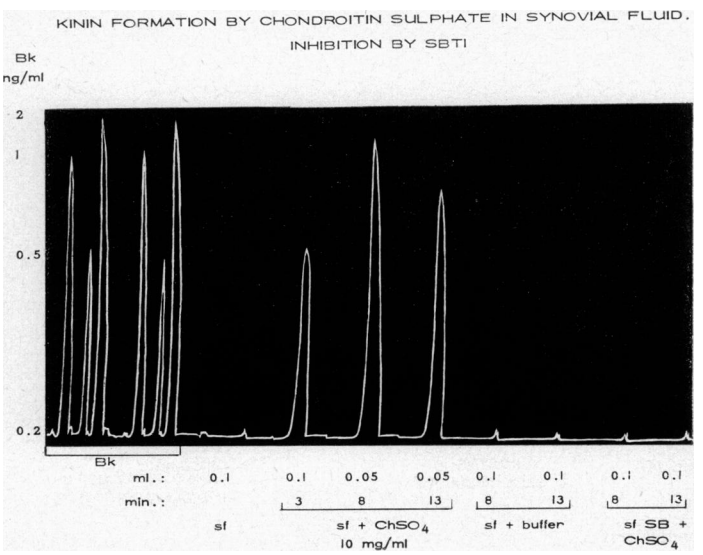

Fig 3 *Kinin formation by chondroitin sulphate in synovial fluid. Inhibition by SBTI. Rat uterus;* Bk=*responses to bradykinin 0·2, 0·5, 1·0 and 2·0 ng/ml. Volumes and times of tests as specified. Incubation with chondroitin sulphate* ($ChSO_4$) *led in this synovial fluid* (sf) *to a kinin level of 140 ng/ml at thirteen minutes. The effect was inhibited by soya bean trypsin inhibitor* (SB)

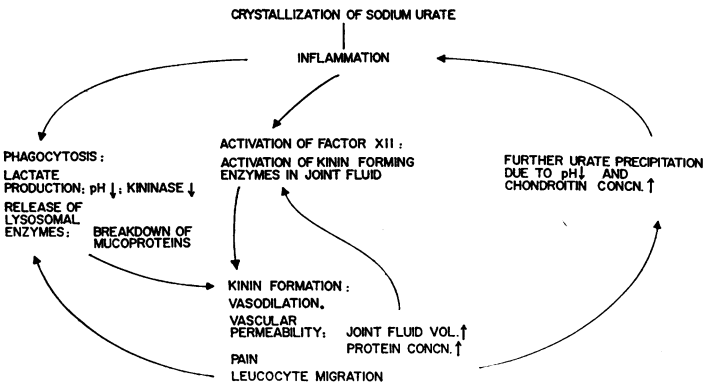

Fig 4 *Role of kinin formation in acute gouty arthritis*

after kinin formation had been stopped by soya bean trypsin inhibitor or by extraction procedures. The active substance had the pharmacological and chemical properties of a plasma kinin. Peak values developed at room temperature in ten to twenty minutes, and at 37° C slightly earlier. Kinin levels induced by 5 mg/ml of monosodium urate in 20 fluids ranged from 50 to as much as 3,000 ng/ml.

The presence and importance of Factor XII was supported by several lines of evidence (Fig 2):
(1) Brief exposure to the antiheparin agent hexadimethrine bromide abolished the capacity of urate crystals to activate Factor XII. The crystals resembled in this respect glass, kaolin and other activating substances (Eisen 1964).
(2) Like all activating surfaces, urate crystals firmly adsorbed Factor XII. Crystals which had adsorbed Factor XII induced kinin formation in Hageman trait plasma which does not respond to clean crystals. This plasma lacks Factor XII, but contains all other intrinsic kinin-forming agents.
(3) The presence of Factor XII in synovial effusions was confirmed by their response to ellagic acid, a specific activator of Factor XII (Ratnoff & Crum 1964).

Phagocytosis

How does phagocytosis by leucocytes influence the formation and destruction of bradykinin in synovial fluid? In the course of phagocytosis, enzymes are released from lysosomes into the cytoplasm and into the environment of the cell. The cell itself is often killed by the action of the released enzymes (Weissmann 1964). One of the lysosomal enzymes which could induce kinin formation in synovial effusions is cathepsin B whose bond specificity resembles that of trypsin. Admittedly, the pH optimum of cathepsins is 5·5 or less, but kinins are effective in such small amounts that even very low forming activity would be significant. We found that lysosomal fractions from rat liver and human leucocytes induced kinin formation in synovial fluid, and also contained a potent kinin-destroying factor.

Furthermore, lysosomal proteases and the plasmin activated by them digest the mucoproteins in cartilage and connective tissue (Lack 1964). Soluble mucopolysaccharides are released into the surrounding fluid, and might also promote kinin formation. Fig 3 shows that purified chondroitin sulphate, one of the principal polysaccharides of cartilage, induces kinin formation in synovial fluid.

In Fig 4, the discussed kinin-forming mechanisms have been summarized and added to the scheme of pathogenesis of acute gouty arthritis proposed by Seegmiller *et al.* (1963). Precipitated urate crystals lead to phagocytosis and to activation of Factor XII. Both processes result in kinin formation. The kinins bring about an influx of fluid and proteins which will promote further humoral kinin formation. They also attract leucocytes which will foster phagocytosis. Further precipitation of crystals will be induced by the acidity of lactate, and possibly also by the higher concentration of mucopolysaccharides which, according to Laurent (1964), reduce the solubility of uric acid.

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Dr K Trnavsky (*Piestany, Czechoslovakia*): In connexion with Dr Eisen's study I should like to say a few words about the possibility of influencing the tissue inflammatory reaction to sodium urate in rats by means of various anti-inflammatory and uricosuric drugs.

In experimental pharmacology the sterile host response to various irritants is frequently used to evaluate anti-inflammatory activity. The inflammatory response to synthetic microcrystalline sodium urate, as described originally in animals by Freudweiler, and by Faires & McCarty, and in humans by Seegmiller and collaborators, has not been used in experimental pharmacology except for the study of Wilhelmi on uric acid peritonitis in rats. In our laboratory we injected 0·2 ml of 10% microcrystalline sodium urate suspension, prepared according to the method of Seegmiller and collaborators, under the plantar fascia of rats, and in another experiment subcutaneously in the scapular region. In both applications we provoked sterile inflammation which was manifested after five days by a cellular reaction of leucocytes and histiocytes surrounding the clumps of sodium urate. Sometimes necrosis could also be seen.

The inflammatory response in the acute stage was inhibited significantly, according to our calculations by more than 40%, by phenylbutazone, ketophenylbutazone, demecolcine and hydrocortisone. Inhibition by sulphinpyrazone was not statistically significant. Of particular interest was the relatively good counteraction of promethazine and chlorpromazine upon the acute œdema.

We evaluated the granulomatous reaction according to dry weight of the granulomatous tissue after seven days of treatment, and also according to content of collagen. The quantity of granulomatous tissue as well as the collagen content was inhibited fairly well by hydrocortisone and demecolcine, and to a lesser extent by ketophenylbutazone and sulphinpyrazone. These anti-inflammatory agents may exert an action by blocking kinin-like substances, perhaps bradykinin or histamine, which are indispensable for both œdematous and fibroproliferative reactions.

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Professor C A Keele (*London*): There are two points that I would like to mention:
(1) The access of these substances to the appropriate nerve endings, presumably either in the synovial membrane itself, or possibly in the capsule which is more highly innervated. In such cases there might be diffusion of the substance formed in the main bulk of joint fluid back into the synovial membrane, and in the inflammatory condition diffusion would be much easier than normal. But it might well be true that the actual changes would take place within the synovial tissue, and therefore the substances would act directly on the surrounding nerve endings.
(2) The variability in response to the contact between these urate crystals and the different samples of synovial fluid. When we first started to study this particular reaction we tried it on plasma and it did not appear to work, but then we found later that it did seem to work on these synovial fluids (which, of course, are essentially comprised of exuded plasma). These inflammatory exudates certainly have reacted on the whole very favourably, but very variably.

That leads on to a point concerning the presence of these very unstable substances, plasma kinins, which are formed in a rather complicated way and can be destroyed by this plasma kininase very quickly so that to get significant levels production must exceed destruction. It is therefore a very dynamic situation; it may be that several of these factors could vary and that this variability might be an important matter, not only in relation to this particular form of gouty arthritis, but also in other forms of arthritis.

Dr R Penny (*Sydney, Australia*): Under the appropriate stimulus for inflammation polymorphonuclear leucocytes (PMN) in the small vessels first adhere to the endothelium, then they migrate through it and adhere to the objects of their prey, following which phagocytosis occurs. In the post-phagocytic phase, neutrophil granules discharge into the phagocytic vacuole.

At the Postgraduate Medical School, London, I have been studying two aspects of polymorph function with reference to the effects of colchicine:
(1) PMN adhesiveness which is determined by allowing whole blood to pass through a glass bead column under standardized conditions (Garvin 1961). The adhesiveness index, AI, which is the ratio of the absolute number of PMSs/c.mm entering the column to the absolute number of PMNs/c.mm emerging from the column, probably reflects the ability of PMNs to adhere to the vascular endothelium (a vital prerequisite for emigration) and/or to the particle to be phagocytosed.
(2) PMN phagocytosis of yeast particles (Brandt 1965) which is obtained quantitatively. The median number of yeast particles phagocytosed by each cell (counting 100 cells) is termed the phagocytic index, PI.

In this study, 3 non-gouty subjects were studied. Blood was taken initially and the AI and PI obtained. Colchicine (in an amount equivalent to 10 mg distributed in the subject's blood volume) was added *in vitro* to this sample and AI and PI repeated. The subjects then took 5·5 mg of colchicine orally over the next eight hours. Further blood samples were taken at four, eight, and in one case twenty-four, hours after commencement of colchicine.

No significant changes in PI were obtained either when the subjects took oral colchicine or in the samples to which colchicine was added *in vitro*.

However, quite significant changes were obtained in the AI. In the *in vitro* study, the AI was reduced by 50% or more in all cases (i.e. PMN adhesiveness was reduced by at least one-half). In 2 of the 3 subjects, after taking colchicine orally, the AI progressively fell again by 50% or more at eight hours, to a figure very close to that obtained with *in vitro* colchicine. In one of these 2 subjects, the AI had returned to normal at twenty-four hours. In the third subject, the AI fell by about 50% at four hours (after 3·5 mg colchicine) but had returned to original levels at eight hours.

One concludes from this preliminary study that colchicine both *in vivo* and *in vitro* causes a marked reduction of PMN adhesiveness but shows no effect on phagocytosis in the system and with the doses of colchicine used. No study has been made on the post-phagocytic phase to which Dr Eisen has referred.

It appears, therefore, that colchicine may interfere with the inflammatory response by reducing the initial phase of leucocyte emigration which is vitally dependent on the phenomenon of adhesion. Whether the site of action is on serum or other factors (e.g. kinins) or due to a direct effect on leucocytes is being further investigated.

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Dr J Seegmiller (*Bethesda, USA*): It is gratifying to see that you could get some effect with that particular dose of colchicine. We were unable to be sure that we had got an effect with orally administered colchicine in our studies on leucocyte metabolism, so we gave it intravenously, which results in a substantially higher concentration of colchicine in the systemic circulation.

Dr Goldfinger, working in our laboratory, has been studying the effects of colchicine on certain aspects of leucocyte metabolism. This involves the effect on the uricolysis of sodium urate-6-^{14}C, which presumably reflects some aspect of the phagocytic action or lysosomal destruction that accompanies phagocytosis of the urate crystals. At the doses he was using *in vitro*, which were just a few micrograms, and which we felt were comparable to the doses produced by the intravenous administration of the therapeutic dose, he did find some effects on this particular parameter. He also felt that there was a definite effect on phagocytosis of urate crystals themselves. What the difference in phagocytosis of urate crystals and the phagocytosis of yeast particles might represent, I am not prepared to say.

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Professor E G L Bywaters (*Taplow*): Dr Penny's work rather suggests that colchicine puts the skids under the polymorph, making it more difficult for it to travel along the tissue planes. It might be a question of polymorph mobility which would not really be operative in a free solution with yeast cells just floating round, where there is no transport problem.

Dr R W E Watts (*London*): A point I would like to raise is the role of pH changes in perpetuating the response in acute gouty arthritis. This does seem to be rather critical from the point of view of the cyclic scheme that Dr Seegmiller has evolved, and also it might affect the kinin formation. What information do we have on the pH changes during the initiation and evolution of acute gouty arthritis, or indeed in the experimental urate arthritis?

Dr Seegmiller: At a recent symposium on gout Dr D S Howell of the University of Miami had some very interesting data in which he had shown – using extremely small micro glass electrodes – a lower pH of cartilage than that of the surrounding tissues. This is what one would expect, since cartilage is deprived of blood supply and must function under relatively anærobic conditions. Conversion of glucose to lactic must provide the energy of these tissues. We have done some studies using leucocytes obtained from a synovial effusion in which there was also a large number of urate crystals. An aliquot of this was placed, without being exposed to the air, in an incubator with an immersed glass electrode so that we could follow the pH changes. Other aliquotes were incubated and removed at varying times to measure lactic acid production. We found that there was indeed a marked increase in lactic acid production and a decline, as one would expect, in pH accompanying several hours incubation of this leucocyte urate crystal suspension.

I suspect that our early attempts to demonstrate a definite effect on pH in synovial fluid were limited by the sensitivity of our pH measuring apparatus. It is also complicated by the fact that within this acute inflammatory process, with exudative processes going on, there is undoubtedly a very rapid turnover of the fluids within the synovial cavity. So I should expect that lactic acid might be washed out at a fairly rapid rate from these tissues.

The problem of the physical chemistry of precipitation in sodium urate as a function of pH is one which we have investigated from time to time, and there is no doubt that the solubility of urate in a buffer solution is enhanced with alkalinity. The precise way in which pH affects urate solubility at a pH less than 7·4 is complicated by the presence of two molecular species. The pKa of uric acid is 5·7; therefore at pH 7·4 almost all of the molecules are present as urate ions. As the pH is lowered an increasing fraction will be present as undissociated molecules of uric acid and each form of the molecule will have its independent solubility.

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Dr Eisen: Professor Keele's point that kinins might be floating around the nerve endings where it matters, and not be detectable in other parts, is very true, because kinin has an extremely short half life. *In vivo* estimates vary from 1·25 seconds to 30 seconds. This is mainly due to the activity of kininase which, in turn, is significantly affected by physiologically occurring alterations of the pH.

For the responsiveness of synovial fluids we have so far looked at two factors. All the forming factors which you have seen are globulins, and some of the inhibiting or inactivating factors are albumins, so any change in the profile of plasma and proteins towards globulins will favour kinin formation.

Another factor which we tested is hyaluronic acid because that is an important peculiarity of synovial fluid. Although the inflammatory effusion has only about one-tenth or one-twentieth of the hyaluronic acid content of normal synovial fluid, it is still high. However, a removal of hyaluronic acid from synovial effusions by hyaluronidase did not affect their kinin forming response.