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PLASMA KININS IN SYNOVIAL EXUDATES

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SUMMARY.—Kinin levels equivalent to 1–30 $\times$ 10^{-9} M bradykinin were found in 33 out of 82 exudates from rheumatoid joints. Only 2 of 27 fluids from joint effusions caused by osteoarthrosis or trauma, contained detectable levels of kinin (1–5 $\times$ 10^{-9} M bradykinin). The kinin concentrations showed little or no correlation with the white blood cell counts, protein concentrations and lactate dehydrogenase and acid phosphatase activities, in synovial fluids. Data on the pain felt in joints suggested that in higher concentrations kinins contribute to arthritic pain, but that other pain-producing factors are undoubtedly involved.

PLASMA kinins are pharmacologically potent polypeptides which increase the calibre and permeability of small blood vessels, cause pain, and attract leucocytes. They are thus capable of producing the principal signs of inflammation. The 3 plasma kinins of known structure, called bradykinin, lysyl-bradykinin (kallidin) and methionyl-lysyl-bradykinin, are chains of 9, 10 and 11 amino acids, respectively. There are only small quantitative differences in their biological actions. These kinins are released from plasma globulins by specific enzymes (kallikreins) and non-specific enzymes, *e.g.* plasmin, which normally circulate in blood in a preactive state. The plasma kinin-forming enzymes and substrates are also present in synovial exudates, where their concentrations may approach those in plasma (Armstrong, Jepson, Keele and Stewart, 1957; Eisen, 1966; Jasani, Katori and Lewis, 1967). Cells may contain non-specific, or in some glandular cells, specific kinin-forming enzymes.

Melmon, Webster, Goldfinger and Seegmiller (1967) found kinin equivalent to bradykinin 3–44 ng./ml. in 6 out of 6 exudates from rheumatoid joints. The present paper reports synovial kinin levels in a larger series of rheumatoid patients. A short account of this work has been presented (Eisen, 1969).

MATERIALS AND METHODS

Exudates were removed from knee joints with plastic disposable syringes. To extract kinin, 2 ml. of exudate was placed within 2–3 min. into 8 ml. of ice-cold 90 per cent ethanol; to extract 5-hydroxytryptamine, 4 ml. of exudate was placed into 20 ml. of ice-cold acetone The precipitated protein and other matter was removed from ethanol extracts by centrifuging at 0° within 30 min., and from acetone extracts after 24 hr. The supernatants were dried in an air stream, and then redissolved in 1 ml. of Ringer-Locke's solution. Extraction with cold ethanol recovered 60–80 per cent of bradykinin added to synovial exudate, which is similar to the recovery from plasma reported by numerous other workers. Kinin and 5-hydroxytryptamine were measured on the isolated rat uterus suspended from an iso- or auxotonic lever (Eisen, 1963). Kinin was also identified by its relaxing effect on the rat duodenum which was prepared according to Gaddum and Horton (1959). The actions of

histamine on the organs were inhibited by mepyramine maleate 1 mg., of acetylcholine by atropine 0·5 mg., and of 5-hydroxytryptamine by bromo-lysergic acid diethylamide 0·1 mg., per l. of bath fluid.

Protein was measured by the method of Lowry, Rosebrough, Farr and Randall (1951), lactate dehydrogenase by the method of Wroblewski and LaDue (1955), and acid phosphatase by the method of Fishman and Lerner (1953). Before these tests, exudates were centrifuged at 25,000 × *g* for 30 min.

RESULTS

Fig. 1(a) shows the distribution of kinin levels in 82 exudates from 73 patients with a diagnosis of classical or definite rheumatoid arthritis established at the Department of Rheumatology and Physical Medicine, the Middlesex Hospital. Kinin levels equivalent to 1–30 × 10^{-9} M bradykinin were found in 33 of these exudates. The kinin-like nature of the active substance was demonstrated by

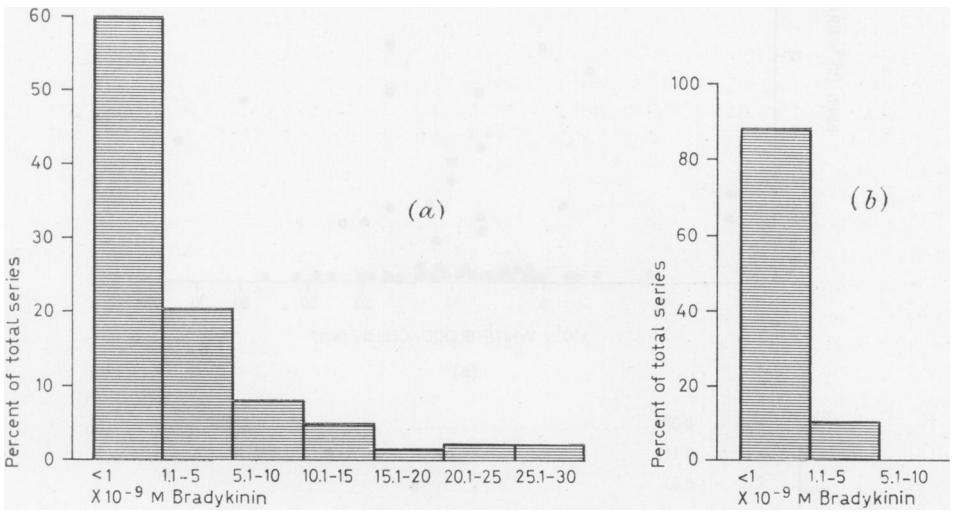

FIG. 1.—Distribution of kinin concentrations (expressed as 10^{-9} M bradykinin) in 83 exudates from 73 patients with rheumatoid arthritis (a), and in 27 exudates caused by oesteoarthrosis or trauma (b). Heights of columns indicate the numbers of exudates with kinin levels of a given range, as a percentage of the total number of exudates.

findings that it contracted the rat uterus and relaxed the rat duodenum; these actions were not abolished by pretreatment of the assay organ with inhibitors of histamine, acetylcholine and 5-hydroxytryptamine. The substance was promptly inactivated by kinin-destroying enzymes like chymotrypsin and plasma kininase (carboxypeptidase N), but not by trypsin. No attempt was made to determine which plasma kinin was present in the exudates, and its concentration was measured in terms of bradykinin. In a series of 27 effusions caused by osteoarthrosis or trauma, kinins were found only in 2 fluids, and in concentrations of less than 5 × 10^{-9} M bradykinin (Fig. 1(b)). It is unlikely that the difference was due to the lower protein concentrations in the non-rheumatoid fluids, since dilution of plasma often favours kinin formation (Miles, 1964; Schachter, 1956). In the rheumatoid series, kinin levels were not correlated with protein concentrations ($r = 0{\cdot}123$, s.e. $= 0{\cdot}143$, $2\alpha > 0{\cdot}1$).

No 5-hydroxytryptamine was detected in the acetone extracts of these exudates.

Intracellular, in particular lysosomal, enzymes released from leucocytes are regarded as one of the causes of the inflammation and tissue damage in the rheumatoid joint (Page Thomas, 1967). To assess whether such enzymes were

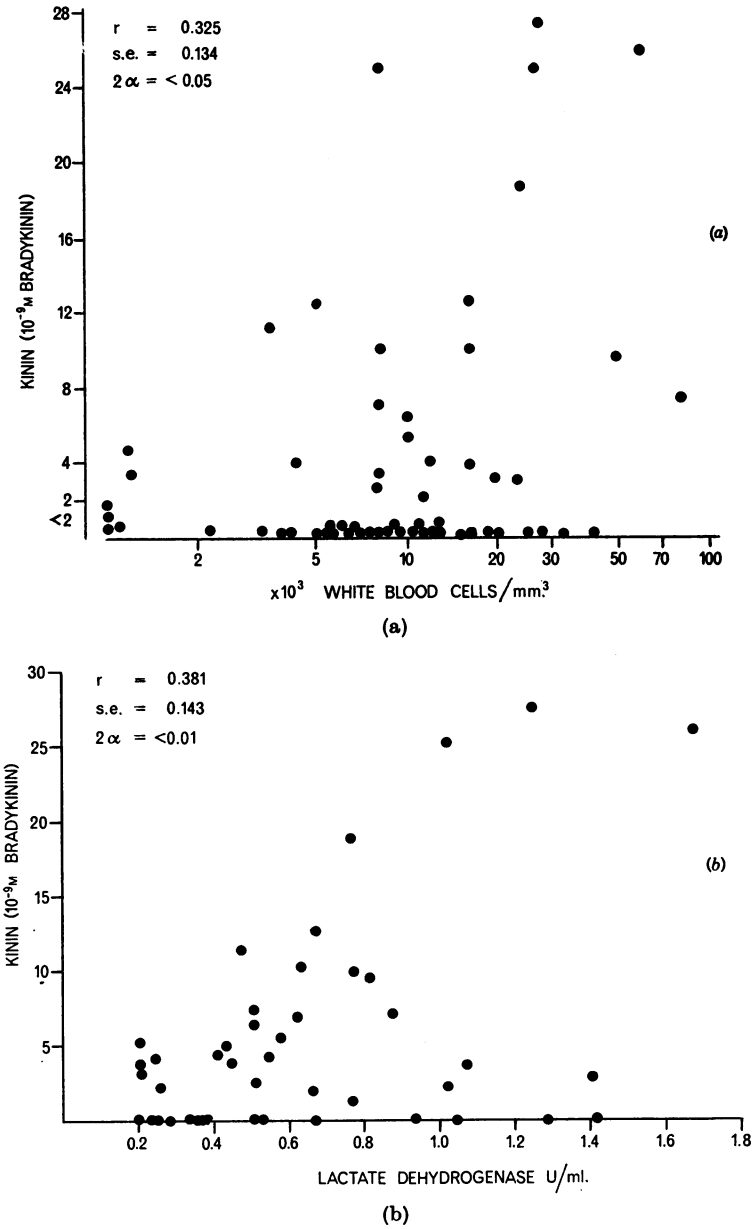

FIG. 2.—Correlation of kinin levels in exudates with white blood cell counts (a) and lactate dehydrogenase (b). r = correlation coefficient.

also responsible for synovial kinin formation, the kinin levels were plotted against cell counts, and against 2 intracellular enzymes, *viz.* the cytoplasmic lactate dehydrogenase (LDH) and the largely lysosomal acid phosphatase. Kinin concentrations were slightly correlated with cell counts and with LDH levels, (Fig. 2(a), (b)) but not with acid phosphatase levels ($r = 0{\cdot}028$, s.e. $= 0{\cdot}129$, $2\alpha = > 0{\cdot}1$).

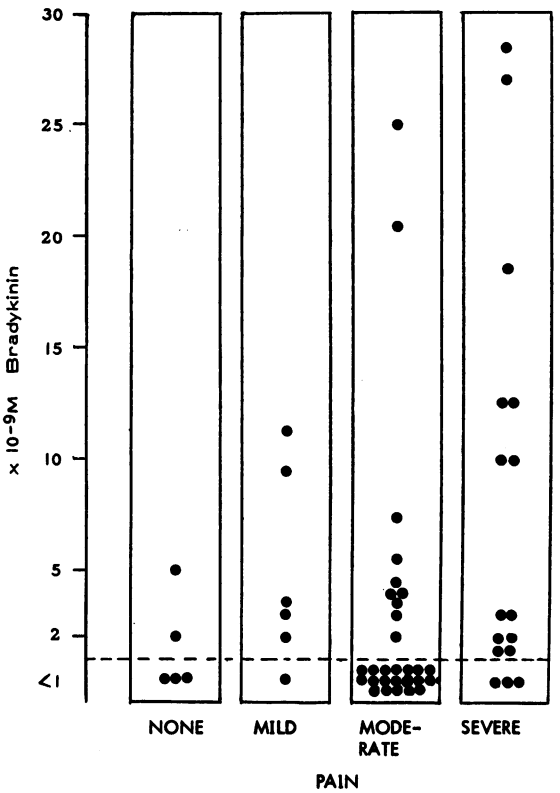

FIG. 3.—Distribution of kinin levels in exudates according to the pain felt in the rheumatoid joints before fluids were removed from them.

Kinins and pain in joints.—Plasma kinins are amongst the most potent algogenic substances occurring in the mammalian body (Keele and Armstrong, 1964). Before exudates were removed, patients with rheumatoid arthritis were asked whether they had no, mild, moderate or severe pain in the joint. The kinin levels in 56 exudates from 50 patients were distributed according to the patients' replies (Fig. 3). Many fluids from painful joints contained no kinin. However, most of the higher levels were associated with pain in the joints.

DISCUSSION

The calibre and permeability of small vessels are increased by 10^{-9}–10^{-8} M bradykinin; pain receptors are stimulated by 10^{-7}–10^{-6} M solutions. It seems

probable therefore that the detected kinins, particularly in the higher concentrations, contributed to the swelling, heat, effusion, and pain in the rheumatoid joints.

In the present work, kinins were detected in a lower proportion of exudates and mostly in lower concentrations, than was the case in the 6 patients studied by Melmon *et al.* (1967), who used a different extraction procedure. Because of the great ease with which kinins are both formed and destroyed in plasma and exudates, artificial generation and/or loss during extraction are difficult to exclude.

Kinin-forming as well as kinin-destroying factors have been described in synovial exudate, in the cells contained in it, and in synovial tissues (Armstrong *et al.*, 1957; Eisen, 1966; Greenbaum and Yamafuji, 1966; Zachariae, Malmquist and Oates, 1966; Melmon *et al.*, 1967; Jasani, *et al.*, 1967, 1969). The kinin levels found in synovial exudates are the result of dynamic equilibria between all these factors. It is not yet known whether removal of the fluids from the joints shifts the overall equilibrium towards the formation of kinins or towards their destruction. The half life of bradykinin in human plasma is about 6–7 min. (Frey, Kraut and Werle, 1968), and probably longer in synovial exudates (Jasani *et al.*, 1967). Bradykinin circulating in blood *in vivo* has a much shorter half life, estimated at 30 sec. in man (Saameli and Eskes, 1962) and 17 sec. in dogs and cats (Vane, 1969). Thus uptake and destruction by tissues plays a much more important role in the removal of kinins, than does digestion by plasma kininases. It is therefore probable that the true kinin levels in the synovial exudates were reduced during collection and extraction by less than 50 per cent. On the other hand, it seems unlikely that the detected kinins were the result of artifacts during collection and extraction of the exudates, because no kinin was found in the large majority of exudates. Kinins equivalent to $1–5 \times 10^{-9}$ M bradykinin were present only in 3 out of 14 ethanol extracts prepared from human plasmas. However, in the experience of Talamo, Haber and Austen (1969), extraction of blood by ethanol resulted in higher levels, *viz.* 4–33 ng./ml. (approx. $4–33 \times 10^{-9}$ M bradykinin).

The pain felt by patients in the joints before removal of the exudates, suggested that in higher concentrations kinins may contribute to arthritic pain. There was no doubt, however, that other pain-producing substances were involved.

The attempts to correlate the kinin levels with the white blood cell counts in the exudate, and with the levels of two enzymes mainly derived from these cells, provided no support for the view that synovial kinins are formed by enzymes released from the leucocytes. Jasani *et al.* (1967, 1969) found only traces of kinin-forming activity in cell pellets from centrifuged synovial exudates. They concluded that any kinin present in synovial exudates would be the result of enzyme activities in the fluid phase. Kinins are therefore possibly formed in the rheumatoid joint by modes which are independent of cellular factors. One possible mode is considered in the following paper (Eisen and Smith, 1970).

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