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LETTERS TO THE EDITOR

Total and free-plasma tryptophan concentrations in rheumatoid disease

It has been proposed (McArthur, Dawkins & others, 1971) that human connective tissue diseases may arise from modifications in the binding characteristics of the plasma proteins. McArthur, Smith & Freeman (1972) presented evidence that human serum contains a substance that possesses anti-inflammatory activity and is bound to circulating proteins. Furthermore, it has been suggested that, in patients with active rheumatoid arthritis, the anti-inflammatory substance is bound to an abnormal extent to the plasma proteins and that the clinically useful antirheumatic drugs act by re-establishing the bound: free ratio to that in the normal subject (Smith & Dawkins, 1971). There is evidence that the behaviour of L-tryptophan mimics that of the hypothetical substance which protects susceptible tissues against chronic inflammatory insults. All the clinically useful antirheumatic agents were found to displace L-tryptophan from human plasma *in vitro* (McArthur, Dawkins & Smith, 1971) but not so other drugs which bind to plasma proteins to an equivalent extent but have no demonstrable antirheumatic effects (Smith, Dawkins & McArthur, 1971). In patients with rheumatoid arthritis receiving drug therapy the percentage of L-tryptophan bound to plasma proteins is significantly reduced but increases when drug administration is stopped (McArthur, Dawkins & others, 1971).

We now wish to report an investigation into total and free tryptophan concentrations in the plasma of patients with "untreated" rheumatoid arthritis and the displacement of the amino-acid from patient's plasma *in vitro* by antirheumatic drugs and oestrogens. Twelve male and 12 female patients aged 24–56 years with classical or definite rheumatoid arthritis (American Rheumatism Association Criteria—ARA; Ropes, Bennett & others, 1959) of at least one year's duration were studied. Twenty were receiving therapy with one or more of the following drugs until two weeks before measurements were undertaken: aspirin, indomethacin, prednisolone, alclofenac and aloxiprin. During the 14-day period following withdrawal of antirheumatic drug therapy the patients were allowed paracetamol only (1 g-6 hly; mean daily dose 3·9 g), which possesses analgesic but not anti-inflammatory activity (Boardman & Hart, 1967). Four patients had received no antirheumatic agents for four weeks before the study. Corresponding control groups were 14 men (aged 28–51 years) and 12 women (aged 20–60 years). None of the healthy controls had taken drugs (including oral contraceptives) for at least 14 days before the study. All subjects had been fasting overnight before blood was obtained by venepuncture into dry lithium heparin tubes. Tryptophan levels in plasma were measured by the method of Wapnir & Stevenson (1969). Free tryptophan was separated using centrifugation with Amicon CF-50 membrane cones.

Table 1 shows that there is a significant increase in total plasma-tryptophan in patients with rheumatoid arthritis compared with corresponding control groups. Furthermore, both the concentrations and percentage of free-tryptophan are significantly lower in the groups of patients than in the control groups. There is no difference in plasma-tryptophan levels between the groups of normal subjects and although there was a tendency to greater total levels in male compared with female

Table 1. *Plasma total and free* L-*tryptophan concentrations in patients with untreated rheumatoid arthritis and corresponding control subjects.* "Untreated" is arbitrarily defined as clinically active disease treated by paracetamol alone for at least two weeks before the experiment.

	Age (yr)		Plasma-tryptophan levels					
			Total (μg ml^{-1})		Free (μg ml^{-1})		% free	
	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Female controls (n = 12)	42·6	1·3	11·92	0·39	0·86	0·09	7·2	0·55
Female rheumatoid patients (n = 12) ..	49·4	2·1	14·13	0·98*	0·71	0·11†	5·0	0·49†
Male Controls (n = 14)	42·4	1·6	12·46	0·97	0·91	0·10	7·4	0·72
Male rheumatoid patients (n = 12) ..	50·2	2·8	15·11	1·01*	0·72	0·08†	4·7	0·89†

Significance of difference from normal: $P < 0{\cdot}001$*
$P < 0{\cdot}05$†

patients, this was not significant ($P > 0{\cdot}05$). No correlation was found between age and plasma-tryptophan levels in any groups. That paracetamol itself was not responsible, is suggested by our finding that in a group of 7 females and 4 males with osteoarthritis alone who had paracetamol 3–4 g daily for up to 21 days, plasma tryptophan concentrations were not significantly different from those of healthy controls. Furthermore in 3 patients with polyarthritis (defined by the ARA) who had not taken anti-inflammatory analgesic drugs for 13 days, plasma tryptophan levels were significantly increased ($P = 0{\cdot}03$) compared with controls while free tryptophan was significantly ($P < 0{\cdot}05$) lower.

Plasma samples from patients and corresponding normal plasma were exhaustively treated by ultrafiltration to remove any amino-acids and drugs present and then

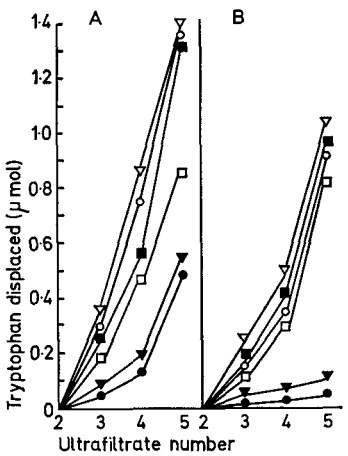

FIG. 1. Displacement of L-tryptophan from serum of patients with rheumatoid arthritis, and from serum of normal controls by anti-rheumatic drugs and natural oestrogens. The results have been plotted as the cumulative displacement of the amino-acid against the ultrafiltrate number. The second ultrafiltrate was obtained after the initial exposure of the cleared serum to 0·5mM tryptophan, the third, fourth, and fifth ultrafiltrates were obtained after the subsequent addition of 100, 200 or 300 μg ml^{-1} of the antirheumatic drugs, or 2, 4, or 6 ng 10 ml^{-1} of the oestrogens.
A, Normal serum; B, Serum of patients with rheumatoid disease; O, indomethacin; □, phenylbutazone; ■, salicylate; ▽, alclofenac; ▼, piperazine oestrone sulphate; ● conjugated equine oestrogens.

exposed to increasing concentrations of L-tryptophan by the method of McArthur & others (1971). The procedure described by McArthur & Dawkins (1969) was used to measure the displacement of L-tryptophan from the cleared plasma of normal controls and patients by antirheumatic drugs. In addition, however, the procedure was repeated successively with 10 ml quantities of buffer containing 2 ng of piperazine oestrone sulphate, then 2 ng of conjugated equine oestrogens.

In the presence of salicylate, indomethacin, phenylbutazone, alclofenac, or the natural oestrogens, the amounts of unbound tryptophan in the ultrafiltrates are greater than in the absence of these agents. However, the displacement of the bound tryptophan by all the antirheumatic agents was significantly greater ($P < 0{\cdot}01$) for the normal plasma whilst there was also a significant difference ($P = 0{\cdot}05$) in the amounts of unbound tryptophan in the ultrafiltrates of normal plasma and patients plasma in the presence of the oestrogens (Fig. 1). These drugs therefore displace the protein-bound amino-acid, the effect being greater as the serum -|- bound tryptophan becomes exposed to increasing concentrations of the antirheumatic agents.

The present work suggests that in patients with active rheumatoid disease who are not receiving antirheumatic drugs, an abnormally high proportion of L-tryptophan is bound to the circulating proteins which also manifests as increased total-tryptophan, and decreased free-tryptophan concentrations when compared with values in the plasma of corresponding healthy controls. These findings support the hypothesis of McArthur, Dawkins & Smith (1971), that the plasma-proteins in rheumatoid disease exhibit an altered binding affinity for small molecules compared with normal plasma proteins. The present work also shows that the ability to displace L-tryptophan *in vitro* from its protein binding sites is shared, to a lesser degree, by the natural oestrogens at the concentrations used. The latter observations may, in part, explain the raised free tryptophan concentrations found in pregnancy (McArthur, Dawkins & others, 1971), a condition known to be associated with remissions in rheumatoid arthritis (Hench, 1949).

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