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ADDENDUM—Since completion of this manuscript, it has been found that the pH 4·0 chloroform extraction step can be omitted if the Dowex 1 columns are eluted with 0·5M acetate buffer, pH 4·0. This gives an even cleaner gas chromatogram than Fig. 1B. Recovery and reproducibility have not yet been determined.

Effect of two non-steroidal anti-inflammatory agents on alkaline and acid phosphatases of inflamed tissue

SIR,—A large increase of phosphatase was found by histological methods to take place in inflamed tissue (Monis & Rutenburg, 1960; Georgiev & Bachvarova, 1962), but no biochemical data have been presented. Continuing our observations on naphthypramide [α-isopropyl-α-(2-dimethylaminoethyl)-1-naphthylacetamide], a new anti-inflammatory agent (Coppi & Bonardi, 1968), we report in this paper on its activity, compared to phenylbutazone, on alkaline and acid phosphatases of inflamed tissue.

Inflammation and treatments with anti-inflammatory drugs were as previously described (Coppi & Bonardi, 1968). The inflamed tissue from paw pads of animals, killed 48 hr after kaolin and 24 hr after carrageenan subplantar injection

TABLE 1. EFFECT OF NAPHTHYPRAMIDE AND PHENYLBUTAZONE ON ALKALINE AND ACID PHOSPHATASES OF INFLAMMED TISSUE OF RATS

Treatment	Dose of drug mg/kg (oral)	Alkaline phosphatase μg of P/mg N				Acid phosphatase μg of P/mg N			
		Rats No.	Mean ± s.e.	P*	P**	Rats No.	Mean ± s.e.	P*	P**
Kaolin oedema									
Normal control	—	12	71 ± 3	—	—	14	175 ± 10	—	—
Inflammation	—	16	202 ± 21	<0·001	—	9	309 ± 30	<0·001	—
Inflammation + naphthypramide	100 × 4	13	116 ± 5	<0·001	0·001 < P <0·01	8	246 ± 11	<0·001	>0·05
Inflammation + phenylbutazone	50 × 4	12	110 ± 6	<0·001	0·001 < P <0·01	8	218 ± 8	<0·001	0·01 < P <0·02
Carrageenan oedema									
Normal control	—	13	73 ± 4	—	—	9	168 ± 37	—	—
Inflammation	—	10	250 ± 16	<0·001	—	10	284 ± 28	0·02 < P <0·05	—
Inflammation + naphthypramide	80 × 3	10	173 ± 15	=0·02	<0·001	10	207 ± 12	>0·05	0·02 < P <0·05
Inflammation + phenylbutazone	40 × 3	10	134 ± 8	0·02 < P <0·05	<0·001	10	178 ± 15	>0·05	0·001 < P <0·01

* Statistical significance of difference between treated and normal controls.
** Statistical significance of difference between treated and inflamed controls.

was homogenized in water at +4°. Alkaline and acid phosphatases (Quastel, 1961) and nitrogen according to Kjeldhal were assayed on the 10% homogenate. In addition, we assayed nitrogen, alkaline and acid phosphatases in the non-inflamed paws of treated rats, on inflamed paws of untreated animals and on non-inflamed paws of untreated animals.

The results (Table 1) show that both phosphatases, assayed biochemically, were much increased in the inflamed tissue; naphthypramide and phenylbutazone reduced both of these phosphatases, phenylbutazone being more active than naphthypramide. The anti-inflammatory drugs tested had no activity on phosphatases of non inflamed tissues.

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Iron carbohydrate complexes

SIR,—Iron complexes of carbohydrates are used in the treatment of iron-deficiency anaemia. No satisfactory chemical formula can be assigned to these substances but there are many possibilities. In a previous investigation of the iron dextran complex (Ricketts, Cox & others, 1965) it was found that ferric iron linked together dextran molecules of molecular weight about 5,000 into large aggregates having a particle size of 3 to 4 μ.

Molecular sieving by gel filtration on columns of Sephadex was useful in examining the iron dextran complex and the same technique has now been applied to the iron-sorbitol-citrate complex (Jectofer, Astra-Hewlett) (Lindvall & Andersson, 1961).

Gel filtration. Columns of various types of Sephadex in saline 48·5 cm long and 1·5 cm diameter were used; 0·5 ml of a 1 : 5 dilution of the complex in saline was applied to the column and eluted into 2 ml fractions at a rate of 8 ml/hr. The iron colour was measured at 430 mμ; Sephadex with spherical grains gave good recoveries of iron. Using Sephadex G-15, 91·5% of the iron was recovered, all in the excluded volume. This indicated that no complex with a molecular weight of less than about 1,500 (the approximate exclusion limit for this type of Sephadex) was present. From Sephadex G-25, 99·2% of the iron was recovered and although most of the iron was again in the excluded volume there was some (14·8% of the whole sample) in later fractions from the column, indicating the presence of some iron complex with a molecular weight less than 5,000.

Optimum fractionation was observed on Sephadex G-50, the iron complex being present in all fractions, as shown in Fig. 1a. The appearance of two peaks does not necessarily indicate the presence of two distinct components, simply that material with molecular weight greater than the exclusion limit is accumulated in the excluded volume. Fraction 13 and 14 (10·8% of the whole sample) were concentrated by ultra filtration and re-chromatographed; the peak was in the same position, demonstrating the validity of the gel filtration. In this experiment it was evident from the position of the sucrose control that the iron-sorbitol-citrate complex was of much greater molecular weight than any of its constituents. The