proteases [9]. The trypsin-inhibiting capacity (T.I.C.) of normal male rat blood was found to be 3.5–3.8 μmole/min/ml (Parrott, unpublished results) and the T.I.C. value of the daily globulin dose administered to the adjuvant arthritic rats was approximately equivalent to 5 ml of rat blood.

It has been suggested that protective substances against inflammation are synthetised as a result of the inflammation. Certainly certain conditions e.g pregnancy [10], viral hepatitis [11] result in the remission of symptoms in rheumatoid arthritis. Liver injury in the rat, induced by the administration of dimethylnitrososamine [12], also leads to a remission of adjuvant-induced arthritis. When other rats with adjuvant arthritis were treated with saline extracts of the livers from animals treated with dimethylnitrososamine a similar anti-inflammatory action was observed. It was suggested that the damaged livers produced substances with anti-inflammatory properties as a result of the damage since saline extracts of normal livers did not possess anti-inflammatory properties [12].

The protective substances present in the proteins used in our experiments were from normal rat plasma, and it may well be that protective substances are present in blood but are elevated as a consequence of inflammatory disease. During inflammatory disease inflammatory mediators are also produced and the course of the disease might depend on the resulting balance of inflammatory and non-inflammatory factors.

In other experiments we treated rats daily by intraperitoneal injection with 1 ml of our protein preparation (40 mg/kg body wt) for 30 days and we observed no ill effects. The major organs of the animals appeared normal on post-mortem examination.

Acknowledgement—The Arthritis and Rheumatism Research Council and the Royal Society are thanked for financial support.

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Biochemical Pharmacology, Vol. 25, pp. 1436–1437, Pergamon Press, 1976. Printed in Great Britain.

The presence of EDTA in commercial preparations of isocitrate dehydrogenase

(*Received* 22 *July* 1975; *accepted* 29 *August* 1975)

We communicate this note merely to call attention to the presence of EDTA in Sigma Type IV isocitrate dehydrogenase preparations (IV-ICD) and certain consequences thereof. In isolated microsomal preparations, iron-stimulated lipid peroxidation results in degradation of phospholipids and is accompanied usually by a reduction in mixed function oxidase activity [1]. EDTA, in micromolar concentrations, inhibits lipid peroxidation and usually enhances microsomal enzymatic activity [2, 3]. In addition, cytosol, upon recombination with the microsomal pellet, inhibits lipid peroxidation and enhances the oxidation of substrates [4].

Most investigations involving the hepatic microsomal mixed function oxidase complex have employed either glucose-6-phosphate dehydrogenase or isocitrate dehydrogenase in the NADPH generating system. The commonly utilized IV-ICD has recently been found to contain a factor, separable from the dehydrogenase-protein by gel filtration, which inhibits microsomal lipid peroxidation and enhances microsomal mixed function oxidase activity [5]. Certain properties of the factor in IV-ICD, such as its apparent molecular weight (10,000 daltons) upon gel filtration, its heat stability, and its effects on lipid peroxidation and mixed function oxidase activity, suggested possible identity with the hepatic cytosol factor currently under investigation in different laboratories [5–7].

Discussion with Sigma Chemical Company, St. Louis, Mos., revealed that IV-ICD is prepared by Method II of the procedure of Siebert *et al.* [8] through and including Step 5 which involves dialysis against 10 mM EDTA. Most investigators utilize an assay system for mixed function oxidase activity that involves approximately a 1:2500 final dilution of IV-ICD. If the original EDTA concentration were 10 mM, final concentrations would approximate 4 μM, a concentration well within the range in which EDTA has been found to influence lipid peroxidation and mixed function oxidase activity.

Spectral data indicate the expected presence of at least 10 mM, and more likely ca. 40, mM EDTA in IV-ICD (Fig. 1). The absorption spectra (600–800 nm) of EDTA cupric ion complex and of mixtures of cupric ion and IV-ICD, before or after boiling and centrifugation to remove denatured protein were identical. The inclusion of glycerol with EDTA, as in IV-ICD, did not alter results. The effects of IV-ICD and EDTA on microsomal ethylmorphine *N*-demethylation and lipid peroxidation are compared in Fig. 2. Upon serial dilution, the maximal effects of IV-ICD and EDTA on both processes were identical, but IV-ICD was about twice as potent as 10 mM EDTA. Dialysis, but not heat denaturation, of IV-ICD resulted in the complete loss of effect on *N*-demethylation, lipid peroxidation, and chelate spectral properties. The gel filtration characteristics of the active factor in IV-ICD preparations may reflect the recently reported anomalous behaviour of EDTA upon Sephadex G-25 gel filtration [12].

These data suggest that the action of IV-ICD on the forementioned aspects of mixed function oxidase activity reflects the presence of EDTA. The higher potency of IV-ICD, as compared to 10 mM EDTA, could indicate an additional mode of action, but the spectral chelate data

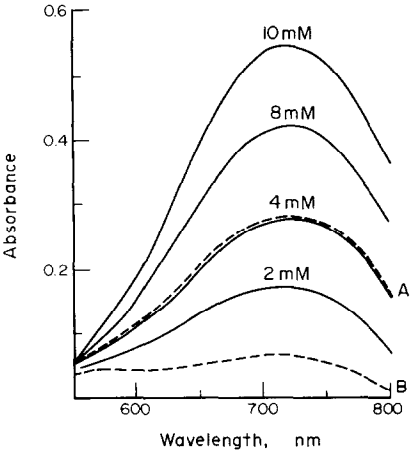

Fig. 1. Absorption spectra of EDTA–Cu^{2+} (——) and IV-ICD–Cu^{2+} (– – – –) complexes. Each cuvette contained 20 mM cupric sulphate and either EDTA at the final concentrations indicated or a 1:10 dilution of IV-ICD (Curve A). Curve B is the spectrum obtained with a 1:10 dilution of IV-ICD after extensive dialysis. In both A and B, the protein was heat denatured and removed by centrifugation prior to addition of copper.

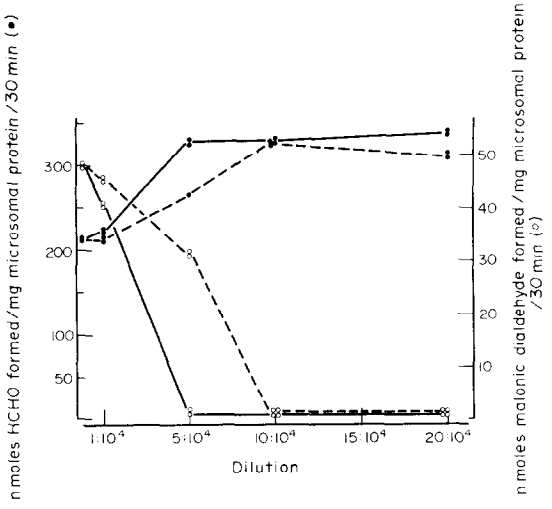

Fig. 2. Effects of serial dilution of IV-ICD (——) and 10 mM EDTA (– – – –) on microsomal lipid peroxidation (○) and ethylmorphine *N*-demethylation (●). Incubation mixtures consisted of sodium phosphate buffer, 30 mM, pH 7.4; NADP, 0.5 mM; glucose-6-phosphate, 20 mM; glucose-6-phosphate dehydrogenase, Sigma, 2 units; Mg^{2+}, 25 mM; micotinamide, 5 mM; ethylmorphine, 2 mM; and microsomes, 1 mg protein. Semicarbazide-HCl, 10 mM, was included only when *N*-demethylation was measured. Incubations were conducted at 37° for 30 min. *N*-Demethylation was assayed by measurement of formaldehyde production [9] and lipid peroxidation by malonic dialdehyde production [10]. Microsomes were prepared as described previously [11] and were used without further washing.

(Figure 1) suggest a concentration of EDTA in IV-ICD of about 40 mM. Kotake *et al.*† have confirmed our findings of EDTA in the commercial preparations of ICD used in their laboratory and conclude that these preparations contain sufficient EDTA to account for the inhibition of microsomal lipid peroxidation seen in their published studies [5]. An awareness of its presence could serve to decrease some inter-laboratory differences with respect to studies of lipid peroxidation and microsomal mixed function oxidase.

Acknowledgements—This research was supported in part by the Medical Research Council of Canada, Grant MA-5162. The technical assistance of H.-Y. Yang is acknowledged.

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