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

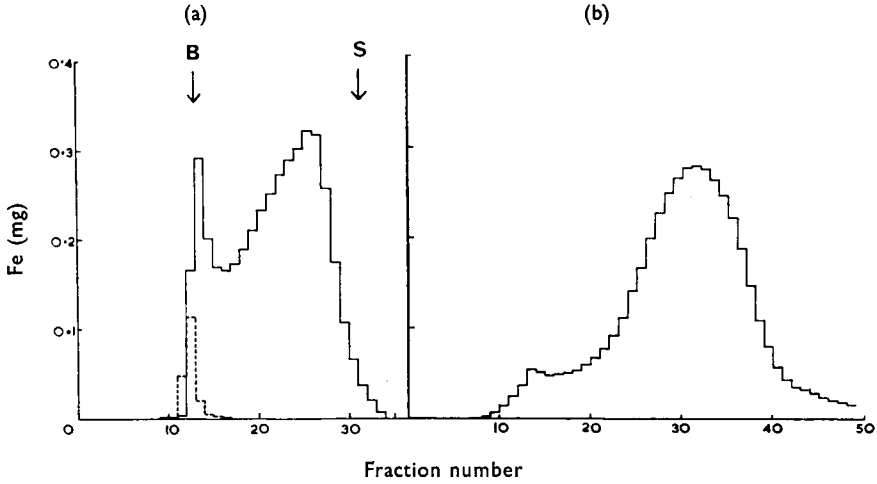

FIG. 1a. Gel filtration on Sephadex G-50 of iron-sorbitol-citrate complex with added sucrose as reference substance. Solid line denotes mg iron in each fraction. Fractions 13 and 14 were combined and re-run; the peak came in the same position as shown by the dotted line. Sucrose peak at S. Blue dextran peak at B denotes excluded volume of column.

FIG. 1b. Gel filtration on Sephadex G-75 in M sodium sulphate solution of iron-sorbitol-citrate complex. Under the same conditions the iron-dextran complex showed a sharp peak containing iron at fraction 10 (Ricketts & others, 1965), no iron being present in fraction 14 and subsequent fractions.

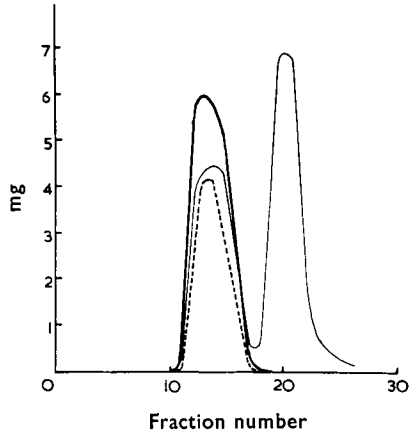

FIG. 2. Gel filtration on Sephadex G-15 of iron-sorbitol-citrate complex. Iron (thick line), sorbitol (thin line), and citrate (broken line) were determined in each fraction. About 43% of the sorbitol and all the citrate was bound to iron.

position of the peak on Sephadex-G50 corresponds with a Stokes radius of less than 10 Å (Laurent & Killander, 1964) i.e., a particle diameter of less than 2 mμ.

Comparison with the iron dextran complex. A column of Sephadex G-75 in M sodium sulphate solution running at 18 ml/hr was used (Ricketts, & others, 1965) to fractionate the iron-sorbitol-citrate complex. Under these conditions 90·3% of the iron was recovered. The main peak was in fraction 32 with a minor peak in fraction 14, as shown in Fig. 1b. In contrast, the iron-dextran complex chromatographed under the same conditions, showed a single peak at fraction 10. Thus the iron-sorbitol-citrate complex was of smaller particle size than the iron-dextran complex.

Reproducibility of particle size distribution. Five different preparations of the iron-sorbitol-citrate complex were submitted to gel filtration on columns of 5 g of Sephadex G-75 (1·3 cm diameter by 55–61 cm long) operating in saline solution. Two ml fractions were collected and the iron colour was measured at 430 mμ. The shape of the elution curve was reproducible.

Separation of free sorbitol. The iron-sorbitol-citrate complex was submitted to gel filtration on Sephadex G-15 in saline solution. Iron, sorbitol and citrate were determined in each fraction, with the result shown in Fig. 2. Two peaks of sorbitol content were found, the first peak coincided with the peak of iron colour; the second peak was free from iron. About 43% of the sorbitol was bound to iron and the remainder was free in solution. All the citrate was bound to iron.

It was of interest to see whether the free sorbitol was exchangeable with the sorbitol bound to iron. An experiment using [^{14}C]sorbitol was designed to test this. About 10 μc of [^{14}C]sorbitol was mixed with 1 ml of the complex and allowed to stand at 20° overnight. A similar mixture was autoclaved at 15 lb/inch^2 for 15 min. The mixtures were subjected to gel filtration on Sephadex G-15 as before. The radioactivity of the fractions was measured with correction for quenching of the liquid scintillator used. Only 2% of the added sorbitol was found in the iron-containing fractions after equilibration at room temperature. In the autoclaved mixture the proportion was 8%.

Gel filtration shows that the iron-sorbitol-citrate complex contains a range of particle sizes, most of the particles being about 2 mμ in diameter. The dispersion about this average value is reproducible in different preparations made in the same way. Free sorbitol is present in the injection solution but this is not to any appreciable extent in equilibrium with the sorbitol bound to iron. The experiments provide evidence for the existence of a complex of iron with sorbitol and citrate and it seems likely that atoms of ferric iron link together molecules of sorbitol and citrate ions to form an aggregate. The particle size of the iron-sorbitol-citrate complex is smaller than that of the iron-dextran complex and this could cause differences in the distribution of these complexes in the body after injection.

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