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Effect of Chelating Agents on the Copper – Protein Bond in Liver

IN man copper can be mobilized from the body by the intramuscular or intravenous administration of BAL (dimercaptopropanol)[1,2] or by the oral administration of penicillamine (dimethyl cysteine)[3]. Further, it has been shown that penicillamine brings about a fall in the plasma level of copper which later rapidly returns to normal[4]; the plasma copper presumably being made good from tissue stores. There is no direct evidence as to the effect of either of these chelating agents on the copper–protein bonds in tissues. We have investigated the ability of BAL and penicillamine to render liver copper available for filtration through a semipermeable membrane.

Extracts were made from 'fresh' post-mortem normal human liver by homogenizing finely chopped liver in 1 per cent potassium chloride in the proportions 2 : 5 w/v, at 4° C. Centrifugation yielded 75 per cent supernatant fluid with a protein nitrogen content of between 4 and 5 mg/ml. and a copper content varying from 6·2 to 19·1 μg/ml. Penicillamine hydrochloride was added at a concentration of 5 mg/ml.; this increased the acidity by not more than 1 *p*H unit. Ultra-filtration was carried out through Hudes collodion membranes at a negative pressure of 7·5 cm mercury. In the control filtrations, to which no penicillamine had been added, 0·2–0·6 per cent of the total copper was recovered in the protein-free filtrate; the addition of penicillamine did not result in a great increase in the mobilization of the metal; the recovery varied from 0 to 3 per cent.

Using high specific activity copper-64 we were able to make a more detailed study of the uptake and binding of copper by liver proteins. Two rabbits of weight 1,690 and 1,945 g were each injected with 2·82 μc. copper-64 in isotonic saline into an ear vein. One animal was killed 2 h later and the other 24 h later. The animal killed at 2 h had a liver weight of 78·57 g and the organ contained 1·2 μc. copper-64 (calculated at zero time), that is, 45 per cent of the injected dose. An extract was made of 19·726 g liver in 49 ml. 1 per cent potassium chloride; after centrifugation there was a 20 per cent solid residue. The supernatant fluid contained 0·19 μc. and the insoluble residue 0·086 μc., a ratio of 2·2 : 1. 5-ml. aliquots of supernatant were taken for filtration through Hudes collodion membranes; to one aliquot was added 25 mg penicillamine hydrochloride. In the control ultra-filtrate there was no detectable activity; this remained entirely in the non-filterable protein fraction within the membrane. In the aliquot to which penicillamine had been added the counting rate in the filtrate was 7 per cent above the background counts, equivalent to a filtration of 2 per cent of the total activity, but with a counting rate so near background this observation is of doubtful significance. The second animal, killed at 24 h, had a liver weight of 105·650 g with a calculated copper-64 content of 0·61 μc. or 22·7 per cent of the injected dose. The homogenate (63·25 g liver, 52 ml. potassium chloride) showed the same distribution of activity between supernatant and insoluble residue, 0·315 μc. in the soluble fraction and 0·139 μc. in the residue, a ratio of 2·27 : 1. Filtration studies were carried out by the same technique to that used previously except that BAL, 5 mg/ml., was used as the chelating agent instead of penicillamine. Neither in the control filtrate nor the infiltration fraction to which BAL had been added was there any detectable activity.

We conclude that copper is rapidly taken up by the liver and is then firmly bound to protein although the efficiency of the concentration mechanism does not appear to be so great in the rabbit as in man[5]. Unlike plasma (from which copper-64 is more readily mobilized at 2 than at 24 h)[6] the liver copper–protein bond appears to be equally strong at each of these time-intervals and the resistance of this bond to the two —SH chelating agents investigated suggests binding to —SH groups in the liver protein, an observation in keeping with Morell, Shapiro and Scheinberg's[7] findings for human liver copper protein; these workers also concluded that the bond was probably to an —SH radicle.

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Macromolecular Ageing *in vivo*

VERY little work has been done in the investigation of possible changes in biologically occurring macromolecules as a function of their age. Research has been done on elucidating chemical alterations of collagen on ageing[1], but this structural protein is a special case involving, among other things, the presence of some unusual amino-acids. The question is whether proteins (and nucleic acids), in general, exhibit some chemical changes on ageing which, by some applied criterion, differentiate them from younger molecules of the same species. Such an alteration, depending on its nature, may or may not affect the molecule's biological activity.

The development of techniques for the separation of young and old erythrocytes by centrifugation[2,3] or of their lysates by a serial osmotic hæmolysis procedure[4] has made the red blood cell a particularly attractive system to investigate. Young and old hæmoglobin and erythrocytic enzymes may be obtained in this fashion and examined. Thus, evidence was recently obtained for the alteration of hæmoglobin, a soluble protein, as it aged *in vivo*.

Hæmoglobin on ageing has been found to increase its electronegativity since it migrates more rapidly on electrophoresis[5] and on column chromatography[6]. It has further been found to lower its oxygen dissociation[7], and to bind less chromium[8] as it becomes older. The structural involvement of hæmoglobin in the hæmoglobin–chromium interaction is underscored by the work of Meijering and Huisman[9], who found that different hæmoglobins bind chromium to differing extents. It would seem that when hæmoglobin ages a structural change occurs so that chromium binding does not occur to the same degree as in the young protein.