The Demonstration of a Liver Factor Stimulating the Sulphate Exhange of Chondroitin Sulphuric Acid

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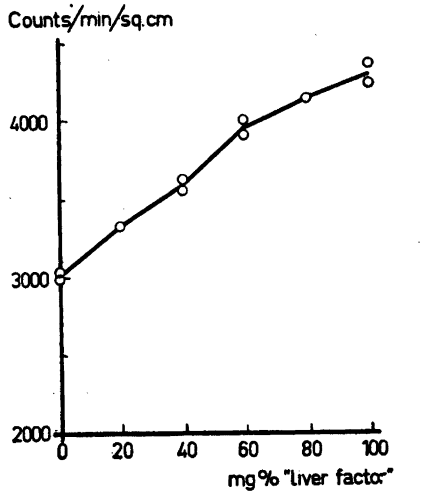
Recently, we discussed some problems concerning the sulphate exchange in chondroitin sulphuric acid of cartilage on the basis of a series of experiments with a slicing technique [1,2]. Slices of cartilage suspended in a Krebs-Ringer-bicarbonate solution containing S^{35}-labelled sulphate were incubated at 37° C for some hours. A considerable S^{35}-incorporation in the ester sulphate group of chondroitin sulphuric acid occurred, as demonstrated after isolation of this compound and splitting off its sulphate group through acid hydrolysis. This *in vitro* sulphate fixation did not occur in slices heated to 47° C, in frozen and subsequently thawed slices or in homogenized cartilage. Moreover, this reaction could be inhibited by different enzyme inhibitors, amongst which the SH inhibitors were found to be the most active.

In subsequent experiments we found that the reaction studied was strongly stimulated by the presence of small amounts of a liver homogenate. It was also found that the active "liver factor" was thermostable and could easily be extracted from fresh liver by water. In the present paper, a preliminary report is given of these observations.

The liver from a newly killed suckling calf was washed in tap water, freed from the large vessels and cut in ½ – 1 cm thick slices. The slices were gently stirred in 1 liter of distilled water for 1 hour at room temperature. After removal of the liver slices by pouring the mixture on a Büchner funnel (without filter paper) the fluid was filtered through hyflo supercel. The clear, red-coloured filtrate was then freeze dried and yielded 3 g of a reddish, light powder.

The ability of this powder to stimulate the sulphate exchange of chondroitin sulphuric acid of costal cartilage of young calves *in vitro* was tested by means of the slicing technique previously described [2].



In one series of experiments, different amounts of the powder (0 – 100 mg %) were added to the Krebs-Ringer-bicarbonate solution, in which the slices of cartilage were suspended, 30 minutes before the addition of S^{35}-labelled sodium sulphate. Incubation at 37° C for 2 hours. The result of these experiments is recorded in Fig. 1. It is seen from the course of the curve that there was a successive increase in the incorporation of S^{35} in the ester sulphate group of chondroitinsulphuric acid with an increase in the amount of liver powder added to the slices. In those samples incubated in the presence of 100 mg % of the liver powder, the S^{35} uptake was about 45 % higher than in the control samples.

In another series of experiments, it was shown that heating of the liver powder suspended in a small volume of water to 100° C did not destroy its ability to stimulate the S^{35} incorporation in sulphuric acid.

As shown previously, the reproducibility of the *in vitro* technique is extremely good [2]. Thus, the response obtained in the present experiments is highly significant. The true nature of the stimulating liver factor is unknown. However, the fact that it is thermostable may indicate that it represents some kind of co-factor interacting with the esterifying enzyme system.

1. Boström, H. *Arkiv Kemi* **6** (1953) 43.
2. Boström, H., and Månsson, B. *Arkiv Kemi* **6** (1953) 23.

Received August 5, 1953.