SHORT COMMUNICATION

SOMATOMEDIN (SULPHATION FACTOR)-LIKE ACTIVITY OF HOMOCYSTINE

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SUMMARY

1. Homocystine (10 mg/100 ml) has a significant effect on the incorporation of inorganic sulphate by rat costal cartilage in vitro.
2. The characteristic skeletal changes associated with homocystinuria due to defective cystathionine synthetase could therefore be caused by the elevated plasma concentration (1–10 mg/100 ml) of homocystine present in this disease.

Key words: homocystine (and homocystinuria), cartilage, sulphate.

An excess of homocystine in the urine may be found in a number of conditions. The commonest is due to cystathionine synthetase deficiency, leading to increased concentrations of both homocystine and methionine in urine and plasma (Mudd, Finklestein, Irreverre & Laster, 1964). This condition, homocystinuria, is inherited as an autosomal recessive (McKusick, 1966). Patients are normal at birth but become progressively more affected with age (Cusworth & Dent, 1969). Clinical manifestations include skeletal abnormalities, dislocation of the lenses and a thrombotic tendency. A major characteristic skeletal change is the marked widening of the metaphysis and enlargement of the epiphysis of the long bones; this commonly occurs at the knee and shoulder joints which are therefore large and abnormally shaped (Brenton, Dow, James, Hay & Wynne-Davies, 1972). These changes provide an easily recognized diagnostic sign for homocystinuria. Such changes are similar to those that occur in embryonic and post-foetal mouse long bones that have been cultured in the presence of somatomedin (sulphation factor) (Leong, Herring & McConaghey, 1972). Somatomedin is a factor in plasma which is believed to be the agent by which growth hormone exerts its effects on the skeleton (Tanner, 1972). One action of somatomedin is to stimulate the production of cartilage matrix glycosaminoglycans, such as chondroitin sulphate, an index of which is $[^{35}S]$sulphate incorporation by rat costal cartilage cultured in vitro (Salmon & Daughaday, 1957).

We have studied whether homocystine and methionine, at the concentrations found in the plasma of untreated patients with homocystinuria, have somatomedin-like activity.

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MATERIALS AND METHODS

Medium
Waymouth's MB 752/1 TC medium was used. There is no homocystine or homocysteine in this medium but it contains, per 100 ml, 5 mg of methionine, 1.5 mg of cystine and 9 mg of cysteine as well as 50 mg of S as inorganic sulphate. Bovine serum albumin (fraction V) was added to give a concentration of 0.25% (w/v). After dissolving the required additional amino acids (homocystine and/or methionine) the medium was sterilized by filtration through a Millipore filter (HAW PO 2500).

Animals
Male 3-week-old Sprague–Dawley rats, weighing 50 g were used in all experiments.

**Fig. 1.** Stimulation of $[^{35}S]$sulphate incorporation into the organic matrix of rat costal cartilage by homocystine and methionine. Values are the means from at least three separate experiments, each carried out as described in the Methods section. The $P$ value for (methionine + homocystine) as compared to methionine alone is $0.02-0.05$. The vertical bars indicate ± SEM.

Assay of $^{35}S$ uptake
The uptake of $^{35}S$ into segments of rat costal cartilage was measured by the method of Salmon & Daughaday (1957). Five pieces of cartilage, one piece from each of five rats, were placed in 3 ml of control or test medium in a sterile 25 ml Erlenmeyer flask; controls and test media were set up in triplicate for each assay. $^{35}S$ was added to each flask to give a final concentration of 0.77 μCi/ml medium. The cartilage segments were incubated at 37°C in air for 48 h in a shaking incubator. After incubation, enzyme activity was stopped by immersing the segments in boiling water for 10 min. The segments were then soaked for at least 3 h in saturated Na$_2$SO$_4$ to remove as far as possible $^{35}S$ present as inorganic sulphate (Bostrom & Mansson, 1952), washed with tap and distilled water; any remaining soft tissue was then removed, weighed, and hydrolysed in 23 M-formic acid before liquid-scintillation counting for $^{35}S$.

RESULTS
The results (Fig. 1) show that homocystine added to the chemically defined culture medium at a concentration of 10 mg/100 ml (0.37 mM) caused a significant increase ($P < 0.01$) in the
amounts of $^{35}\text{S}$ incorporated in the rat costal cartilage segments. Lower concentrations of homocystine (1.0 mg and 0.1 mg/100 ml) were also stimulatory but not significantly so. Methionine at a concentration of 20 mg/100 ml (as compared to the 5 mg/100 ml normally present in the medium; i.e. $\times 4$) had either no effect, or a slight inhibitory effect.

**DISCUSSION**

The experiments show that homocystine stimulates the incorporation of $[^{35}\text{S}]$sulphate into a fixed form by rat costal cartilage. In this respect homocystine has an action analogous to the somatomedin that is produced *in vivo* by the action of growth hormone on the liver (McConaghey, 1972) and the kidney (McConaghey & Dehnel, 1972).

McCully (1971) showed that homocysteic acid derived from homocysteine/homocystine will react with ATP to form phosphoadenosine phosphosulphate, the enzymically active precursor of the esterified sulphate of glycosaminoglycans. These results led him to suggest that homocysteine could act on cartilage *in vivo* similarly to somatomedin. However, any preferential use of homocystine sulphur rather than inorganic sulphate for phosphoadenosine phosphosulphate formation would, in the present experiments, be expected to lead to a decrease of $^{35}\text{S}$ fixed by cartilage rather than the increase observed. Our results suggest therefore that homocystine may have effects on cartilage glycosaminoglycan formation in addition to the transulphurylation pathway proposed by McCully. The apparent inhibition of sulphate uptake from $[^{35}\text{S}]$sulphate by increasing the methionine concentration with or without added homocystine may result from preferential use of methionine-S as a source of activated inorganic sulphate.

The influence of other disulphides, such as cystine, was not tested in these experiments as there are already high concentrations of cystine/cysteine in the medium which are required for the maintenance culture of the cartilage. However, previous investigators (Salmon & Daughaday, 1958; Hall, 1970) have found that cystine does not appear to have the stimulatory effect shown here for homocystine, but further work is required to substantiate this.

These results may provide an explanation for the skeletal abnormalities found in homocystinuria due to cystathionine synthetase deficiency. The concentrations at which homocystine has its effect in the assay for sulphate incorporation *in vitro* (1–10 mg/100 ml, Fig. 1) are similar to those found in the plasma (1–10 mg/100 ml) of homocystinuric patients (McKusick, 1966). Whether similar skeletal abnormalities occur in the other rarer forms of homocystinuria has not been reported. The way in which either homocystine or somatomedin (Tanner, 1972) produces its effects on cartilage remains obscure. Their similarity of action in the *in vitro* assay used here does not necessarily imply that they act by a common mechanism.

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**REFERENCES**


