THE GROWTH HORMONE DEPENDENT INCORPORATION OF SULPHATE INTO THE COSTAL CARTILAGE OF OBESE-HYPERGLYCAEMIC MICE OF DIFFERENT AGES

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ABSTRACT
The in vivo incorporation of sulphate into costal cartilage was measured in both lean and obese-hyperglycaemic mice of different ages. In 2–10 months old obese mice the sulphate incorporation was found to be markedly increased as compared with lean controls. After a peak value at the age of 4–5 months there was a gradual fall in the sulphation activity. In the lean litter mates a decrease in the sulphation activity was already observed after the first month of life. These findings are considered against the background of the metabolic changes characteristic of the syndrome and the current concepts of the actions of insulin and growth hormone. A possible role of growth hormone in the aetiology of the obese-hyperglycaemic syndrome is discussed.

Mice with the obese-hyperglycaemic syndrome (genotype obob) are characterized by i.a. pronounced over-weight, hyperglycaemia, greatly increased serum insulin levels and marked insulin resistance (Stauffacher et al. 1967; Westman 1968). In addition certain acromegaloid signs such as increased organ weights (Marshall et al. 1957) and hyperostosis of the skull bones (Herbai, unpubl. observation) have been recorded. Despite a great deal of research the primary metabolic lesion of these animals has not yet been elucidated. The development of the disorder has been analyzed in a recent study (Westman 1968), which indicates that the first signs of a deranged metabolism are

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already apparent at the time of weaning, when the animals display an abnormal fat deposition and increased insulin resistance. The syndrome first becomes fully developed in 4–5 months old animals. Furthermore, many of the features characteristic of the syndrome disappear in older mice. It was suggested that the obese-hyperglycaemic syndrome in mice may be related to some factor(s) active during a limited period of life. All these observations conform with the idea that a circulating agent, possibly of hormonal nature, might be of pathogenetic significance.

It is well established that the hypersomatotrophism in acromegaly is associated with insulin resistance and markedly increased serum insulin levels (Beck et al. 1965; Karam et al. 1965; Luft et al. 1967; Liebermeister et al. 1968). It has also been shown that growth hormone administration to dogs induces a diabetic state and a marked increase in serum insulin levels (Campbell & Rastogi 1966). Furthermore, raised levels of circulating growth hormone in rats with the MT-W15 tumour resulted in a marked hyperplasia of the pancreatic islets with a considerable elevation in the serum insulin concentration (Martin et al. 1968). These observations support the hypothesis that an increased production of growth hormone might be of significance for the development of the obese-hyperglycaemic syndrome in mice.

Stimulation of sulphate incorporation by growth hormone into costal cartilage has previously been used as a bioassay for somatotrophic activity (Daughaday & Kipnis 1966). A new in vivo method has recently been developed for studies of the growth hormone dependent sulphation of chondroitin sulphate in the rib cartilage of mice (Herbai, in press). This method seemed particularly suitable for studies on the sulphation activity in the obese-hyperglycaemic syndrome, since it allows a correction to be made for the large differences between the sizes of the inorganic sulphate pools in the obese and lean mice (Herbai, in press). The aim of the present investigation was to evaluate the sulphation activity of costal cartilage in obese-hyperglycaemic and lean mice of various ages.

**MATERIAL AND METHODS**

Altogether 78 male obese-hyperglycaemic mice and 45 of their lean litter mates were used. After weaning the animals were allowed free access to water and pelleted food with the following composition (percentage analysis of dry matter): crude protein 20, crude fat 5; ash 5; fibre 3; nitrogen-free, extractable substances 67. The water content was 8 per cent. At the time of the experiment the animals were between 30 and 540 days old.

A detailed description of the method used in the present study has been given elsewhere (Herbai, in press), and a summary of the procedure is given below. The method is based on a double isotope assay, which allows a simultaneous determination of the inorganic sulphate pool and a calculation of the total amount of sulphate ions in-
incorporated into a defined region of the mouse costal cartilage during the experiment. A mixture of \(^3\)H-labelled phenol and \(^35\)S-labelled sodium sulphate (\(^{35}\)SO\(_4\)) is injected intravenously into each animal. Phenol is rapidly excreted into the urine after conversion to phenyl sulphate. Thirty minutes after the isotope injection a urine sample is taken from the bladder and its phenyl sulphate content isolated by thin layer chromatography (TLC). After this urine sample has been withdrawn, the animals receive an ip injection of a large amount of carrier sodium sulphate in order to decrease the specific activity of the circulating \(^{35}\)SO\(_4\) in the blood. About 4 hours later the animals are killed and the five lower pairs of the ribs attached to the sternum are removed. An anatomically defined part of the cartilage (Herbai, in press) is dissected out and digested in acid. Finally the \(^{35}\)SO\(_4\) content of the cartilage samples and the \(^3\)H/\(^35\)S ratio of the phenyl sulphate from the TLC are measured. Sulphate pools and amounts of incorporated sulphate ions are calculated from these data. The sulphation values are expressed as nanogram sulphate incorporated into 5 pairs of ribs during the period of 30 min. This method of expression has been chosen because marked regional differences in the sulphation activity have previously been demonstrated between different parts of the costal cartilage (Herbai, in press).

RESULTS

The sulphate incorporation recorded in the two types of animals is summarized in Table 1. It is evident that the pattern of sulphation activity differed between obese-hyperglycaemic and lean mice. In the lean litter mates the highest sulphation activity was found in the youngest age group, whereas from the age of about 2 months it decreased markedly and then remained unchanged in the older animals.

The one month old obese-hyperglycaemic mice displayed a sulphate incorporation activity of the same magnitude as the lean litter mates. However, in the older age groups the obese-hyperglycaemic animals showed a markedly increased activity, which reached a peak value in the five months old animals and then gradually decreased. Levels of the same order as in the control group were not recorded until the age of about 17 months. In Fig. 1 the concentrations of immuno-reactive serum insulin obtained in a previous study on obese-hyperglycaemic mice of various ages are also given (Westman 1968). As can be seen in Fig. 1, the peak value for insulin was reached somewhat later than that for the sulphation activity.

DISCUSSION

It emerged from the present results that the rate of sulphate incorporation into costal cartilage of the obese-hyperglycaemic mice remained elevated throughout a prolonged period of life. By contrast, the sulphate uptake found in the lean litter mates decreased rapidly after their first month of life, until at the age of about 5 months when it reached a steady state. At the same time the body
Table 1.
Sulphate incorporation into costal cartilage (ng sulphate/5 pairs of ribs/30 min) of obese-hyperglycaemic and normal mice of different ages. The number of animals in each group is indicated within brackets. Mean values ± sem.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Animals</th>
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<tbody>
<tr>
<td></td>
<td>Obese</td>
</tr>
<tr>
<td>30–34</td>
<td>130.7 ± 12.8</td>
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<tr>
<td></td>
<td>(6)</td>
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<tr>
<td>45–70</td>
<td>130.3 ± 9.7</td>
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<td></td>
<td>(13)</td>
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<tr>
<td>100</td>
<td>150.4 ± 11.4</td>
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<td></td>
<td>(2)</td>
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<tr>
<td>120–150</td>
<td>169.3 ± 10.5</td>
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<tr>
<td></td>
<td>(16)</td>
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<tr>
<td>170–195</td>
<td>142.6 ± 12.1</td>
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<td></td>
<td>17</td>
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<tr>
<td>240–270</td>
<td>95.5 ± 0.8</td>
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<td></td>
<td>(9)</td>
</tr>
<tr>
<td>300</td>
<td>107.5 ± 10.1</td>
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<tr>
<td></td>
<td>(5)</td>
</tr>
<tr>
<td>510–540</td>
<td>76.4 ± 9.8</td>
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<td></td>
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Growth of the animals ceased. Since the sulphate incorporation into chondroitin sulphate of cartilage tissue is stimulated by growth hormone it would appear from these results that the endogenous growth hormone activity of the obese animals was raised. However, insulin shares many of the effects of growth hormone on the protein metabolism and its possible significance in the increased sulphate incorporation in the obese mice must therefore be considered. The influence of insulin on the sulphate metabolism in cartilage has previously been analysed both in vivo and in vitro. Studies on the $^{35}$SO$_4$ uptake of costal cartilage from hypophysectomized rats in vitro showed that the addition of insulin in physiological concentrations to the incubation medium had no effect (Salmon & Daughaday 1957; Salmon 1960), whereas high insulin levels enhanced the sulphate incorporation (Salmon et al. 1968). The action of growth hormone on cartilage is, however, not dependent on the presence of serum insulin as evidenced by the stimulating action of growth hormone on the sulphation activity in hypophysectomized alloxan diabetic rats (Salmon 1960). Likewise, the nitrogen retention caused by growth hormone in hypophysectomized-pancreatectomized rats was found to be independent of insulin ad-
Fig. 1.
Sulphate incorporation (M ± SEM) into costal cartilage (ng sulphate/5 pairs of ribs/30 min) of obese-hyperglycaemic (●——●) and lean (○——○) mice of different ages. The broad cross hatched curve represents the variation with age of the mean serum insulin level (± SEM) in obese-hyperglycaemic mice; the corresponding variation for the lean litter mates is represented by the thinner curve at the bottom of Fig. 1. When the SEM is not indicated it was smaller than the symbols representing the mean value.

ministered in therapeutic doses (Scow & Chernic 1960). It was, however, claimed that the epiphyseal width of the tibia was increased in immature female rats given 10 U of insulin per kg body weight (Ailabouni et al. 1966). Whether this action was mediated through an increased circulating growth hormone level remains to be established.

Previous reports indicating that growth hormone both in animals and humans causes hyperinsulinaemia with insulin resistance (Beck et al. 1965; Luft et al. 1967; Liebermeister et al. 1968; Campell & Rastogi 1966) agree with the view that a pituitary factor with at least some growth hormone-like activity may be the cause of the increased sulphate incorporation into the cartilage of obese-hyperglycaemic animals. The present observation that the peak value of the serum insulin concentration appeared later than that of the sulphation activity is also in line with this view. It is noteworthy in this context that a recent study of mutant diabetic mice of the C57BL/KS strain indicated that the pituitary content of growth hormone was lower than that of normal mice during the initial stage of the syndrome (Dejardins 1969). The results were interpreted as suggesting an abnormally high release of growth hormone in the young diabetic animals. Like the obese-hyperglycaemic mice these diabetic mice show both an increased fat deposition and insulin resistance (Coleman & Hummel 1967).

It appears from the data referred to above that the interactions between
insulin and growth hormone are by no means as yet clarified. Hence the results of the present in vivo assay may be interpreted as depending both on an increased growth hormone activity and a stimulation by insulin of the sulphate incorporation into the costal cartilage. The fact that there is a similar sulphate uptake in both the obese and lean mice at one month of age is still difficult to explain, since at this age there is already a significantly increased serum insulin level in the obese animals (Westman, in press).

REFERENCES


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