Changes in serum somatomedin A and its binding to kidney membranes in growing rats

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Abstract. Changes in serum somatomedin A levels and \[^{125}\text{I}]\text{somatomedin A}\ binding to membrane fractions from kidney were studied in rats 1–80 days of age. The mean level of serum somatomedin A was 0.80 U/ml at birth and increased with age; at 80 days the mean level was 7.41 ± 0.67 U/ml. There was a close correlation between serum levels of somatomedin A and body weight. Labelled somatomedin A binding to membrane fractions from kidney was highest at birth and decreased with age up to 50 days. In Scatchard analysis of the data the affinity constant did not show a clear change with age, but the binding capacity decreased with age up to 30 days. An inverse correlation was observed between serum somatomedin A levels and labelled somatomedin A binding to membrane fractions from kidney. Compared to changes in circulating somatomedin A, the change in tissue binding was modest. This observation suggests that other circulating growth factors not measured by this radioreceptor assay or altered post-receptor sensitivity to somatomedins may be involved in growth.

Normal growth is achieved by a complex interaction between a variety of hormonal factors and the responsiveness of their target organs. The first step in the action of a polypeptide hormone is binding to specific receptors on the surface of target cells (Roth 1972). The growth-promoting actions of growth hormone are believed to be mediated by somatomedin (Salmon & Daughaday 1957; Daughaday et al. 1979). It has been reported that serum somatomedin levels are low at birth and in early childhood in spite of rapid growth (Almqvist & Rune 1961; Van den Brande & Du Caju 1974; Takano et al. 1976b; D'Ercole et al. 1977; Hall et al. 1980). It is postulated that increased sensitivity of the target tissues may be responsible for the rapid growth. Binding sites for somatomedin A have been identified in membrane fractions prepared from rat kidney, lung, liver, testis, epididymal fat, pancreas, spleen, brain, heart, cartilage, and thymus (Takano et al. 1976a) as well as from human placenta (Takano et al. 1975).

This study was undertaken to investigate changes in serum somatomedin A and somatomedin binding to membrane fractions from kidney in growing rats.

Materials and Methods

Somatomedin A used as the labelled and unlabelled hormone was purified by Dr. L. Fryklund at Recep Polypeptide Laboratory, AB Kabi, Stockholm. The somatomedin A used as labelled and unlabelled hormone had an activity of 1200 U/mg and 12 U/mg, respectively, as determined by the sulphate incorporation bioassay using chick pelvic leaflets (Hall 1972). Somatomedin A was iodinated by Na\[^{125}\text{I}\] to a specific activity of 20–30 \(\mu\text{Ci/\mu g}\) by the peroxidase method described by Takano et al. (1976b). Iodinated somatomedin A was purified on carboxymethylcellulose in a pH gradient from 4.1–6.8 in 0.1 M ammonium acetate buffer.

Serum somatomedin A was determined by the radioreceptor assay using human placental membrane (Takano et al. 1975, 1976b). Pooled serum from two healthy human males and two healthy females between 20 and 25 years of age was used as arbitrary reference. One unit (U) of somatomedin A is defined here as the content in one
ml of this pooled serum. The data presented are expressed as potency relative to this local arbitrary unit.

Male Wistar rats were divided into eight groups according to age: 1, 10, 20, 30, 40, 50, 60, and 80 days. Each group had 5 rats except the groups of day 1, 10, and 20 which had 15, 10, and 10 rats, respectively. After delivery, all rats were caged in a light-controlled room with the lights on from 0800 to 2000 h, and allowed free access to MF chow (Oriental Yeast Co. Ltd.) and water. Rats were weaned at 21 days of age. They were killed between 0900 and 1100 h. Blood was obtained from the neck after decapitation in rats at day 1 and day 10, and from the inferior caval vein, during ether anaesthesia in the remaining rats. Serum was stored at −20°C. Somatomedin A levels were determined in serum samples from individual rats except when it was necessary to pool the sera in order to have a sufficient sample for testing i.e. day 1 group of 15 rats and day 10 group of 5 rats.

The kidneys were removed immediately after sacrifice, kept at −70°C, and homogenized in ice-cold 0.25 M sucrose. Tissues from the younger rats were pooled as follows: day 1, 15 rats/pool, day 10, 5 rats/pool, and day 20, 2 rats/pool. The particulate membrane fractions were prepared by stepwise centrifugation as previously described for rat liver, kidney, and human placental membrane fractions (Cuatrecassas 1972; Takano et al. 1975, 1976a).

The fractions obtained from centrifugation at 12 000 × g and 100 000 × g were used in the binding assay. The protein content of the membrane fractions was determined by the method of Lowry et al. (1951).

The binding assay is the same as that developed for the radioreceptor assay of somatomedin A using human placental membrane (Takano et al. 1975, 1976b).

The ingredients for the binding assay are as follows: 100 μl 0.05 M Tris-HCl buffer, pH 7.4 and 1% human serum albumin with or without unlabelled somatomedin A, 100 μl membrane fraction (200 μg protein), and 100 μl labelled somatomedin A (15 000 cpm). After 16 h of incubation at 4°C, bound and free hormone were separated by centrifugation at 6 000 × g for 15 min and membrane-bound labelled somatomedin A in the pellet was counted in a well-type gamma spectrometer. The binding studies were done in duplicate. Nonspecific binding is defined as binding in the presence of an excess of unlabelled somatomedin A (10 μg/tube). Specific binding was determined by subtracting nonspecific binding from total binding. The radioreceptor assay using human placental membrane measures not only somatomedin A but also biologically related polypeptide such as somatomedin C (Hall et al. 1975), multiplication stimulating activity (Hall et al. 1976), and insulin like growth factors I and II (Hall et al. 1980) with more or less equipotency. Hence the binding sites of somatomedin A in the membrane fraction from rat kidney might not be specific for somatomedin A. However, because we use labelled somatomedin A in the radioreceptor assays using human placental membrane and rat kidney membrane we refer to the values obtained as the levels of somatomedin A and the binding sites of somatomedin A.

The binding affinity and capacity of somatomedin A binding site in kidney was determined by the method of Scatchard (1949). In the present study, because somatomedin A with a potency of only 12 U/mg was used as

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**Fig. 1.**

Changes of body weight and levels of somatomedin A in rats 1 to 80 days of age. Vertical lines indicate M ± SEM.
Fig. 2.
Relationship of [125I]somatomedin A binding to age of rat. The specific binding of [125I]somatomedin A to membrane fractions from kidneys \((\% = M \pm \text{SEM}, \,* = P < 0.05, \,** = P < 0.005).\)

the unlabelled hormone in contrast to the highly purified preparation (1200 U/mg) used as labelled hormone, the moles of somatomedin A bound to the membrane fractions were calculated for Scatchard analysis assuming that highly purified somatomedin A with 1200 U/mg was used as unlabelled hormone and molecular weight was 7000.

Correlation coefficients were calculated by the least squares method. Student's *t*-test was used for statistical analysis.

Results
In Fig. 1 we show that serum somatomedin A levels measured by the radioreceptor assay using human placental membrane increased with age up to 80 days in the rats. The body weight of the rats also increased with age, and there is a positive correlation \((r = 0.94)\) between serum somatomedin A levels and body weight.

Binding sites for somatomedin A were identified in membrane fractions prepared from kidney. The competition of labelled somatomedin A by unlabelled somatomedin A was dose dependent in the tissues. The characteristics for binding of somatomedin A in the rat kidney are similar to those described for human placental membrane i.e. pH 7.4, at 4°C for 16 h (Takano et al. 1975). Fig. 2 shows changes in somatomedin A binding to membrane fractions from kidney in rats. The specific binding of labelled somatomedin A to membrane fractions from kidney of the day 1 groups was 7.2% and decreased with age until 50 days; no further significant change was observed at 60 and 80 days.

Scatchard analysis of the data was used to determine whether changes in binding of somatomedin A were due to an alteration in binding affinity or binding capacity in the kidney. The Scatchard plots of binding of somatomedin A to kidney membrane fractions are shown in Fig. 3. We assume two classes of binding sites and in Table 1 we compare the affinity constant and the binding capacity of high affinity binding sites in kidney membrane.
fractions at different ages. It is apparent that the affinity constant showed no clear change, but binding capacity gradually decreased with age until 30 days and the small change in binding capacity after 30 days does not appear to be of note.

The relationship between serum somatomedin A levels and somatomedin A binding to membrane fractions from kidney was determined. As shown in Fig. 4, an inverse correlation between these two values was observed. The correlation coefficient (r) is −0.65 (P < 0.005).

Discussion

In this study we used a radioreceptor assay to demonstrate changes in serum somatomedin A levels in growing rats. The level of serum somatomedin A was low at birth and gradually increased with age up to 80 days. Sara et al. (1980) also reported the serum levels of immunoreactive somatomedin A increased with age in growing rats and a similar observation has been reported in man (Almqvist & Rune 1961; Van den Brande & Du Caju 1974; Takano et al. 1976b; D’Ercole et al. 1977; Hall et al. 1980). However, the possibility that these changes in serum somatomedin A may partly be due to changes in serum somatomedin binding protein cannot be excluded.

Binding sites for somatomedin A in the rat kidney were reported by Takano et al. (1976a). In the present study, we observed changes in somatomedin A binding to the kidney membrane frac-

**Table 1.**

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Binding affinity (M⁻¹ × 10⁹)</th>
<th>Binding site capacity (fmole/mg protein)</th>
</tr>
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<tr>
<td>1</td>
<td>0.92</td>
<td>1.77</td>
</tr>
<tr>
<td>10</td>
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</tr>
<tr>
<td>20</td>
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</tr>
<tr>
<td>40</td>
<td>1.07</td>
<td>0.85</td>
</tr>
<tr>
<td>50</td>
<td>1.28</td>
<td>0.63</td>
</tr>
<tr>
<td>60</td>
<td>0.96</td>
<td>0.76</td>
</tr>
<tr>
<td>80</td>
<td>1.06</td>
<td>0.72</td>
</tr>
</tbody>
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tions of growing rat. Binding of labelled somatomedin A to a given amount of protein of kidney membrane fraction was highest at birth and decreased with age. Similar changes occur in lung and costal cartilage membrane fractions from rats (data not shown).

In order to determine whether the decrease in somatomedin A binding to kidney membrane fractions with age was due to the decrease of binding affinity or capacity of binding sites, Scatchard analysis of the data was used. The binding affinity for somatomedin A was relatively constant with age, but binding capacity decreased up to 30 days. We conclude that decreased binding of somatomedin A with age is due to decreased binding capacity.

Another possibility which might explain our observations is that the proportion of cell-types in the organs, i.e. the amount of connective tissue and the amount of non-membrane protein, may change with age. Because we used a crude membrane fraction between 12 000 × g and 100 000 × g, changes of somatomedin A binding due to these factors cannot be excluded. Contamination due to endogenous somatomedin A in membrane fractions could also influence the results because serum somatomedin A rose about 8 fold from day 1 to day 80. Two experiments were done to examine whether somatomedin A remained bound to cells
during membrane preparation. In the first experiment we used labelled somatomedin A and found that about 90% of labelled somatomedin A was removed during membrane preparation. In the second experiment labelled somatomedin A was incubated with the membrane fraction, more than 90% of labelled somatomedin A bound to the membrane fraction was released into the medium by 1 M acetic acid. The somatomedin A concentration in the supernatant after incubation of the membrane fraction in 1 M acetic acid was negligible. Therefore, under these conditions the possibility of somatomedin A contamination seems to be unlikely.

It is interesting to note that there is an inverse correlation between serum somatomedin level and its binding to membrane fractions from kidney. This relationship of high circulating somatomedin A with low binding sites in the kidney is consistent with hormone regulation of its own receptor i.e. 'down regulation' as reported for insulin and human growth hormone binding sites (Kahn et al. 1973; Gavin et al. 1974; Lesniak & Roth 1976).

The question has been raised as to why growth is rapid in spite of low levels of serum somatomedin in early childhood. There are several possible explanations. An increased affinity and/or capacity of binding sites may play a role in rapid growth. It has been reported that cartilage from young animals is more sensitive to the sulphate uptake when compared with cartilage from old animals. (Heins et al. 1970; Herington et al. 1976; Mosier et al. 1977). In this study, we demonstrated that somatomedin A binding to membrane fractions from kidney is highest at birth and diminishes with age, due to the decreased binding capacity. A similar observation in the human neonate is that circulating mononuclear cells have increased receptor sites when compared to adult cells (Rosenfeld et al. 1979). It may be reasonable to postulate that the higher binding of somatomedin to infantile rat tissue is responsible in part for rapid growth in early life in spite of low serum somatomedin levels. Compared to change in circulating somatomedin A levels the change in binding to tissue was however modest. Therefore, factors other than high binding of somatomedin to tissue may play a role during rapid growth; e.g. other circulating growth factors not measured by this radioreceptor assay and/or altered post-receptor sensitivity to somatomedin may be important for rapid growth. Further studies regarding these factors are required.

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