CHAPTER VI

EFFECT OF CHRONIC ADMINISTRATION OF GLUCAGON ON THE CONCENTRATION OF COLLAGEN AND ELASTIN
In the previous chapters, the effect of chronic administration of glucagon on the metabolism of glycosaminoglycans and glycoproteins in the liver has been studied. The hormone has been found to have significant effect on the metabolism of these macromolecules. Administration of glucagon for seven days has been found to result in significant decrease in the concentration of total glycosaminoglycans in the liver and of the individual glycosaminoglycan fractions except heparin. This decrease has been found to be due to the increased degradation of the glycosaminoglycans caused by the hormone, as is evident from the increased activity of the enzymes involved in their degradation. The hormone also causes alteration in the concentration of carbohydrate components of the glycoproteins in the liver. The concentration of total hexose, fucose and sialic acid in the liver glycoproteins has been found to decrease significantly in the rats administered the hormone. This decrease has again found to be due to the increased
activity of glycohydrolases caused by the hormone.

Collagen is another important macromolecular component of the extracellular matrix. It occurs intimately associated with other macromolecules which include proteoglycans and glycoproteins such as fibronectin. There are five different types of collagen each containing three polypeptide chains of about 1000 residues per chain. The polypeptide subunits may have the same sequence or a highly homologous sequence in which every third residue is glycine. The three chains are coiled about a common axis to form triple stranded helical molecules. After intracellular synthesis, the collagen molecules are secreted from the cell, and then assembled extracellularly to form highly ordered arrays, termed fibrils. The collagen fibrils then aggregate to form larger fibers. The fibrils and fibers in different tissues, however, vary considerably in their thickness, mode of aggregation and association with noncollagenous substances in the extracellular matrix.

The different types of collagen are formed by subunit polypeptide chains of different sequence. More than five types have been identified.

Each type contains three subunits, some contain three identical subunits, whereas others contain two or three
different subunits. Type 1 collagen exists in two forms: One has two different chains with the subunit structure $[\alpha 1(I)]_2 \alpha 2(I)$, and the other is a trimer of identical $\alpha 1(I)$ chains, $[\alpha 1(I)]_3$. Types II and III have three chains of the same subunit designated $\alpha 1(II)$ and $\alpha 1(III)$, respectively. Type IV contains $\alpha 1(IV)$ and $\alpha 2(IV)$ chains, designated $\alpha 1(V)$, $\alpha 2(V)$ and $\alpha 3(V)$. Types IV and V appear to have three chains per molecule, but the exact stoichiometry of the kinds of subunits has not been established. The amino acid composition of these subunits is remarkable in that about one-third of the amino acid residues are glycine. Proline plus the 3- and 4-hydroxyprolines (Hyp) provide another 21 to 23 per cent. 4-Hydroxyproline is the major hydroxyimino acid. 5-hydroxylysine (Hyl) is also present in small amounts. The different types of subunits vary in the amount of different amino acids.

As mentioned above, different types of collagen are found in different tissues, but a single tissue may have several different types. Type I predominates in the more dense tissues that are not distensible, e.g., bone, intervertebral disks and tendon. Type II is found in cartilagenous structures and type III is found in
tissues that require more compliance and less rigidity than cartilage. Type IV is generally found in basement membranes, in vascular tissues, and in the skin. Type V is found in basement membranes as well as in cartilage and bone. The most abundant collagen in the liver has been found to be type I and type III.

Collagen forms the essential framework of the tissues and organs. Many cells have been found to rest in a collagen containing basal lamina or within a collagenous matrix. Cell-collagen interactions are an essential feature of cell movement during development in the embryo and under a variety of circumstances in the adult. Collagen has been shown to have a specific binding site for fibronectin, an important glycoprotein widely distributed in the extracellular matrix. It has also been reported to bind to other macromolecules. Collagen thus plays a very important role in cell-cell interactions.

Elastin, like collagen, is another macromolecular component of the connective tissue particularly of tissues with elastic property. Like collagen, glycine accounts for about one-third of the total amino acids in elastin. Proline plus hydroxyproline content, however, is lower.
Very little information is available on the effect of hormones on the metabolism of collagen and elastin. It has been found in this laboratory that administration of insulin showed significant decrease in the concentration of collagen and elastin in the aortic tissue in diabetic animals. No information seems to be available on the effect of glucagon on the metabolism of collagen or elastin. In view of these, the effect of chronic administration of glucagon on the concentration of collagen and elastin in the liver tissue has been studied.

Materials and Methods

Male albino rats (Sprague-Dawley Strain, weighing 80-100 g) were divided into two groups of twelve rats each.

1. Control rats
2. Experimental rats given glucagon

Glucagon (0.1 mg/100 g body weight, subcutaneously) in physiological saline was given twice daily to the rats of group 2 for seven days while the control rats received the same volume of physiological saline. After the experimental period, the rats were killed and blood and tissues collected into ice-cold containers. Procedures for the estimation of acid soluble, insoluble and total
collagen are described in chapter II. Procedure for the
determination of elastin are also given in this chapter.

Results

1. Rats administered glucagon showed, as before, elevated blood glucose and plasma free fatty acid.

2. Concentration of total, acid soluble and insoluble collagen in the liver.

Results are given in table 46.
t values are given in table 46a.

The concentration of total collagen showed significant increase in the liver in the rats administered glucagon when compared to control rats. Concentration of acid soluble collagen showed a decrease while insoluble collagen showed significant increases.

3. Concentration of elastin in the liver.

Results are given in table 47.
t-values are given in table 47a.

Concentration of elastin in the liver showed significant increase in the rats administered glucagon when compared to control rats.
<table>
<thead>
<tr>
<th>Groups</th>
<th>Total (mg hydroxyproline/g dry defatted tissue)</th>
<th>Acid Soluble</th>
<th>Insoluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>5.87 ± 0.18</td>
<td>1.05 ± 0.04</td>
<td>4.82 ± 0.14</td>
</tr>
<tr>
<td>2. Experimental</td>
<td>7.11 ± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.85 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.26 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM for 6 rats.
Group 1 has been compared with group 2.
<sup>a</sup> - p < 0.01

**TABLE 46a**

't' VALUES TO TABLE 46

<table>
<thead>
<tr>
<th>'t' between groups</th>
<th>Total</th>
<th>Acid soluble</th>
<th>Insoluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 and 2</td>
<td>4.03</td>
<td>4.00</td>
<td>5.52</td>
</tr>
</tbody>
</table>
**TABLE 47**

**ELASTIN CONTENT IN THE LIVER**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Elastin (mg N/g dry defatted tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>7.53 ± 0.23</td>
</tr>
<tr>
<td>2. Experimental</td>
<td>9.65 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM for 6 rats
Group 1 has been compared with group 2
<sup>a</sup> - p < 0.01

**TABLE 47a**

't' VALUES TO TABLE 47

<table>
<thead>
<tr>
<th>'t' between groups</th>
<th>Elastin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 and 2</td>
<td>5.16</td>
</tr>
</tbody>
</table>
DISCUSSION

The results obtained indicate that glucagon has significant effect on the concentration of collagen and elastin in the liver tissue. Concentration of total and insoluble collagen increased in the liver on administration of the hormone whereas that of the soluble collagen showed a decrease. Concentration of elastin also increased in the liver in the rats administered glucagon.

In this connection the effect of this hormone on the concentration of these substances appears to be opposite to that of insulin. Insulin administration has been found in this laboratory to produce significant decrease in the concentration of collagen and elastin in the aorta in diabetic animals.

Glucagon thus may have an influence on cell-cell interactions which involves interactions between collagen, proteoglycans and specific glycoproteins particularly fibronectin.