CHAPTER IV

EXPERIMENTAL RESULTS.

The sorption and desorption of HCl experiments were conducted on lyophilised deaminated collagen and spray frozen collagen at various temperatures and they are presented section wise in this Chapter.

SECTION I

Lyophilised deaminated collagen-HCl system:

Earlier studies (1) in this laboratory were concerned with the HCl sorption on modified lyophilised collagens (esterified and acetylated). The reactivity of HCl over the samples were compared with that of lyophilised collagen. The data on the interaction of HCl on lyophilised deaminated collagen seemed to be lacking in these interesting studies. It is the purpose of this section to present the results on lyophilised deaminated collagen with HCl and compare the reactivity of the polar gas with those on lyophilised collagen and lyophilised modified collagens.

Comparison of the amounts of HCl sorption on different modified collagens:

The Table I gives the amounts of HCl sorption on lyophilised deaminated collagen together with those (1) of lyophilised samples of the original, the esterified and the
acetylated collagens (earlier reported from this laboratory). It is seen that the initial rate of HCl sorption, i.e., upto 30 minutes, is fastest, 117.3 ml/gm, on the esterified specimen, faster on the acetylated sample, 108.7 ml/gm, less fast on the deaminated sample, 89.78 ml/gm, and a little slower on the original collagen, 87.28 ml/gm, of collagen. The higher rates on the esterified and acetylated collagens compared to those on the original protein were attributed to the effect of the chemical modification of the carboxyl and hydroxyl groups. These groups are known to be interlinked (2) along the chains of the protofibril through hydrogen bond, the bonds may have been ruptured during the chemical treatment, resulting in a more open structure for the esterified and acetylated collagens. The deaminated collagen also must have undergone breaking of hydrogen bond up to some extent which would explain its sorption by slightly higher than that of pure native collagen. The HCl sorption data also suggest that esterification of carboxyl groups might have produced some other changes leading to a more open structure than acetylation of hydroxyl groups thus accounting for the higher amounts of HCl sorbed.

In case of deaminated collagen, there is also an increase of 6.2 ml/gm between 24th and 27th hour. No change in sorption between 6-24 hours has been observed for deaminated collagen, whereas for the acetylated and the esterified powders there is a noticeable amount of gas sorbed, 5.6 ml and 11.7 ml/gm.
### TABLE

Influence of chemical modification of amino, hydroxy, and carboxy groups of collagen on HCl sorption.

Temperature: 29°C  
Pressure: 49 cm Hg

<table>
<thead>
<tr>
<th>Time</th>
<th>Original</th>
<th>Deaminated</th>
<th>Acetylated</th>
<th>Esterified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 min</td>
<td>30.68</td>
<td>-</td>
<td>62.82</td>
<td>78.41</td>
</tr>
<tr>
<td>2 &quot;</td>
<td>-</td>
<td>61.63</td>
<td>75.27</td>
<td>99.76</td>
</tr>
<tr>
<td>3 &quot;</td>
<td>42.84</td>
<td>-</td>
<td>81.98</td>
<td>96.92</td>
</tr>
<tr>
<td>4 &quot;</td>
<td>-</td>
<td>66.45</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5 &quot;</td>
<td>57.46</td>
<td>-</td>
<td>89.30</td>
<td>103.50</td>
</tr>
<tr>
<td>10 &quot;</td>
<td>62.96</td>
<td>76.56</td>
<td>97.88</td>
<td>119.40</td>
</tr>
<tr>
<td>20 &quot;</td>
<td>77.85</td>
<td>84.26</td>
<td>105.20</td>
<td>115.10</td>
</tr>
<tr>
<td>30 &quot;</td>
<td>87.28</td>
<td>89.98</td>
<td>118.70</td>
<td>117.30</td>
</tr>
<tr>
<td>60 &quot;</td>
<td>103.00</td>
<td>96.79</td>
<td>113.40</td>
<td>120.26</td>
</tr>
<tr>
<td>120 &quot;</td>
<td>116.60</td>
<td>104.50</td>
<td>117.20</td>
<td>121.80</td>
</tr>
<tr>
<td>180 &quot;</td>
<td>122.20</td>
<td>-</td>
<td>119.10</td>
<td>122.70</td>
</tr>
<tr>
<td>210 &quot;</td>
<td>-</td>
<td>111.70</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.79 hrs</td>
<td>127.50</td>
<td>117.50</td>
<td>121.80</td>
<td>124.20</td>
</tr>
<tr>
<td>20.59 &quot;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>129.60</td>
</tr>
<tr>
<td>24.77 &quot;</td>
<td>131.10</td>
<td>117.50</td>
<td>127.40</td>
<td>135.20</td>
</tr>
<tr>
<td>27.77 &quot;</td>
<td>-</td>
<td>123.77</td>
<td>-</td>
<td>139.80</td>
</tr>
</tbody>
</table>

respectively, when compared to 3.6 ml/gm sorbed by the original sample. In view of the higher amounts of sorption by the
chemically modified specimens, much less than 3.6 ml/gm would be expected to be sorbed by the unmodified collagen. Hence, it has to be inferred that the side reaction of HCl with the acetylated and esterified groups must have been taking place even during the period 6–24 hours, whereas the -OH groups replacing amino groups in deaminated collagen must be assumed to have reacted with HCl only after 24 hours.

A comparison of the amounts of HCl sorbed by the four samples during the first 6 hours shows that the original, the acetylated and the esterified samples sorbed 127.5 ml/gm, 121.8 ml/gm and 124.2 ml/gm respectively, more or less the same amount, whereas the deaminated collagen sorbed a slightly less amount 117.5 ml/gm. At equilibrium i.e., after 24 hours the first three namely, the original, the acetylated and the esterified samples sorbed around 130 ml/gm at 47 cm Hg pressure of the gas showing the maximum capacity of binding the HCl and the deaminated collagen sorbed at equilibrium only 117.5 ml/gm.

**Amounts of HCl firmly bound:**

The total amounts of HCl sorbed at 29°C reversibly and firmly bound at 47 cm pressure by the lyophilised samples of the original, the acetylated, the esterified and the deaminated collagen are compared in Table II.
TABLE II

Effect of chemical modification of collagen on firm HCl bound HCl.

<table>
<thead>
<tr>
<th>Collagen</th>
<th>Temperature 29°C</th>
<th>Amount of HCl bound ml HCl/gm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Reversibly</td>
</tr>
<tr>
<td>Original</td>
<td>133.5</td>
<td>92.5</td>
</tr>
<tr>
<td>Acetylated</td>
<td>129.8</td>
<td>96.8</td>
</tr>
<tr>
<td>Esterified</td>
<td>139.8</td>
<td>103.6</td>
</tr>
<tr>
<td>Deamnated</td>
<td>136.7</td>
<td>113.6</td>
</tr>
</tbody>
</table>

From Table II, it is seen that the deaminated collagen binds the least amount of HCl (23.6 ml/gm) reflecting the absence of amino groups with which HCl reacts more strongly.

When the desorption run was carried out at 89°C on the deaminated sample which had been in equilibrium with HCl at 29°C, the permanently bound gas was found to be the same, i.e., 23.6 ml/gm as at 29°C shown in Table III. For acetylated and esterified collagens it was reported (1) that the permanently (cf Table III) bound HCl at 89°C (sorbed and desorbed at 89°C) decreased from 33.0 ml/gm to 15.1 ml/gm and 36.2 ml/gm to 27.9 ml/gm respectively.
### TABLE III

**Effect of temperature on the firmly bound HCl.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Firmly bound HCl ml NTP/gm at 29°C</th>
<th>Firmly bound HCl ml NTP/gm at 80°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylated</td>
<td>33.0</td>
<td>15.1</td>
</tr>
<tr>
<td>Esterified</td>
<td>36.2</td>
<td>27.9</td>
</tr>
<tr>
<td>Deaminated</td>
<td>23.6</td>
<td>23.6</td>
</tr>
</tbody>
</table>

This shows, the binding of HCl to deaminated collagen seems to be stronger than the binding to the remaining two modified collagens, even though one would expect HCl to bind more strongly to the amino groups of the acetylated and esterified collagens. This might mean that some of the hydroxyl groups of deaminated collagen are actively involved in binding HCl strongly at the temperatures studied.

**Distribution of firmly bound HCl in the original and the deaminated collagens:**

HCl was sorbed on the deaminated collagen at 29°C containing 23.6 ml/gm firmly bound gas. The results with those on fresh sample are given in columns 6 and 5 respectively in Table IV. The difference between the amounts adsorbed on fresh and used sample in a given interval is shown in the
7th column. These values are compared with the similar data on original collagen (1) represented in columns 2, 3 and 4 (cf Table IV).

**TABLE V.**

**Distribution of firmly bound HCl from sorption as es in the lyophilised samples of original and deamated collagens.**

<table>
<thead>
<tr>
<th>Time</th>
<th>Amount of HCl sorbed by original collagen ml NTP/gm.</th>
<th>Amount of HCl sorbed by deaminated collagen ml NTP/gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh sample</td>
<td>Jaed sample</td>
</tr>
<tr>
<td></td>
<td>Jaded sample</td>
<td>Difference</td>
</tr>
<tr>
<td></td>
<td>contain due to firmaly bound HCl gm HCl</td>
<td>contain due to firmaly bound HCl gm HCl</td>
</tr>
<tr>
<td>1 min</td>
<td>39.68</td>
<td>22.93</td>
</tr>
<tr>
<td>2 &quot;</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3 &quot;</td>
<td>42.84</td>
<td>33.77</td>
</tr>
<tr>
<td>5 &quot;</td>
<td>57.96</td>
<td>47.33</td>
</tr>
<tr>
<td>10 &quot;</td>
<td>62.96</td>
<td>51.95</td>
</tr>
<tr>
<td>20 &quot;</td>
<td>77.85</td>
<td>62.96</td>
</tr>
<tr>
<td>40 &quot;</td>
<td>94.97</td>
<td>74.76</td>
</tr>
<tr>
<td>60 &quot;</td>
<td>103.01</td>
<td>87.97</td>
</tr>
<tr>
<td>120 &quot;</td>
<td>116.70</td>
<td>88.69</td>
</tr>
<tr>
<td>180 &quot;</td>
<td>122.20</td>
<td>91.79</td>
</tr>
<tr>
<td>240 &quot;</td>
<td>124.60</td>
<td>92.41</td>
</tr>
<tr>
<td>360 &quot;</td>
<td>127.50</td>
<td>93.71</td>
</tr>
<tr>
<td>24 hrs</td>
<td>131.10</td>
<td>96.80</td>
</tr>
</tbody>
</table>

Pressure : 47 cm Hg
In the case of unmodified collagen, the difference of amounts adsorbed between fresh and used samples, i.e., the firmly bound HCl increases steadily. For example, it increases from about 8 ml at the first minute to about 10, 14.9, 27.9 and 33.8 ml/gm at the 5th, 27th, 127th and 367th minute respectively.

On the other hand, the difference in the amounts of adsorption on the used and fresh sample of the lyophilised deaminated collagen is fairly constant within 6.3 ml and 8.9 ml/gm up to the 47th minute. From 60th to 180th minute the firmly bound HCl increases from 11.01 to 22.60 ml/gm, probably because some of the sites which were hidden in the inner regions not available for adsorption before the 60th minute became available after the 60th up to 180th minute. It may be remarked in this connection that in the case of collagen treated by solvent displacement technique, the steady increase (1) in the firmly bound HCl with time was attributed to the availability of the adsorbing sites from the interior parts of collagen. After the 180th minute the firmly bound HCl for deaminated collagen remains constant around 23.9 ml/gm.

Comparison of the reversibly bound HCl at 29°C before and after desorption at 80°C:

The effect of intermediate HCl desorption at 80°C on the reversibly bound gas on deaminated collagen at 29°C
is shown in Table V along with the date on acetylated and esterified collagens (earlier work from this lab). Columns 1 and 2 (cf Table V) represent the amounts of reversibly bound gas from the acetylated and the esterified collagens and column 3 represents the values of the deaminated collagen.

**TABLE V**

*Effect of intermediate HCl desorption at 80°C on the reversibly bound gas at 29°C.*

<table>
<thead>
<tr>
<th>HCl desorption at 80°C</th>
<th>Amount of HCl reversibly desorbed from the collagen sample at 29°C (ml NTP/gm)</th>
<th>Acetylated</th>
<th>Esterified</th>
<th>Deaminated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td></td>
<td>92.04</td>
<td>93.54</td>
<td>95.40</td>
</tr>
<tr>
<td>After</td>
<td></td>
<td>93.24</td>
<td>87.47</td>
<td>96.30</td>
</tr>
</tbody>
</table>

The acetylated and the deaminated collagens bind reversibly constant amounts around 92 ml/gm and 95 ml/gm respectively, whereas the esterified collagen binds reversibly 11.14 ml/gm less than the amount 93.54 ml/gm bound before desorption at 80°C. This difference in the reversibly bound amounts in the esterified collagen was explained as due to the activation of hydroxyl groups at 80°C.

If it is so, the deaminated collagen containing more hydroxyl groups than esterified collagen must also bind
reversibly less amount of HCl. But, as already mentioned, it is binding more or less the same amount before and after desorption at 80°C, which means the above explanation does not hold good in this case. This could be shown with greater certainty, if adsorption had also been carried out on deaminated collagen at 80°C just as the other two modified collagens.

REFERENCES

1. N. Venkateswara Rao; Ph.D., 'Thesis', Madras University 1962
2. K. Kuhn and E. Gebhardt; Z. Naturforschung, 15a, 23 (1960).
CHAPTER IV.
SECTION 2.

Spray frozen collagen-HCl system:

Collagen is apparently not soluble in water, but can be made soluble in different ways, as for instance by heating it in water at 60°C - 70°C for long periods of time, i.e., 12 hours. Collagen seems to shrink at its temperature in water due to the rupture (1) of hydrogen bonds between the neighbouring peptide chains. This is generally known as the hydrothermal shrinkage of collagen. HCl sorption at several temperatures was studied earlier (2) in this lab on the hydrothermally shrunk hide powder which is insoluble in water at 60°C and its reactivity was compared with that of the hide powder not subjected to hydrothermal shrinkage.

In order to examine how the reactivity of collagen towards HCl is affected by solubilization, experiments were carried out on the soluble sample, at 29°C to 80°C and the results are detailed in this section.

Desorption runs at 28.6°C, 40°C, 60°C and 80°C:

Equilibrium pressures were measured at several stages during the desorption of HCl, corresponding to sorption runs. The actual procedure employed in such cases was described in Chapter II.
The equilibrium pressures and the amounts of HCl bound at each pressure at various temperatures are shown in Fig. 6. The lower curve is the adsorption isotherm and the upper one is the desorption isotherm. They are marked by arrows accordingly. Isothermal data on other proteins were used by Benson (3) and on hide powder by Rao (2), (4) to verify compound formation between protein and HCl. An isobar in the isotherm was interpreted to indicate compound formation whereas its absence was taken to mean the formation of a complex.

In agreement with the previous studies on hide powder (2,3) the desorption isotherms of HCl on hide powder (cf Fig. 7) reveal no isobaric regions and therefore rule out any compound formation.

However, it is of interest to see whether the HCl bound would vary linearly with the equilibrium pressure. Such a behaviour is predicted by the classical Freundlich equation

$$\frac{x}{m} = kp^n$$

where \(x\) is the amount of gas adsorbed by \(m\) gm of adsorbing material at pressure \(p\), and \(k\) and \(n\) are constants for the given system at a given temperature. On taking logarithms, it becomes

$$\log \frac{x}{m} = \log k + \frac{1}{n} \log p$$
FIG. 7
DESORPTION ISOTHERM ON SPRAY FROZEN COLLAGEN - HCl SYSTEM

○ - 26°C
△ - 40°C
● - 60°C
▲ - 80°C
Fig. 8

FREUNDLICH PLOTS OF DESORPTION DATA ON STRAY FROZEN COLLAGEN - HCl SYSTEM

○ - 28.6°C

● - 40°C

log V

log p

-2.0 -1.0 0.0 1.0 2.0
so that a plot of log x against log p should be linear. The Freundlich plots of the desorption equilibrium data at temperatures 28.6° and 40°C are presented in Fig. 8. The plots are reasonably linear, indicating that within the pressure range studied, compound formation does not occur.

In spite of the absence of any evidence for compound formation, it is interesting to note that a considerable amount of HCl remains bound by collagen, even at low pressures of 10^{-6} cm. For example as much as 33.9 ml/gm remains bound at 28.6°C, 40.42 ml/gm at 40°C, 49 ml/gm at 60°C and 49.7 ml/gm at 80°C. Therefore, it seems certain that a part of the total amount of HCl sorbed is rather strongly bound by some reactive groups of collagen, most probably by the basic amino groups. It can also be seen from Tables (VI, VII) that two thirds of the HCl bound at higher pressures gets easily removed at 28.6° and 40°C on lowering the pressure considerably, and it does so about half of the amount bound at higher temperatures 60° and 80°C as shown in Tables (VIII, IX).

None of the desorption isotherms in Fig. 7 show any isobaric break indicative of compound formation in the pressure range 40 - 0.1 cm of HCl. Whether a pressure of 0.1 cm is sufficient or not for the appearance of an isobar in the isotherms at 28.6°, 40°C, it should be quite adequate at least at 60° and 80°C to reveal such an isobaric region if that has been actually present but missed at low temperatures. It may
### Table VI.

Desorption of HCl from *S. collana.*

<table>
<thead>
<tr>
<th>Temperature 28.6°C</th>
<th>Vol. of HCl bound in ml/gm at NTP</th>
<th>Equilibrium pressure P cm Hg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>138.7</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>136.2</td>
<td>29.9</td>
<td></td>
</tr>
<tr>
<td>131.9</td>
<td>19.9</td>
<td></td>
</tr>
<tr>
<td>122.3</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>112.3</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>93.6</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>79.1</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>44.4</td>
<td>6.7 x 10^{-3}</td>
<td></td>
</tr>
<tr>
<td>34.0</td>
<td>10^{-6}</td>
<td></td>
</tr>
</tbody>
</table>

### Table VII.

Desorption of HCl from *S. collana.*

<table>
<thead>
<tr>
<th>Temperature 40°C</th>
<th>Vol. of HCl bound in ml/gm at NTP</th>
<th>Equilibrium pressure P cm Hg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>119.4</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>116.2</td>
<td>29.9</td>
<td></td>
</tr>
<tr>
<td>110.5</td>
<td>19.9</td>
<td></td>
</tr>
<tr>
<td>101.9</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>93.7</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>76.2</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>58.3</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>42.9</td>
<td>5.2 x 10^{-3}</td>
<td></td>
</tr>
<tr>
<td>40.4</td>
<td>10^{-6}</td>
<td></td>
</tr>
</tbody>
</table>

### Table VIII.

Desorption of HCl from *S.F. collana.*

<table>
<thead>
<tr>
<th>Temperature 60°C</th>
<th>Vol. of HCl bound in ml/gm at NTP</th>
<th>Equilibrium pressure P cm Hg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>104.8</td>
<td>39.0</td>
<td></td>
</tr>
<tr>
<td>100.1</td>
<td>28.4</td>
<td></td>
</tr>
<tr>
<td>97.2</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>95.5</td>
<td>14.6</td>
<td></td>
</tr>
<tr>
<td>91.9</td>
<td>10.1</td>
<td></td>
</tr>
<tr>
<td>85.8</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>74.0</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>61.9</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>49.0</td>
<td>10^{-6}</td>
<td></td>
</tr>
</tbody>
</table>

### Table IX.

Desorption of HCl from *S.F. collana.*

<table>
<thead>
<tr>
<th>Temperature 80°C</th>
<th>Vol. of HCl bound in ml/gm at NTP</th>
<th>Equilibrium pressure P cm Hg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>88.3</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>86.2</td>
<td>29.7</td>
<td></td>
</tr>
<tr>
<td>86.2</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>82.2</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>79.0</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>70.4</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>62.9</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>49.7</td>
<td>10^{-6}</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 9

FREUNDLICH PLOTS OF ABSORPTION DATA ON SPRAY FROZEN COLLAGEN - HCl SYSTEM.

- 60°C

- 80°C
be stated in support of this argument that in the egg albumin-HCl system investigated by Benson and Srinivasan (3), the isobar showed up in the neighbourhood of 7.01 cm at 52°C although it was not detected at 32°C with a pressure as low as 0.9991 cm. Therefore, it is reasonable to presume that the isobar is not present in the isotherm at 28.6°C or 40°C even at very low pressures. The second possibility is that the isotherms at 60°C and 80°C do not represent correctly the nature of the isotherms at relatively low temperatures, if some other type of reaction such as the activated HCl sorption were to occur at these high temperatures.

In order to see if further clarification could be obtained regarding the compound formation, Freundlich equation was also applied to the desorption data at 60°C and 80°C, and the plots are shown in Fig. 9. They are found to be reasonably linear in the pressure range 40 to 7.1 cm. Thus, the evidence seems to be rather against the formation of a compound between collagen and HCl, under the experimental conditions.

Heats of adsorption for reversibly bound HCl:

The equilibrium pressures $p_1$ and $p_2$ at the same amounts of adsorption of HCl were read off from two adsorption isotherms in Fig. 6 and substituted in the Clausius-Clapeyron
Fig. 10
DIFFERENTIAL HEAT OF ADSORPTION OF HCl ON SPRAY DRIED COLLAGEN

- 28.6 - 40.6°C
- 40.6 - 60°C
- 60 - 80°C

ΔH Kcal/mole

amount adsorbed, ml/gm
\[ \Delta H = 2.303 \times R \times \log \frac{p_1}{p_2} \]

where \( R \) is a gas constant, \( p_1 \) and \( p_2 \) are equilibrium pressures at two different temperatures \( T_1 \) and \( T_2 \) respectively and \( \Delta H \) is the heat of adsorption.

The heats thus obtained at different coverages of HCl are shown in the Fig. 10. The heat of adsorption in the temperature interval 20.6°C - 40.0°C appears to have a maximum value of 15 k cals/mole at an adsorption of 97 ml/gm of protein. Essentially similar heats were reported by Benson and Srinivasan (3) from the isotherm of HCl on egg albumin. The fall in the heats with the amount of adsorption, must be due to the adsorption on the less active groups.

At 40.0°C - 67°C, the maximum heat is 16 k cals/mole which is more or less equal to that in the lower temperature range and the heat curve is gradually falling with the amount of adsorption.

The values at still higher temperature range seem to be abnormally low, 6 k cals/mole, for inexplicable reasons.

**Foot of temperature on irreversible sorption of HCl and the distribution of it in the array frozen so lase**.

Since the HCl firmly bound by a protein is normally assumed to have entered into chemical combination with its side chain amino groups, it should be possible to get some
idea about their spatial distribution in the protein molecule by finding out how the permanently bound gas is distributed.

It is assumed that the HCl bound at the desorption equilibrium pressure of 10^-6 cm is more or less strongly held by the reactive groups of collagen. From the results in Table X, the amount would be on average 2.08 \pm 0.57 m mole/gm.

**TABLE X**

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Permanently bound gas in ml/gm at NTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.6</td>
<td>33.9</td>
</tr>
<tr>
<td>40.0</td>
<td>40.4</td>
</tr>
<tr>
<td>60.0</td>
<td>49.0</td>
</tr>
<tr>
<td>80.0</td>
<td>49.7</td>
</tr>
<tr>
<td>40.0</td>
<td>59.7</td>
</tr>
</tbody>
</table>

It is generally believed that only the side chain free amino groups of arginine, lysine and histidine in the protein bind HCl strongly by valancy or chemical forces. This is supported by the investigations of a number of people, and especially Benson and Seehof (5,6). Collagen contains 0.93 m mole/gm of these basic amino groups and therefore can
account only for 0.95 m mole of the 2.08 m mole/gm of the firmly bound HCl. The remaining 1.13 m mole/gm cannot be accounted for, even though it is assumed that the 0.2 to 0.3 m mole/gm of amide groups also bind HCl in the same way. In this connection, it may be said that, since spray frozen collagen is denatured during the preparation, more polar sites must have been exposed to HCl gas. For this reason, it might be binding firmly higher amounts.

As is seen from the Table XX, the firmly bound HCl is increasing with the temperature unlike HCl on insulin (7). This increase might be due to the opening of the closely packed structure, thereby exposing more polar sites.

Another interesting observation is, when the protein was cooled to 40°C from 80°C and the sorption experiment was performed, the amount of irreversibly bound HCl was found to have increased to 59.4 ml/gm at NTP. This confirms the observations made earlier in this lab (2) and clearly shows that there is some irreversible change in the groups accessible to the polar gas.

A mention may be made that the white spray frozen collagen turned orange-brown after sorbing HCl at 60° and 80°C. The appearance of this colour was not so distinct in experiments at 29° or 47°C. The colour produced at 60° or 80°C did not disappear even after desorbing as much of HCl
as possible and hence appears to be a permanent change.
Collagen is known to contain small amounts of carbohydrate
material (8) and the latter may react with HCl gas parti-
cularly at higher temperatures such as 60° or 80°C to give
rise to the orange-brown colour observed.

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Fig. 11

OXYGEN ISOTOPES OF WATER ON POLY-L-PROLINE II.

- △ - 30°C
- ○ - 40°C
- ■ - 60°C