THE HISTOCHEMISTRY OF THE ADRENAL GLAND

I. THE QUANTITATIVE DISTRIBUTION OF VITAMIN C

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Because the adrenal glands contain a higher concentration of vitamin C than most other parts of the body (1, 2), they have been a focus of attention for those interested in the metabolism of the vitamin, and the pathological conditions resulting from its deficiency. At the present time, a number of attempts to establish the histological distribution of the vitamin in the adrenals have been made. Thus, Giroud and Leblond (3), Westergaard (4), Siehrs and Miller (5), and Bessey, Menten, and King (6), separately employed the silver nitrate staining reaction to indicate the distribution of vitamin C in the adrenal glands. However, as pointed out by Harris and Ray (7), and Gough and Zilva (8), this staining reaction is unreliable in some instances.

Since the introduction of a direct chemical method for quantitative estimation of the vitamin C in microtome sections of tissue (9), it is possible to determine the distribution of this substance with much greater accuracy than the silver staining reaction permits. Accordingly, this method has been employed in the present investigation, so that the number and type of the cells in the adrenal gland can be correlated with the presence of the vitamin.

EXPERIMENTAL

Beef adrenals were employed for the present investigation, since the normal glands could be obtained in a fresh state, and they are large enough to be handled conveniently. Furthermore, the beef adrenal has essentially the same histological character as the
human gland. The adrenals were removed from freshly killed animals, transported at once to the laboratory in a box with a separate ice compartment, stripped of all fat, and stored in closed Petri dishes at \(-5^\circ\). The glands were used within 1 to 2 days, though preliminary experiments showed that there was no appreciable loss of the vitamin after 4 days.

The procedure followed in the sampling and sectioning of the tissue was patterned after the method described by Holter and Linderstrøm-Lang (10), and the histological studies were made in a manner similar to that employed by Linderstrøm-Lang, Holter, and Søeberg-Ohlsen (11). The titration of the vitamin was carried out with 2,6-dichlorophenol indophenol as previously described (9). A sharp cork borer, having an internal diameter of 4.5 mm., was used to remove a cylinder of tissue through the smallest axis of the frozen adrenal. This cylinder was placed at once on the freezing microtome (rotary type), and sections 30 μ thick were removed, placed in tubes containing 50 c.mm. of 9 per cent acetic acid, and the tubes were immersed in a salt-ice mixture until ready for titration as described by Glick (9). The titrations were carried out in two series. In the first series every other tube was titrated, and in the second, the remaining tubes. The tubes of the second series were titrated approximately 1 hour after those of the first. In this manner it was shown that this tissue, as already demonstrated for rabbit liver (9), is completely extracted of all its vitamin almost at once, and there is no loss due to standing in the freezing mixture, since adjacent slices, titrated 1 hour apart, fit on the same curve (Fig. 1).

Before the histological studies were undertaken, a section of tissue was fixed in Orth's fluid and paraffin sections were stained with hematoxylin to identify the medullary cells by the chromaffin reaction. Once these cells were identified, they were easily recognized in all other tissue preparations.

Immediately after the vitamin titration, the tubes were filled with 95 per cent alcohol, and in a few minutes the sections were placed in 10 per cent formalin, stained with hematoxylin and eosin, and mounted. This procedure, in which first alcohol and then formalin is employed, was found to be the best of a number of combinations tried, since it enabled a flattening out of the sections so that they might be more easily handled. Of course,
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FIG. 1. Curve A. The points marked ○ were obtained by titration conducted after the tissue remained in acetic acid solution approximately 1 hour longer than for the titration indicated by the points marked +. 1 c.mm. of dye = 0.150 microgram (γ) of vitamin C. Owing to the fact that the microtome used was calibrated wrongly, the ordinates for Curve B should be multiplied by 2.26; the ordinates for Curve C should be divided by 2.26.
these sections are not as good for histological study as the paraffin sections, but they do enable a distinction of the type of cell present. In this way the correlation between the titrations and the histological picture could be determined.

As a check on the preceding method of correlating the chemistry and histology, the following procedure was employed. The block of tissue surrounding the removed core, that was used for vitamin C analysis, was fixed in 10 per cent formalin, dehydrated in graded alcohols, and imbedded in paraffin in the customary manner. Serial sections 8 μ thick were taken, and every seventh section was mounted and stained with hematoxylin and eosin.

A comparison was made of the number of cells in the various zones of the cortex and in the chromaffin region of the medulla, in order to estimate the relative amount of vitamin C contained in the different types of cells. Furthermore, each of these zones was composed of practically one type of cell. The areas in which cells were counted were free of nerves, large blood vessels, and thick bundles of connective tissue.

For cell counting, a slice of the adrenal, representing a cross-section of the gland, was fixed, imbedded, sectioned 4 μ thick, and stained with iron hematoxylin (Masson's technique). It was found impractical to make cell counts on the 8 μ sections, since the simpler staining technique employed for them did not bring out the nuclei with sufficient clarity.

The cell counts were carried out with a 15 X ocular and an HI 90, 1.30 oil immersion objective (Zeiss) on a binocular microscope. The area of the field under observation was found to be 0.012 sq.mm., calculated from the diameter measured with a micrometer slide. Every parenchymal nucleus in the field was counted.

Results

The results of the titrations were represented graphically by plotting the vitamin C content of each slice against its distance from the upper surface of the gland. The curve so constructed shows the variations of the vitamin content of the cells through the various layers of the adrenal. One curve, of the five obtained, is included (Curve A, Fig. 1) as representative of the typical vitamin C distribution.
To facilitate comparison of the chemical findings with the histology, a sketch of the cross-section of the adrenal, expanded to the same scale as the curve, has been included. This sketch is based on the histological picture obtained by examination of the serial sections actually used for titration and checked by comparison with the paraffin sections, after due allowance had been made for the shrinking of the tissue block while being fixed and imbedded. To get the distance from the surface of the gland of any paraffin section, it was found necessary to multiply the distance of the section from the surface by the factor 1.28. This factor has been obtained by dividing the length of the hole in the tissue before fixation by the length after imbedding.

**TABLE I**

*Cell Counts in Various Portions of the Adrenal Gland*

The readings represent the number of cells per $72 \times 10^{-3}$ $\mu l$ of stained tissue, equivalent to $0.15 \mu l$ of fresh tissue.

<table>
<thead>
<tr>
<th>Glomerulosa</th>
<th>Zona fasciculata</th>
<th>Mixed fascicular and reticular region</th>
<th>Chromaffin cells nearest cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nearest glomerulosa</td>
<td>Central portion</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>115</td>
<td>77</td>
<td>112</td>
</tr>
<tr>
<td>80</td>
<td>107</td>
<td>86</td>
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<td>88</td>
<td>106</td>
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<td>89</td>
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<td>97</td>
<td>124</td>
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<td>97</td>
</tr>
<tr>
<td>88</td>
<td>114</td>
<td>79</td>
<td>118</td>
</tr>
<tr>
<td><strong>Average...90</strong></td>
<td><strong>115</strong></td>
<td><strong>87</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

The average of the counts for six different areas in each region (Table I) was used to calculate a point in Curve B, Fig. 1. The volume of the group of cells included in each count was $0.012$ sq.mm. $\times 0.006$ mm. $= 72 \times 10^{-6}$ c.mm. or $72 \times 10^{-3}$ $\mu l$. Since the tissue shrank, the original volume was $72 \times 10^{-3} \times (1.28)^3 = 0.15 \mu l$. Hence in the glomerulosa there are $90/0.15 = 600$ (cells/$\mu l$). In the fasciculata nearest the glomerulosa $115/0.15 = 766$ (cells/$\mu l$). In the central portion of the fascicular $87/0.15 = 580$ (cells/$\mu l$). In the mixed fascicular and reticular region $100/0.15 = 666$ (cells/$\mu l$). And in the medulla $85/0.15 = 566$ (cells/$\mu l$).
The volume of the sections used in the vitamin C determination was 0.030 mm. \( \times (\pi/4 \times (4.5)^2) \) sq.mm. = 477 \( mL \).

From the foregoing data the number of cells per 30 \( \mu \) slice was calculated and represented graphically in Curve B, Fig. 1.

The ratio of the micrograms of vitamin per slice, to the number of cells per slice, gives the micrograms per cell, which were represented in Curve C, Fig. 1.

**DISCUSSION**

The cross-section of the adrenal gland demonstrated in Fig. 1 shows that the central medullary portion is flanked on each side by the cortex. The gland is encapsulated by a thin layer of fibrous tissue (C) which extends somewhat into the adjacent glomerular zone (G) of the cortex. The glomerular region gives way rather abruptly to the zona fasciculata. The outer portion of the fasciculata (F1) is composed of densely packed cells surrounding small sinusoids. These sinusoids become larger, and the cells less numerous in the region F2. The fasciculata is gradually displaced by the reticularis (R), where the cells again become rather compactly arranged. The border between the reticularis and the medulla (M) is sharp but irregular. The medulla is composed of chromaffin cells (K) among which are foci of sympathetic nerve cells (S). In this region there are nerves (N) and large vascular spaces (V).

It will be seen in Fig. 1 that the highest peak in the curve corresponds to the fascicular region (F1). The sharp drop in F2 can be explained by the change in the number, not the type, of cells in this region (Curve B, Fig. 1).

A practical difficulty was encountered with some of the frozen section slices, especially in the medulla, since the occurrence of large sinusoids and vessels, which could be seen macroscopically at times, displaced normal adrenal tissue. The chief deviations of points from the curve could be explained on this basis.

The right half of Curve A is lower than the corresponding left half, probably because the right end of the cylinder of adrenal tissue was set in a drop of water on the microtome block and immediately frozen to make a firm attachment. This procedure no doubt caused some extraction of the vitamin and resulted in the decrease in concentration.

As shown by Curve A, Fig. 1, the concentration of vitamin C in
the most active fasciculata is about $1\frac{1}{2}$ times as great as that in the medulla. This corresponds with the results of Harris and Ray (7). These workers, however, made their determinations on a macro scale, on the entire cortex or medulla of the adrenal of the ox. They report 1.1 to 1.2 mg. of the vitamin per gm. of medulla while our results, converted to the same units, show 1.2 to 1.3 mg. per gm.

From a comparison of Curves A and B, Fig. 1, it will be seen that the most active fascicular zone corresponds to the greatest concentration of cells, while the least active medulla contains the smallest concentration of cells. However, the smaller fascicular cell still contains more vitamin than the medullary cell (Curve C, Fig. 1).

SUMMARY

A histochemical study was made of the quantitative distribution of vitamin C in the beef adrenal by means of a technique enabling chemical determination of the vitamin in microtome sections of tissue.

The concentration of vitamin C in the various zones of the gland, the relative number of the different cells in these zones, and the vitamin content per cell were estimated.

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BIBLIOGRAPHY

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