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VIDEO SCANNING FOR DETERMINATION OF THE PROPORTION OF CORTICAL TISSUE IN THE AVIAN ADRENAL GLAND*

By

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VESTERGAARD, K. and P. WILLEBERG: *Video scanning for determination of the proportion of cortical tissue in the avian adrenal gland.* Acta vet. scand. 1978, *19*, 331—340. — The use of a video scanning apparatus (Leitz, T.A.S.) for the determination of the corticomedullary proportion in histological sections of the avian adrenal gland is described and statistically evaluated. When the video scanning method was applied to material from groups of domestic hens, which had been exposed to different experimental conditions, the results were similar to those obtained through the integrating method as described by *Siller et al.* (1975). The mean values obtained by both methods did not differ significantly, and there was a highly significant correlation between the counts for both methods applied on the same sections. When applying the video scanning method to 16 sections from four adrenals, repeated measurements on each of the sections showed considerable variation. However, this variation was found to be significantly smaller than the variation among the sections. It is suggested that the video scanning method could be made more precise by improvement of the staining procedure. However, on relatively large samples it seems to give reliable results, and it has a great advantage in reducing the tedious work involved in other available methods.

avian; adrenal; video scanning; texture analyzing system; image processing.

Assessment of the relative amount of medullary and cortical tissue in the adrenal gland of birds is possible by determination of area sizes in histological sections. However, this is intrigued by the complex interwoven arrangement of the tissues. One of the available methods implies projection on paper, tracing, cutting out and weighing of the respective components (*Latimer*

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& Landwer 1924—25, *Sauer & Latimer* 1931, *Miller & Riddle* 1942, *Kar* 1947 and *Miller* 1967). Another method used by *Bareham* (1972) is based on projection on graphed paper and counting of the mm squares containing predominantly cortical or medullar tissue.

The third and so far most reliable method is the "integrating" method described and carefully evaluated by *Siller et al.* (1975). The present paper describes and examines a method based on automatic area determination by a video scanning equipment. Results obtained by this method and by the integrating method are compared.

MATERIALS AND METHODS

General

The experimental hens were white leghorn bought from a commercial unit when 18 weeks old. The hens had been beak-trimmed on Day 1 and were raised in big flocks on deep litter. At the day of purchase the hens were randomly allocated to four experimental groups each consisting of 116 hens. Two of the flocks were housed on sloped wire floors, the other two on deep litter. The two flocks on each of the floor types had stocking rates of seven and 14 hens per m^2 respectively. All other factors which might influence the behaviour and/or physiology, i.e. space at feeders and waterers, number of nests, illumination etc. were similar. The flocks were housed in the same room, but visually isolated.

Extensive behavioural studies and assessment of the humoral corticosteroid level were carried out. The results of these investigations will be published elsewhere.

Both adrenals from 10 hens in each of the four flocks were obtained when the hens were 18 months old. The hens were captured and killed within 5 min. and the adrenals were immediately dissected, cut into two and fixed in Allen's B 15 solution (*Silverton & Anderson* 1961). Thereafter they were embedded in paraffin wax. Sections of 7 μm from the middle part of the gland were cut, mounted on slides and stained by the picro-Mallory method. From a few glands it was impossible to prepare proper sections.

For comparison with the video scanning method the proportion of cortical tissue out of combined cortical and medullary tissues was first assessed by the integrating method as described

by *Siller et al.* (1975). According to this method the whole section is projected on ¼ inch (here ½ cm) squared paper so that each section covers approx. 2500 grid intersection points. Points with, respectively, cortical and medullary tissue projected on them were counted, and the proportion of cortical tissue to combined cortical and medullary tissue was calculated.

The video scanning method was applied on the same sections that were tested by the integrating method. The measurements were done with the aid of a Leitz texture analyzing system (T.A.S.).

The video scanning method

Measures of areas by the T.A.S. is based on the number of raster elements of the video image. The image is projected on a sensitive layer in a plumbicon tube and scanned line by line. Different brightnesses in the image are then transformed into electrical signals, and the number of raster elements of the different components of the image are thus automatically counted and can be computerized for further investigations (*Serra* 1973). Separation of the different image components is based on the shades of grey of the components. In the present case the sections were projected by the aid of a microscope with a 25 × objective. The image which was analyzed in a single operation comprised 300 × 180 μ ∞ 406,000 raster points. The image was displayed on a video monitor.

Three components on each section were separated: cortical tissue, medullar tissue and empty space, i.e. openings in the section mainly comprising blood vessel lumina. The area of the minor fraction comprising connective tissue etc. was not assessed. In order to enhance the difference in the shades of grey between cortical and medullar tissue two filters were used: red 630 nm and blue 470 nm. Most often the red filter was applied, but sometimes better separation could be obtained by the blue filter. The effect was visually checked on the monitor.

Each of the components of the image was cleaned for minor gaps of different shades in grey, which was mainly due to the cell nuclei. This cleaning was carried out either by the overture process or by the fermeture process which are parts of the T.A.S. programme. The effects of the different steps in the cleaning processes were checked on the monitor which could display the component in question in white colour.

Representation of each of the sections

A single image only covered a small fraction of each section. However, the sections were placed on a scanning stage which automatically stepped to 50 different images according to a preset programme. The counts for each of the images as well as for the total of the 50 images were printed on a paper by a calculating unit. The pattern of steps was meander shaped, and there was overlap between the different pictures. The size of the steps, the stepping pattern and the degree of representation of a section by the video scanning method are depicted in Fig. 1. After each step the area of the component was measured. When the 50 images of a section had been analyzed for the first com-

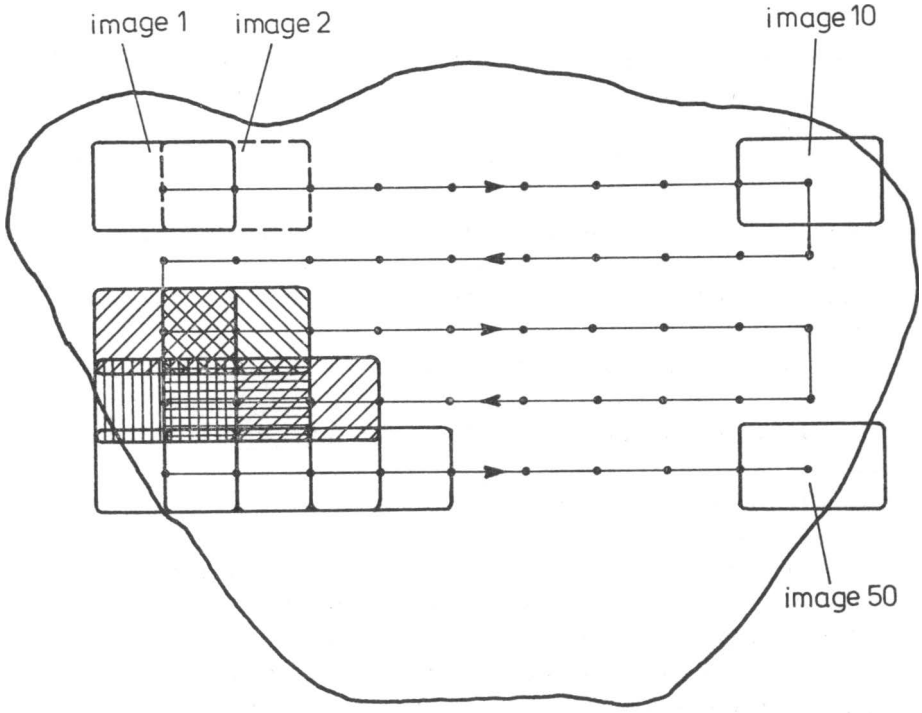

Figure 1. Representation of the adrenal sections by the video scanning method. Some of the areas, where the images overlap, are shown by shading. The dots indicate the centres of the images, and the arrows show the direction of the steps. The outline of the smallest section which had a maximum diameter of 2000 μ is indicated. The largest section had a maximum diameter of 4500 μ.

ponent, the scanning stage automatically stepped back to the starting point and after adjustments (applying the right interval of shades of grey, cleaning the image) on the first image, the area of the second component was assessed. Thirdly the same procedure was carried out for the last component.

As a check of the method the total area for the three components of all images was calculated and compared to the theoretical value which was 20.3 million raster points.

Statistical design and analysis

The counts obtained were transferred on to punched cards along with a coded description of the experimental group from which the animal and its adrenal gland originated. The data were subsequently processed by standard computer programs.

In Experiment 1 analyses of variance were carried out for the proportions obtained by the integrating method and for those obtained by the video scanning method, respectively. Only one "central" section from each adrenal was used. Factors were 1) type of floor (deep litter or sloped wire floor), 2) density (seven or 14 hens per m^2) and 3) side (right or left adrenal). Secondly, the two methods were tested against each other in an analysis of variance where method (integrating or video scanning) was a factor in addition to factors 1 and 2 mentioned above. Thirdly, the correlation between the proportions obtained from 74 sections by the two methods was determined.

In Experiment 2 four glands were randomly selected among the material used in Experiment 1, and from each gland, four sections were examined by the video scanning method. In this experiment the sections were measured four times, each time starting at a new location of the section. For each section within each gland, the mean, standard deviation and coefficient of variation were calculated. Secondly, an analysis of variance was carried out, to test whether the variation within sections differed from the variation among sections. This was done for each of the four glands as well as for the 16 sections combined.

RESULTS

Experiment 1

Generally, the two methods, when applied to the present material, gave similar results (Table 1). By both methods the mean proportions are higher for deep litter hens as compared

Table 1. Comparison of the integrating method and the video scanning method: Adreno-cortical proportion in hens related to housing conditions and side of adrenal.

		Integrating method			Video scanning method		
Factor	Category	Mean ± s	n	significance of difference	Mean ± s	n	significance of difference
Type of floor	Deep litter	0.593 ± 0.072	37	$P < 0.076$	0.594 ± 0.112	34	$P < 0.007$
	Sloped wire floor	0.567 ± 0.061	40		0.528 ± 0.099	40	
Density	7 hens per m²	0.595 ± 0.063	39	$P < 0.035$	0.582 ± 0.110	38	$P < 0.042$
	14 hens per m²	0.563 ± 0.068	38		0.533 ± 0.105	36	
Side	Right gland	0.582 ± 0.072	39	$P < 0.697$	0.550 ± 0.104	37	$P < 0.503$
	Left gland	0.577 ± 0.063	38		0.567 ± 0.116	37	
Total		0.580 ± 0.0257	77		0.558 ± 0.0320	74	
	Significance of difference between methods			$P < 0.126$			

Correlation: $r = 0.50$, $n = 74$, $P < 0.0001$.

to wire floor hens, and also higher values are found for hens kept at low density (seven hens per m²) as compared to high density (14 hens per m²). All differences are statistically significant, except for the effect of type of floor measured by the integrating method. Neither of the methods uncovered significant differences between right and left adrenals. There were tendencies that the counts found for sloped wire floors and for 14 hens per m² were lower when measured by the video scanning method, and thus the effects of these factors seem to be enhanced by the video scanning method. However, as is also seen from Table 1, no significant difference in overall means for the two methods was found. There was a highly significant positive correlation between the proportions obtained by the two methods.

The mean sum of raster points from each group of hens were 1.822 ± 0.266, 1.8307 ± 0.195, 1.829 ± 0.155 and 1.868 ± 0.151 million, respectively, making up 90—92 % of the theoretically expected value.

Table 2. Adreno-cortical proportions by the video scanning method. Variation among and within sections. Statistics based on four replications within each section.

Gland no.	Section no.	Mean	s	Coefficient of variation	F (among/within section)	
	1	0.626	0.178	29.8		
	2	0.442	0.173	39.2		
1	3	0.407	0.068	16.6	2.79	$P < 0.086$
	4	0.643	0.128	19.9		
	Total	0.529	0.171	32.2		
	1	0.531	0.070	13.3		
	2	0.393	0.119	30.4		
2	3	0.609	0.070	11.4	5.42	$P < 0.014$
	4	0.592	0.066	11.2		
	Total	0.531	0.116	21.8		
	1	0.640	0.056	8.8		
	2	0.464	0.121	26.1		
3	3	0.528	0.069	13.1	2.86	$P < 0.081$
	4	0.599	0.106	17.6		
	Total	0.558	0.108	19.3		
	1	0.589	0.061	10.3		
	2	0.477	0.011	2.4		
4	3	0.556	0.060	10.8	3.06	$P < 0.069$
	4	0.461	0.111	24.1		
	Total	0.521	0.084	16.1		
Grand total		0.535	0.121	22.7	2.70	$P < 0.0048$

Experiment 2

From Table 2 it appears that the range in standard deviations for sections was from 0.011 to 0.187. The range in coefficients of variation was 2.4 to 39.2 %. Similarly for the glands, the range in standard deviations was 0.084 to 0.171 and the range in coefficients of variation was 16.1 to 32.2 %. The overall variation within sections was found to be smaller compared to the variation among sections ($P < 0.0048$).

DISCUSSION

Proportions of the cortical and medullary components of the avian adrenal gland have been used in many studies to assess quantitative changes after exposure to different experimental conditions and treatments (*Oakberg* 1951, *Heindle* 1955, *Siegel*

1959, *Bareham* 1972, *Bhattacharya & Ghosh* 1972). Effects of age (*Payne* 1955, *Siller et al.* 1975) and season (*Höhn* 1947–48) have also been evaluated on the basis of the cortico-medullary proportion.

In other studies simply the weight of the adrenals or the weight relative to body weight have been assessed. A complete list of available methods is found in *Siller et al. Oakberg* found that the proportion obtained by the integrating method of *Chalkely* (1943) was independent of the orientation of the section, and *Siller et al.* found that for evaluation by their integrating method, a single "central" section of unknown orientation is sufficient to obtain a reliable result. Therefore in the present study the counts were also based on a single "central" section. The integrating method was found to be much less laborious than the tracing method, but it still involves much tedious work. *Siller et al.* estimated that at least 2500 grid points had to be counted in order to keep the variance due to counting points below 0.0001 (corresponding with a standard error of $\pm$ 0.01). By the video scanning method less than 15 min. is sufficient to assess the proportion for a single section, and there is no tedious counting work.

One of the major differences between the integrating method and the present method is that the former is based on counts from the whole section, whereas the latter is based on counts from only part of a section. This might influence the accuracy of the video scanning method. However, when applied on the material (Exp. 1) which was also tested by the integrating method, similar results were found, and there were also tendencies that the differences found between treatments were enhanced by the video scanning method. It was also found that there was a highly significant positive correlation between the counts obtained by the two methods. Experiment 2 showed that the video scanning method gives a considerable standard deviation and coefficient of variation for measures within sections, but this variation was smaller than the variation among sections from a given gland.

In conclusion the video scanning method seems to be an easy and very quick way of assessing the cortico-medullary proportion in the avian adrenal gland. For further use of this method it is suggested that the variation within sections could be lowered by improvement of the staining procedure to give a better separation in shades of grey of the components.

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SAMMENDRAG

Video scanning til bestemmelse af proportionen af barkvæv i fuglebinyrer.

Artiklen beskriver, hvorledes et video scanning-apparat til automatisk billedanalyse (Leitz, T.A.S.) kan benyttes ved bestemmelse af proportionen af barkvæv i histologiske snit af fuglebinyrer. Metoden blev anvendt på et materiale fra grupper af tamhøner, der havde været udsat for forskellige forsøgsbehandlinger, og resultaterne viste overensstemmelse med de, der kunne opnås ved integrationsmetoden beskrevet af *Siller et al.* (1975). Gennemsnitsværdierne, der opnåedes ved de 2 metoder, var ikke signifikant forskellige, og der var en meget signifikant korrelation mellem talværdier opnået ved begge metoder på de samme histologiske snit. Forsøg, hvor video scanning-metoden blev anvendt på 16 forskellige snit fra 4 forskellige binyrer, viste, at gentagne målinger på hvert af snittene gav betydelig variation i de opnåede talværdier. Denne variation var dog signifikant mindre end variationen mellem de forskellige snit. Den beskrevne video scanning-metode kan formentlig gøres mere præcis ved forbedring af den anvendte farveteknik. Allerede nu kan den imidlertid, anvendt på et relativt stort materiale, give pålidelige resultater, ligesom den er hurtig og arbejdsbesparende sammenlignet med andre metoder.

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