The Comparative Biochemistry of Avian Egg White Proteins*

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(Received for publication, March 7, 1960)

The homologous proteins from the egg whites of different avian species offer excellent opportunities for the study of the comparative and genetic biochemistry of proteins. Comparative studies of avian egg whites have utilized electrophoretic (1-4), immunological (5, 6), and specific biochemical (7, 8) analyses. These studies have all shown significant, and in some instances large, differences between the whites from eggs of different avian species. The principal and more extensive studies, however, have relied on electrophoretic examinations of the whites (1-3). Although these have supplied distinctive patterns which have been valuable in considering genetic aspects, in many cases they are difficult to interpret in terms of the recognized constituents of chicken egg white.

The present study was initiated for the purpose of obtaining more specific biochemical and chemical information on the constituents of the egg whites of a variety of avian species. The specific biochemical and chemical analyses which have been developed for chicken egg white (9, 10) have been employed. These analyses provide information on the presence and relative amounts of the biological activities and chemical groups as found in chicken egg white. They thus give information which is not provided by electrophoretic analyses. In addition, certain of the whites have been fractionated and the purified constituents have been partially characterized. Data have been obtained on the following constituents: ovalbumin, conalbumin, ovomucoid, lysozyme, flavoprotein-apoprotein, sulfhydryl groups, and sialic acid. Important differences have been found for all constituents. The ovomucoids (inhibitors of proteolytic enzymes) were found to exhibit complex differences, varying both quantitatively and qualitatively in their activities, and are difficult to interpret in terms of the recognized representatives of chicken egg white.

The indices of egg whites and egg yolks were determined ally separated within 2 days of refrigeration, and the whites were blended and stored in the frozen state until used. With only a few exceptions, the eggs procured from the San Diego Zoo were examined and the whites frozen by one of the authors working at the zoo, and the frozen samples were shipped to Lincoln. In addition, determinations of conalbumin, lysozyme, and sialic acid were made on representative samples of different whites at the zoo before freezing.

**Analytical—**Specific chemical and biochemical methods of analysis for the components of chicken egg white as routinely used in this laboratory (10) were employed for analyses of the other egg whites. These include the following methods: sulphydryls by the spectrophotometric method of Boyer (12); flavoprotein-apoprotein by the residual and total riboflavin-binding capacity (13); conalbumin by the chromogenic capacity with iron (14); lysozyme by the lytic activity against Micrococcus lysodeikticus employing an automatic recorder (10); avidin by its biotin-binding capacity in the yeast growth assay (15); and sialic acid by the assay of Warren (16) and Feeney et al. (17). The respective purified chicken egg white proteins were used as standards in the methods above. The percentages of each protein in the individual whites in this paper, therefore, indicate the amounts based on the properties of chicken egg white proteins rather than absolute amounts. Crystalline sialic acid prepared from *Escherichia coli* 1 was used for the standard of sialic acid. Bound sialic acid, as it occurs in the egg whites, was liberated by heating with dilute acid or incubating with the enzyme neuraminidase (18).

**Fractionation of Egg Whites and Physical Analyses—**Fractionations of the various egg whites on CM-cellulose2 and DEAE-cellulose (19) were performed essentially as described for chicken egg white (10, 11). Further details are given below.

A Beckman model DU and a Bausch and Lomb Spectronic-20 spectrophotometer were used for spectrophotometric work. Sedimentation analyses were performed with a Spinco model E ultracentrifuge. Paper electrophoretic analyses were performed with a horizontal strip apparatus. Free boundary electrophoretic analyses were performed with an American Instrument Company, Inc., portable electrophoresis apparatus.

The indices of egg whites and egg yolks were determined as previously described (20).

* Published with the approval of the Director as Paper No. 1010, Journal Series, Nebraska Agricultural Experiment Station.

1 The sialic acid used in this study for a standard was a sample of N-acetylneuraminic acid prepared from *Escherichia coli* and was kindly supplied by Dr. Saul Roseman and Dr. D. G. Comb of the University of Michigan.

2 The abbreviations used are: CM-cellulose, carboxymethyl cellulose; DEAE-cellulose, diethylaminoethyl cellulose.

EXPERIMENTAL PROCEDURE

**Procurement of Eggs—**The eggs of the chicken, duck, turkey, and guinea fowl were obtained from the University poultry farm, and those of the pigeon and goose were obtained from other local sources. Eggs of all other birds were obtained at the San Diego Zoo, San Diego, California. All eggs were refrigerated within 24 hours after being laid. The eggs were usually separated within 2 days of refrigeration, and the whites were blended and stored in the frozen state until used. With only a few exceptions, the eggs procured from the San Diego Zoo were examined and the whites frozen by one of the authors working at the zoo, and the frozen samples were shipped to Lincoln. In addition, determinations of conalbumin, lysozyme, and sialic acid were made on representative samples of different whites at the zoo before freezing.

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TABLE I

Compositions of avian egg whites

<table>
<thead>
<tr>
<th>Order</th>
<th>Avian species</th>
<th>Common name</th>
<th>Scientific name</th>
<th>Egg weight</th>
<th>Dry weight</th>
<th>Dry %</th>
<th>Lysozyme %</th>
<th>Catal-bumin %</th>
<th>Flavo-protein %</th>
<th>Sialid %</th>
<th>Sialic %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheiformes</td>
<td>Rhea</td>
<td>Rhea americana</td>
<td>600</td>
<td>110</td>
<td>2.0</td>
<td>3</td>
<td>0.4</td>
<td>20</td>
<td>1.5</td>
<td>0.25</td>
<td>0.11</td>
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<tr>
<td>Caeniformes</td>
<td>Cassowary, double wattled</td>
<td>Casuarius aruensis</td>
<td>650</td>
<td>110</td>
<td>0.5</td>
<td>0.6</td>
<td>20</td>
<td>14</td>
<td>18</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Emu</td>
<td>Dr. comment on n. nova-hollandiae</td>
<td>600</td>
<td>103</td>
<td>0.05</td>
<td>0.9</td>
<td>10</td>
<td>14</td>
<td>18</td>
<td>2.3</td>
<td></td>
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<tr>
<td>Anseriformes</td>
<td>Duck</td>
<td>Anas platyrhynchos</td>
<td>60</td>
<td>132</td>
<td>1.2</td>
<td>2</td>
<td>0.3</td>
<td>25</td>
<td>10</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goose</td>
<td>Anser anser</td>
<td>150</td>
<td>133</td>
<td>0.6</td>
<td>4</td>
<td>0.3</td>
<td>36</td>
<td>10</td>
<td>0.25</td>
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<tr>
<td>Galliformes</td>
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<td>125</td>
<td>3.4</td>
<td>12</td>
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<td>37</td>
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<td></td>
<td>Red jungle fowl</td>
<td>Gallus gallus</td>
<td>35</td>
<td>115</td>
<td>4.2</td>
<td>11</td>
<td>0.4</td>
<td>30</td>
<td>0.25</td>
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<td></td>
<td>Arizona scaled quail</td>
<td>Calilepepla s. palitida</td>
<td>11</td>
<td>115</td>
<td>2.8</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>0.25</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>California valley quail</td>
<td>Lophortyx californica</td>
<td>9</td>
<td>108</td>
<td>3.0</td>
<td>5</td>
<td>10</td>
<td>18</td>
<td>0.20</td>
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<td></td>
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<tr>
<td></td>
<td>Texas bobwhite quail</td>
<td>Colinus v. texanus</td>
<td>9</td>
<td>136</td>
<td>1.9</td>
<td>6</td>
<td>0.7</td>
<td>36</td>
<td>0.31</td>
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<td>Harlequin quail</td>
<td>Coturnix delegorguei</td>
<td>8</td>
<td>111</td>
<td>3.1</td>
<td>15</td>
<td>0</td>
<td>33</td>
<td>0.22</td>
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<tr>
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<td>Philippine button quail</td>
<td>Coturnix c. lineata</td>
<td>4</td>
<td>117</td>
<td>4.3</td>
<td>16</td>
<td>10</td>
<td>-</td>
<td>0.42</td>
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<tr>
<td></td>
<td>Golden pheasant</td>
<td>Chrysolophus pictus</td>
<td>30</td>
<td>104</td>
<td>2.6</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0.22</td>
<td></td>
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<tr>
<td></td>
<td>Lady amherst</td>
<td>Chrysomolus amherstiae</td>
<td>30</td>
<td>108</td>
<td>1.9</td>
<td>16</td>
<td>0.6</td>
<td>27</td>
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<tr>
<td></td>
<td>Reeves' pheasant</td>
<td>Sirmaticus reeves</td>
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<td>128</td>
<td>1.4</td>
<td>11</td>
<td>0.4</td>
<td>24</td>
<td>0.32</td>
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<tr>
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<td>Gray's francolin</td>
<td>Pyrrhitis leucocephalus</td>
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<td>129</td>
<td>1.4</td>
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<td>0</td>
<td>40</td>
<td>0.22</td>
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<tr>
<td></td>
<td>Erckel's francolin</td>
<td>Prunellops erckelii</td>
<td>-</td>
<td>128</td>
<td>2.2</td>
<td>12</td>
<td>0</td>
<td>-</td>
<td>0.27</td>
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<tr>
<td></td>
<td>Helmeted guinea fowl</td>
<td>Numida meleagris</td>
<td>40</td>
<td>134</td>
<td>2.2</td>
<td>9</td>
<td>0.48</td>
<td>34</td>
<td>0.38</td>
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<tr>
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<td>Banded plover</td>
<td>Zosterus tricolor</td>
<td>-</td>
<td>107</td>
<td>0.05</td>
<td>3</td>
<td>0.1</td>
<td>80</td>
<td>0.78</td>
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<tr>
<td></td>
<td>Green Java pea fowl</td>
<td>Pavo muticus</td>
<td>-110</td>
<td>123</td>
<td>2.8</td>
<td>9</td>
<td>0.4</td>
<td>42</td>
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<td></td>
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<tr>
<td></td>
<td>Indian blue pea fowl</td>
<td>Pavo cristatus</td>
<td>100</td>
<td>105</td>
<td>2.3</td>
<td>10</td>
<td>0.6</td>
<td>0.21</td>
<td>0.06</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Turkey</td>
<td>Meleagris gallopavo</td>
<td>80</td>
<td>124</td>
<td>3.1</td>
<td>11</td>
<td>0.4</td>
<td>33</td>
<td>0.97</td>
<td></td>
<td></td>
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<tr>
<td>Columbiformes</td>
<td>Galapagos dove</td>
<td>Nesopelia galapagoensis</td>
<td>7</td>
<td>125</td>
<td>0.1</td>
<td>9</td>
<td>1</td>
<td>24</td>
<td>0.62</td>
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<tr>
<td></td>
<td>Pigeon</td>
<td>Columba livia</td>
<td>18</td>
<td>101</td>
<td>0.1</td>
<td>9</td>
<td>1</td>
<td>-</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psittaciformes</td>
<td>Masked lovebird</td>
<td>Agapornis personata</td>
<td>-</td>
<td>126</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Figures are averages of two or more determinations on whites of several eggs (usually several pooled whites from 2 or more eggs) unless otherwise indicated.

+ Figures are calculated on basis of proteins from chicken white as standards.

" Flavoprotein calculated on basis of total flavoprotein and apoprotein content (13).

b Egg weights not determined. These figures are estimates.

c Single determinations.

d Pavo cristatus was probably the correct name for this pea fowl. The exact origin of the flock, however, was questionable.

RESULTS

General Physical Characteristics—Table I contains a general summary of the quantitative data obtained on eggs from 25 different species or varieties. Eggs varied in weight from less than 10 g to over 600 g and the whites in content of dry matter from 105 mg per ml to 136 mg per ml. In addition to the large variation in size, the eggs also varied in shape and color. General descriptions of these external physical characteristics are available from many sources (21).

Despite the large differences in external physical characteristics, however, the physical structures of the internal contents of the eggs were quite similar. The color of the egg whites varied from clear or slightly chalky to light yellow, as usually found in chicken egg white. As will be discussed below, this difference in color is apparently related to the content of riboflavin. All eggs had thick and thin egg whites which existed in proportions that were similar to those found in fresh chicken eggs, i.e. 50 to 60% thick egg white and 40 to 50% thin egg white. Chalazae existed in all egg whites, although their apparent sizes and opacities varied considerably. For example, the chalaza in the large cassowary egg was nearly transparent and sometimes difficult to observe and could have been classified as merely a slightly more dense and thicker portion of the thick egg white. Other empirical physical characteristics, such as the yolk and white indices, were in general very similar. For example, the yolk and white indices of several cassowary eggs were 0.35 and 0.11, respectively. Average yolk and white indices of a series of chicken eggs were 0.44 and 0.07, respectively. (These indices are the numerical ratios of the heights to the widths when the broken-out egg is placed on a flat surface (20).)

General Chemical Fractionations—The fractionations of egg whites of several avian species including the duck, turkey, goose, red jungle fowl, California valley quail, Texas bobwhite quail,
golden pheasant, cassowary, emu, rhea, franchinol, and painted quail were performed employing CM-cellulose and DEAE-cellulose. Elutions of the proteins from the exchangers were performed employing stepwise changes in pH which had been established to give a separation of chicken egg white into its major constituents (10). It was possible to select intervals of pH which were sufficiently broad to allow for small differences in the isoelectric points of the homologous proteins of the different whites. Initially, the intervals of pH for elution of the proteins from CM-cellulose were at pH 4.3, 5.0, 7.0, 9.0, and 9.0 plus 1 m NaCl. Separations were obtained in all instances, and the respective fractions contained the following proteins as indicated by specific chemical or biochemical assays: unadSORBED at pH 4.3, ovalmusoid and flavoproteins; from pH 4.3 to 5.0, ovalbumin; from 5.0 to 7.0, conalbumin; from pH 7.0 to 9.0, avadin and unidentified proteins; and from pH 9.0 to 9.0 plus NaCl, lysozyme. These fractions included varying amounts of unidentified proteins depending upon the particular fraction and the species.3 In most instances further fractionation with smaller intervals of pH (including essentially zero gradient or starting-buffer development in some cases) for elution from CM-cellulose or with use of DEAE-cellulose was necessary to obtain proteins which were homogeneous by paper electrophoretic analyses. These were performed at pH 6.9 with 0.1 M/2 potassium phosphate buffer for 16 hours at 8 milliamperes. In some cases, however, homogeneous proteins were not difficult to obtain. Several of the ovomucoids appeared homogeneous (11), and the lysozymes of the turkey and the duck were easily crystallized and were found homogeneous.

The corresponding proteins from the various whites are, of course, not eluted under exactly the same conditions as those from chicken white. Variation in the elution pH values from CM-cellulose for some of the isolated ovomucoids from various whites has been reported (11). Even here, variation of the several ovomucoid components in a given species may be greater than from species to species. Slight differences have also been noted in the optimal pH for elution of ovalbumin of the various species from CM-cellulose. However, preliminary observations would indicate that the major proteins of all the whites were eluted from CM-cellulose within approximately 0.2 to 0.4 pH unit of the pH values at which the corresponding proteins from chicken egg white are eluted.

Lysozymes—Lysozyme activities varied from amounts which were essentially undetectable in the Passeriforme egg white to as high as 4.2% in the red jungle fowl egg white. These large differences in lysozyme activity were not correlated with any other characteristics with the possible exception of the amount of turbidity occurring on dilutions of the egg white with water. Egg whites containing smaller amounts of lysozyme generally gave lower turbidities when diluted with 5 volumes of water or dilute buffer as previously reported in comparisons of chicken and duck whites (20).

The lysozymes of turkey and duck white were isolated by the use of CM-cellulose and further purified by crystallization.

3 Patterns similar to electrophoretic patterns are obtainable with gradient elution schemes. This method is adaptable so that patterns of a large number of egg whites may be obtained with little prior information concerning the composition of these whites. Dr. C. G. Sibley of Cornell University has extended his recent electrophoretic studies of egg whites (3) to include gradient elution schemes to provide other comparative patterns of egg whites.

Their properties were compared with chicken lysozyme. All three formed crystals which were in the form of needles and were grossly similar. Specific enzymatic activities were the same within the limits of the error of the enzyme assay as performed in two assays (±10%). The isoelectric points were all > pH 0.0 as determined by paper electrophoretic studies, and only single peaks were observed in each case. Ultracentrifugal examinations also gave similar homogeneous patterns for the three lysozymes. \( k_m \) values for 1% solutions of chicken, turkey, and duck lysozyme were 1.9, 1.7, and 1.8 respectively. The determinations were at \( 52,040 \) r.p.m. for 128 minutes in 0.1 \( \Gamma/2 \) phosphate buffer at pH 6.9.

Conalbumin—The concentration of conalbumin in the various whites did not show the relatively large differences found for lysozyme activity. However, the absolute differences were much greater for conalbumin than for lysozyme. In no cases were any eggs encountered which contained obvious amounts of the iron complex of conalbumin as judged by the absence of any obvious amounts of a salmon-pink color.

Limited studies indicated that the conalbumins from the different species had very similar structures and properties. One series of experiments performed directly on the whites showed that the absorption spectra of the iron complexes were essentially the same for the following whites: chicken, red jungle fowl, turkey, cassowary, emu, Reeves' pheasant, Gray's francolin, Erkel's francolin, and pea fowl. These were determined from 430 \( \mu \mu \) to 510 \( \mu \mu \) at intervals of 10 \( \mu \mu \). More detailed studies were made with conalbumins of the cassowary, golden pheasant, and turkey. Purified preparations of these were found to have chromogenic capacities with iron within 80 to 100% of that of crystalline chicken conalbumin and to be homogeneous by free boundary electrophoresis. The isoelectric points were in the same general range as that of chicken conalbumin but slight differences in mobilities were found. Fig. 1 presents patterns obtained for chicken conalbumin and mixtures containing 1% chicken conalbumin and 0.5% cassowary, turkey, or golden pheasant conalbumin at pH 4.7. The cassowary conalbumin migrated slightly faster and the turkey and golden pheasant conalbumins migrated slightly slower than the chicken conalbumin. In a single ultracentrifugal determination in which chicken and cassowary conalbumins were compared, similar homogeneous patterns were obtained. These were performed with 1% solutions in a buffer of 0.05 m NaCl and 0.05 m glycine.

![FIG. 1. Free boundary electrophoretic patterns of several avian conalbumins. The ascending patterns of chicken conalbumin (1%) and mixtures of chicken conalbumin (1%) with the conalbumins of the other species (0.5%) are given. The buffer was 0.1 m sodium acetate at pH 4.7 and the determinations were for 150 min at 7 milliamperes.](http://www.jbc.org/)

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at pH 8.5 and at 52,640 r.p.m. for 48 minutes. Calculated $s_{20}$ values were 4.8 and 3.3 for the chicken and cassowary respectively. Cassowary conalbumin was also found to give practically identical results to chicken conalbumin in experiments comparing the relative resistances of the iron complex and the iron free protein to hydrolysis by chymotrypsin and to thermal denaturation (14). The iron complex of cassowary conalbumin was also more stable than the iron-free protein to these two treatments.

Flavoprotein-apoprotein—The contents of flavoprotein and apoprotein varied not only as to total amounts of flavoprotein-apoprotein present but also as to the percentages existing as flavoprotein. The percentage of total flavoprotein-apoprotein varied from a low of 0.14% for the banded plover to a high of 0.9% for the emu, whereas the relative amounts of the total existing as flavoprotein varied from a low of 14% for the emu to a high of 48% for the guinea fowl. The flavoproteins were all nonfluorescent. In all cases the titrations with riboflavin to fluorescence gave sharp end points as previously reported with chicken flavoprotein (13). The visually observed yellow color of the whites was approximately proportional to the amount of riboflavin.

Sulfhydryls and Ovalbumins—The concentration of titratable sulfhydryl groups in the egg whites varied considerably as shown in Table I. Emu egg white contained only 14 pmoles per g of dried egg white whereas the California valley quail contained 42 pmoles per g. The probable numbers of titratable sulfhydryl groups per mole of the ovalbumins were calculated from the data in Table I. These were made with the assumptions that the different egg whites contained amounts of ovalbumin similar to chicken egg white (55%), that the different ovalbumins have molecular weights similar to chicken ovalbumin (46,000), and that the different ovalbumins contribute essentially all the titratable sulfhydryls in the different whites as is the case with chicken ovalbumin (8). Results of these calculations are shown in Table II and are compared with the calculated number of sulfhydryl groups for the partially purified ovalbumins. In general, the number of sulfhydryl groups calculated from data on the egg white and isolated ovalbumins agreed. Small differences could easily be due to the variation in the amounts of ovalbumins in the whites, variability in determination of sulfhydryl groups by the method employed, small impurities in the isolated ovalbumins, and the presence of unidentified proteins in the whites which contribute to the sulfhydryl content. In chicken white, for example, a small amount of a protein was isolated with an apparent isoelectric pH of 5.1 to 5.3 and a sulfhydryl content of approximately 66 pmoles per g. The possible presence of this uncharacterized protein in the egg whites of other species has not been investigated.

Table II also gives the average distance moved by the fastest moving component of each partially purified ovalbumin during paper electrophoretic analyses. These analyses were run for 16 hours at 8 milliamperes with potassium phosphate buffer, pH 6.9, 0.1 T/2. Certain of the ovalbumins appeared to move slightly further than chicken ovalbumin such as the duck, goose, and rhea. On the other hand, the golden pheasant ovalbumin moved very slowly compared to the other ovalbumins. In addition to differences in migration on paper, the ovalbumins differed as to how well the components, those probably corresponding to chicken ovalbumin A1, A2, and A3, separated from each other. By visual inspection of the patterns, only the ovalbumin of the Texas bobwhite gave three distinct peaks. Most of the other patterns showed two distinct peaks with the exception of those for duck and goose ovalbumins which tended to smear together. These observations would appear to be in general agreement with those of Bain and Deutsch (1) and Sibley (3) who presented patterns obtained on the unfracti- nations whites and with those of Landsteiner et al. (4).

Ultracentrifugal comparisons of the ovalbumins of the chicken, turkey, and duck were made with 1% solutions in 0.1 T/2 potassium phosphate buffer at pH 6.9. The speed of the rotor was 52,640 and the time was 94 minutes. All three ovalbumins gave similar homogeneous patterns. The calculated $s_{20}$ values were 2.8, 3.0, and 3.0 for the ovalbumins of chicken, duck, and turkey, respectively.

Sialic Acid—Sialic acid appeared to be bound in all the whites. Only low or insignificant amounts of sialic acid were found without preliminary hydrolysis by acid or by enzyme. The rapid rate of release of sialic acid from all the whites by neuraminidase might indicate a similar linkage of the sialic acid to the proteins in the various whites. In addition, the individual sialic acids prepared by enzymatic hydrolysis from the whites of chicken, emu, cassowary, turkey, goose, and guinea fowl had identical $R_f$ values when chromatographed on paper in butanol-acetic acid-water (4:1:5) and in propanol-butanol-water (1:2:1).

In chicken egg white, approximately one-half of the sialic acid occurs associated with the ovomucin fraction (17). An approximately one-fourth greater content of sialic acid in the thick white compared to the thin white correlates with the distribution of ovomucin between these two structures. This difference in the content of sialic acid between the thick and thin white was not found in the cassowary or rhea whites which were high in sialic acid.

Avadin—The biotin-combining activity of the whites of the chicken, turkey, duck, and goose have been examined. On the
basis of a content of 0.05% of avidin in chicken white (10),
turkey, duck, and goose egg whites contained 0.15, 0.02, and
0.005%, respectively.

**DISCUSSION**

Studies on the proteins of different species provide information
useful to the taxonomist and geneticist and also to the
protein chemist who may be able to relate differences in structure
to differences in properties. Several workers have suggested the value of comparative compositional studies on egg whites. McCabe and Deutsch (2) made the following speculation from their electrophoretic studies: "It seems possible that the
physiochemical character of an egg retains more of its incipient phylogeny than the more superficial aspects of the bird's adult morphology." Sibley and Johnsgard (22, 23) have reached essentially similar conclusions. Anfinsen (24) has suggested that proteins with important functional properties might be expected to undergo fewer structural changes during the evolutionary process. Although no definite functions have yet been found for the egg white proteins, they possess unique antimicrobial and antienzymatic activities.

It is hoped that the present study has supplied information which will prove valuable to the geneticist. The comparatively large differences in the contents of lysozyme and sialic acid (>30-fold in several instances) demonstrate the interrelationships that may be encountered. These two substances alone could provide the basis for an exhaustive study. They both can be rapidly determined in egg white by relatively simple methods (10, 16, 17). Differences in the amounts of the individual egg white proteins have been found to exist between different strains of chickens. These differences, however, have been small compared to the differences found between species. For example, Wilcox and Cole (25) found only an approximately 25% difference in the lysozyme contents of "high and low lysozyme" strains of chickens.

Of possibly even greater general importance would be the comparisons of the fundamental structures of the homologous proteins from the different whites. Studies of the comparative properties and structures of homologous proteins from different whites should also prove an important tool in understanding the relationship of structure to the properties and functional activities of the proteins. This general area has recently been receiving considerable attention in studies on ribonuclease, hemoglobin, and several of the proteins or peptides with hormonal activity (24). The recent discovery that the ovomucoids vary quantitatively and qualitatively in their activities suggests the possible value of this approach (11). In like manner, the results of this and a previous (8) study have indicated that ovalbumins with different numbers of sialhydryl groups occur. It would be hoped that detailed comparative studies on the conalbumins and lysozymes would reveal structural differences that could be interpreted in terms valuable to the understanding of observed differences in properties. Lysozyme should receive particular attention because of its comparative ease of preparation and its enzymatic activity. A concerted attack on this problem, however, should probably await the discovery of a more satisfactory noncellular or synthetic substrate.

A study should be made of the roles of sialic acid in the properties of proteins from an egg white high in sialic acid such as that of the emu (17). The presence of large amounts of sialic acid in some of the proteins from emu white might offer a particularly good area for studying the relationship of structure to properties because of the facility with which it can be specifically removed enzymatically from the proteins.

**SUMMARY**

The egg whites of 25 different avian species or varieties were examined by specific biochemical and chemical analyses, by chromatographic separations of the constituent proteins, and by examinations of the properties of several of the purified proteins. The constituents studied included sulfhydryl groups, sialic acid, lysozyme, apoprotein-flavoprotein, conalbumin, and ovalbumin.

Large differences were found in the amounts of various of these substances in the whites from the different birds. In the cases of lysozyme and sialic acid, differences as great as 30-fold were found. Differences in the properties of several of the purified proteins were also found.

The significances and values of the results from the standpoint of comparative and genetic biochemistry were discussed.

**Acknowledgments**—The authors appreciate advice and assistance from Dr. Richard Dam, Dr. Hilmi Pamir, Dr. Robert M. Hill, and Mr. Paul Stageman. Particular appreciation is due Kinston C. Lint, Curator of Birds, and other technical personnel of the San Diego Zoo, San Diego, California, for advice and assistance in obtaining eggs.

**REFERENCES**

The Comparative Biochemistry of Avian Egg White Proteins
Robert E. Feeney, John S. Anderson, Parviz R. Azari, Nelle Bennett and Marvin B. Rhodes


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