lower than that of protein accumulation throughout the growth period and that it has a tendency to reduce its value in the later phase of this period.

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Filter Paper Electrophoresis of Avian Serum Proteins[1]

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The serum proteins of the fowl have been studied by the classical Tiselius' method (*1, 2*), but not, so far as we are aware, by the method of filter paper electrophoresis described by Durrum (*3*) and by Cremer and Tiselius (*4*). The present communication describes preliminary results of studies of fowl sera by filter paper electrophoresis, including studies on the highly lipemic sera of laying and estrogenized birds. Kunkel and Slater (*5*) have pointed out that filter paper electrophoresis may have special advantages for studying lipoprotein components of serum.

The technique was essentially that of Durrum (*3*), as modified by Flynn and De Mayo (*6*). Strips of Whatman 3 MM paper $2\frac{1}{2} \times 16$ in. were used. The Veronal buffer (pH 8.6, ionic strength 0.05) was allowed to rise from both ends of the paper by capillarity until a narrow transverse zone near the anodic end remained unwetted. From 0.1 to 0.2 ml of serum was applied by micropipet to the paper along this zone. The advancing fronts of the buffer were then allowed to come together along the line of application of the serum and before the serum could dry on the paper. This avoided undue diffusion of the proteins, a difficulty which may be accentuated when relatively large amounts of serum (e.g., 0.1–0.2 ml) are applied directly to fully wetted paper strips.

The potential was maintained at 100 v, giving a total current of 8.5 ma when there were 5 strips in the chamber. This current would appear to be smaller than that used by most other workers. A low current reduces trouble from heating and from flow of the buffer. Some (*7, 8*) have described movement of the γ globulin fraction of serum in the opposite direction to the other fractions relative to the place of application. This apparently anomalous movement, due to electroendosomis, is probably accentuated by high currents; it has frequently been absent in this laboratory when using low currents. Control of evaporation and temperature by keeping the papers immersed in a bath of nonaqueous solvent (*4*) was considered inadvisable for studies involving lipoproteins, especially those of the sera of laying or estrogenized fowl. Electrophoresis was usually continued for 24 hr or occasionally longer. Under these conditions the fastest moving major component (the albumin) moved 15–16.5 cm from the zone of application. The drying, staining, and quantitative determination of the dyestuff were performed as described by Flynn and De Mayo (*6*) using bromophenol blue as stain.[3] Lipids were located by exposing the papers to the vapor from a solution of 1% osmic acid in 1% chromic acid. Substances positive to the Liebermann-Burchard reaction were located by immersing papers for a few moments in a reagent made of 10 parts chloroform, 10 parts acetic anhydride, and 1 part concentrated sulfuric acid, and then laying the papers on a glass

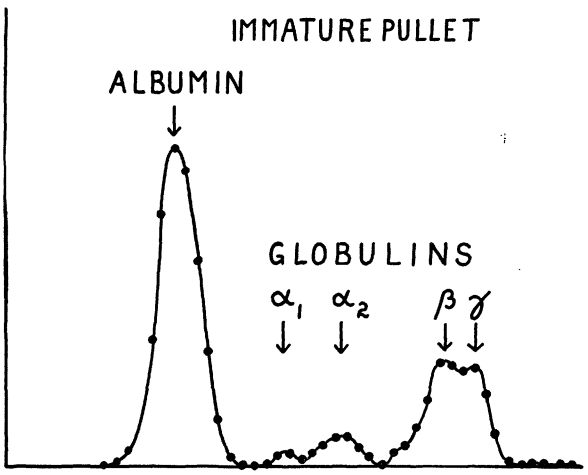

FIG. 1. Quantitative data for paper electrophoresis of avian sera. Vertical axis is optical density. Papers cut in 5-mm transverse strips. A. Immature cockerel, untreated. B. Immature pullet, untreated. C. Turkey hen, nonlaying. D. Normal human.

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[3] *Added in proof.* In our experience, the staining of protein with this dye, and with naphthalene black, was confined to the superficial layers of the paper.

plate. (This technique was somewhat rudimentary, but demonstrated the practicability of using the Liebermann-Burchard reaction for direct examination of the papers.)

Typical results for turkey, fowl, and human sera are shown in Fig. 1. The data show that the sera of the sexually inactive female fowl and turkey yielded at least 5 distinct protein fractions. The disposition and relative intensity of these 5 fractions bore a close similarity to those recorded for human serum (*9*) and for other vertebrate sera (*7*). Accordingly, we have designated these fractions in order of decreasing mobility as albumin, α_1 globulin, α_2 globulin, β globulin, and γ globulin by analogy with human serum fractions.

The following points of similarity and contrast between human and fowl sera are noteworthy (Fig. 1): (*a*) The fast-moving albumin fraction had the deepest stain in turkey, fowl, and human sera. (*b*) The α_1 globulin fraction gave the faintest stain in all three species. The band is particularly weak with sera from the sexually inactive fowl and may be difficult to distinguish if staining is not sufficiently prolonged. The relative mobility of the α_1 fraction appeared to be more variable than that of the other globulin fractions. (*c*) The γ globulin fraction was next to the albumin fraction in intensity of staining in all three species, but moved relatively faster in the avian sera than in human serum. The β globulin fraction stained almost as deeply as the γ globulin fraction, and moved relatively slower in the avian sera than in human serum. (*d*) The α_2 globulin fraction stained more deeply than the α_1 fraction in all three species, but considerably less deeply than the β globulin. β Globulin appeared to move more slowly relative to the albumin in the avian sera than in human serum.

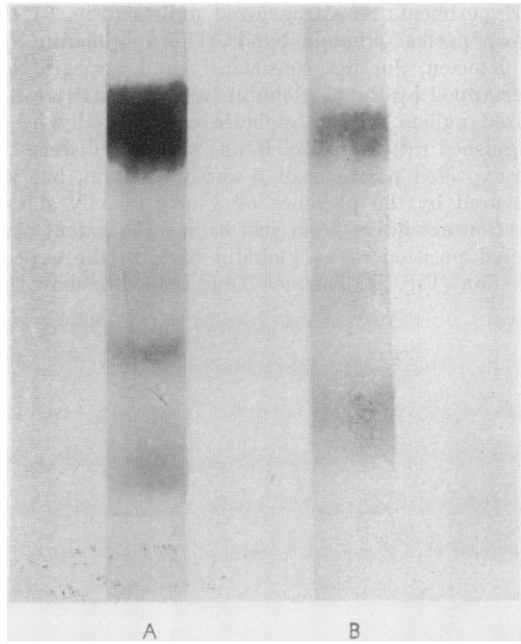

FIG. 2. Comparison of human and immature pullet sera. A. Human: albumin, β globulin and γ globulin clearly visible: α_2 globulin is very faint on photograph. B. Immature pullet: albumin clearly visible, β and globulin so close together that separation is not apparent on photograph; α_2 globulin very faint on photograph.

These observations on relative mobilities are summarized in Table 1.

Several wide papers were run in order to compare the mobilities of the avian serum albumins and human serum albumin. The average results are presented in Table 2.

These observations on mobilities suggest that: (*a*) Sera of male or sexually inactive fowl and turkeys yield 5 protein fractions which may be considered analogous to those of human serum. (*b*) The albumin fractions of human, turkey, and fowl sera move at similar speeds. Fowl serum albumin is probably a little slower than human albumin, but the difference is slight. (*c*) The difference in mobility between β and γ globulins is greater in human serum than in male or sexually immature fowl sera (Fig. 2). (*d*) The α_2 globulin of fowl serum is slower relative to the albumin than in human serum (Fig. 2).

Osmic acid staining demonstrated that some lipid accompanies the albumin in all sera studied, but we have not detected lipid in association with the globulins with any certainty in immature pullets or cockerels. Durrum *et al.* (*9*) have recorded the presence of lipid along with the albumin of human serum, and also in a region extending from the middle of the α_2 globulin band to the middle of the β globulin band in lipemic human serum.

TABLE 1

MOBILITIES OF VARIOUS PROTEIN FRACTIONS OF HUMAN AND AVIAN SERA RELATIVE TO MOBILITIES OF ALBUMIN FRACTIONS*

	Cockerels	Pullets, sexually immature	Turkey hens, inactive	Human	Human (*9*)
Albumin	1.00	1.00	1.00	1.00	1.00
α_1 globulin	0.86	0.73	0.76	0.87	0.83
α_2 globulin	0.60	0.59	0.65	0.70	0.66
β globulin	0.36	0.36	0.46	0.47	0.55
γ globulin	0.30	0.27	0.31	0.17	0.22

* Running time, 40 to 41 hr; Veronal buffer, pH 8.6, ionic strength 0.05.

TABLE 2

MOBILITIES OF ALBUMIN FRACTIONS OF AVIAN SERA RELATIVE TO MOBILITY OF HUMAN SERUM ALBUMIN

Source of albumin	Relative mobility
Human	1.00
Goose	1.00
Cockerel, mature	0.96
Pullet, sexually immature	0.98
Hen, laying	0.98
Turkey, inactive male	0.94

Experiments on estrogenized pullets (Fig. 3) have shown normal albumin bands. The α_1 globulin could be detected, but not consistently and appears to be overlapped by the α_2 globulin in more heavily estrogenized pullets. The α_2 globulin could usually be distinguished satisfactorily. Bands could be discerned in the expected positions of β and γ globulin, but were obscured by the presence of a new heavily staining fraction extending from just behind the patent or expected position of α_2 globulin back to the expected β region (Fig. 3). This new band tended to show "tailing," and this was more severe the more heavily the bird had been estrogenized. Kunkle and Slater (*5*) have associated tailing with the presence of neutral fat. The new region also stained rather deeply with osmic acid and gave a positive Liebermann-Burchard reaction. This lipid-staining region included a narrow zone visible as a yellowish band on the unstained paper and exhibited a blue fluorescence in screened UV light. Kunkle and Slater (*5*) have described a somewhat similar orange band in lipemic human sera.

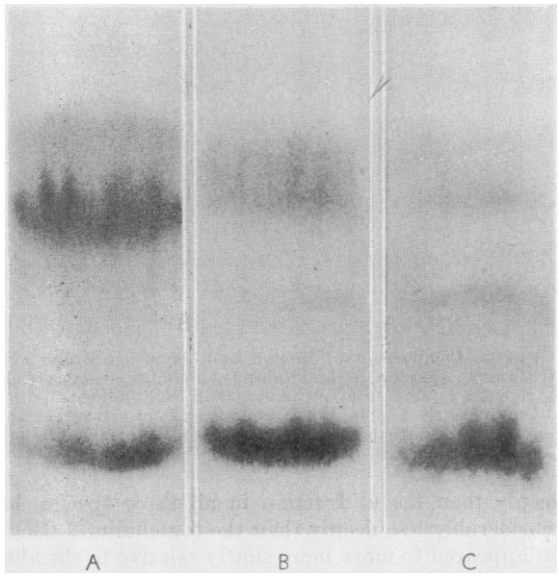

FIG. 3. A. Normal immature pullet. B. Immature pullet, androgenized. C. Immature pullet, estrogenized. Albumin, α_2 globulin, β globulin, and γ globulin visible in A and B. Note appearance of new protein zone in C, where the new fraction obscures the β globulin: this zone displayed tailing and stained deeply with osmic acid. The α_1 globulin is not visible.

The new zone associated with lipid was probably in large part due to serum "lipovitellin" and we have designated it as "presumptive lipovitellin" (PLV). Attempts to resolve this PLV band by electrophoresis were based on the use of buffer made up with 20% methanol instead of water. Using this buffer, sera from cockerels or normal immature pullets gave a pattern similar to the normal pattern using the aqueous buffer, but the papers with sera from estrogenized immature pullets showed a distinct new band near the starting line (Fig. 4). This band gave practically no stain with osmic acid, although lipid was still present in the PLV position. Studies using injections of phosphate tagged with P^{32} revealed a high count in the new slow-moving band obtained by use of the methanolic buffer. This high count was not altered appreciably by extraction with Bloor's mixture, whereas a somewhat lower count in the lipid-staining region was almost entirely removed by extraction with Bloor's mixture. It is possible that this new fraction obtained by the use of methanolic buffer represents a phosphoprotein component separated from phospholipid.

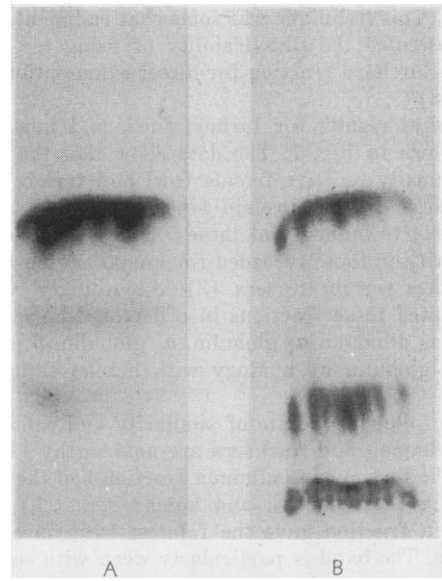

FIG. 4. Electrophoresis using Veronal buffer pH 8.6 made up with 20% methanol. A. Immature pullet, untreated. B. Immature pullet, estrogenized. Note that there is a fraction in about the same position as the presumptive phosphovitellin in Fig. 1B, but that the methanolic buffer has resulted in appearance of a further new slow-moving fraction. The band in the PLV position was associated with heavy lipid staining whereas the new slow-moving band was not. An α_2 band in paper B was present, but does not show on the photograph.

From the standpoint of technique, it is noteworthy that the intense staining of certain bands on the papers obtained from sera of estrogenized birds may have been due in part to staining of lipid, or to important modifications of the staining properties of the protein due to the presence of lipid. Papers were run in duplicate with normal immature pullet serum and with serum from estrogenized immature pullets. One paper of each pair was extracted with Bloor's mixture before staining. The bands on the paper from normal serum appeared to stain with much the same relative intensities whether extracted or not. The unextracted paper from the estrogenized pullet stained deeply in the fashion typical of such sera, but relative intensities of the bands appeared to have been altered on the papers extracted before staining. It would appear that the interpretation of colorimetric data on dye extracted from stained papers in terms of protein may call for caution when lipemic avian sera are being studied, and further studies on this point are in progress.

A more nearly complete account of our experiments will be published elsewhere.

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Book Reviews

Plant Anatomy. Katherine Esau. New York: Wiley; London: Chapman & Hall, 1953. 735 pp. Illus. + plates. $9.00.

Those who have followed Dr. Esau's research papers and scholarly reviews during the past two decades and have enjoyed her magnificent Kodachrome photomicrographs at scientific meetings have eagerly awaited the appearance of *Plant Anatomy.* This volume should find its way into every up-to-date botanical library, as it will undoubtedly serve for many years as the standard reference on the structure of seed plants.

Although the arrangement of the subject matter follows a conventional sequence—cells, tissues, organs (stems, leaf, root, and reproductive structures)—with topical organization patterned after that of Dr. A. S. Foster in his *Practical Plant Anatomy,* the material has been meticulously reworked from a refreshingly dynamic viewpoint in line with the author's research interest in developmental anatomy. Special mention might be made of the sections on the chemistry and structure of the cell wall, on the meristems, and on the longitudinal course of differentiation in stems, and the final chapters on the flower, fruit, and seed. Here and elsewhere, the results of recent research have been critically reviewed.

Outstanding features of the book are (1) the concisely written introductory statements at the beginning of each chapter in which concepts are outlined and terms defined, (2) the extensive, carefully selected, up-to-the-minute bibliographies on each topic treated, and (3) the superb illustrations. It is somewhat unfortunate that the cost of book manufacture has necessitated grouping all the halftones in plates immediately following the text. This does not seriously impair the usefulness of the volume, however, but it does bring together in a concentrated dose of 90 pages the finest series of photomicrographs of plant structure yet assembled. The majority of these are the author's original photographs of her own preparations or of slides obtained from others. The text figures are also of the highest quality, most of them having been drawn by the author from original material or from published photomicrographs.

To botanists confronted with the problem of selecting a textbook for use in an introductory course in the structure of higher plants, this book poses a difficult problem. On the one hand, the excellence of its coverage and approach points up the serious shortcomings of available texts in the field; on the other, the advanced and comprehensive nature of the book will probably make it difficult reading even for the graduate student. We badly need a moderately priced, illustrated text suitable for the undergraduate, that presents the structure of vascular plants from a dynamic point of view with emphasis on the developmental and physiological aspects of the subject. This reviewer hopes that Dr. Esau can be persuaded to turn her masterly hand to such an assignment.

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Clinical Allergy. French K. Hansel. St. Louis, Mo.: Mosby, 1953. 1005 pp. Illus. $17.50.

This volume upon a complex and rapidly expanding subject has been prepared for the student and the general practitioner, as well as for the interested layman. It embraces the various phases of clinical allergy which involve the respiratory, cutaneous, intestinal, neural, and vascular systems. There are presented not only the ideas of the author but also, without bias, those of many other writers and investigators. Consequently, the lists of references which are placed at the close of the chapters are unusually complete and comprehensive.

The first four chapters are devoted to the terminology and basic considerations of allergic conditions; the next seven to discussions of the multitude of etiologic factors of consequence in allergic diseases. One chapter contains much detailed information on the hay fever-producing pollens as found in the different seasons and in the various parts of the United States. This information would have been more immediately accessible to the reader had it been included in tabular form as well, assembled according to seasonal occurrence and sectional distribution.

Adequate space is devoted to various specific and nonspecific diagnostic and treatment procedures as applied to the several clinical forms of allergy. Less than two pages, however, are devoted to the preparation of the allergenic extracts of such vital impor-