

(9) The academy went on record as strongly favoring the efforts being made by the committee on conservation to secure lake shore lands for park purposes.

The academy held one general scientific session at which Dr. Paul Popenoe, of the Institute of Family Relations at Los Angeles, Calif., gave a very fine address on "Heredity and Education," and the state director of education, Mr. E. N. Dietrich, spoke on the "Training of Science Teachers."

For real zeal and enthusiasm, the annual dinner on Friday, May 6, was the climax. This fine gathering was presided over in a most delightful manner by Professor Karl Ver Steeg, of the College of Wooster, who had rendered such fine service as chairman of the local committee. Professor Ver Steeg proved himself a real past master as a toastmaster. More than 300 persons sat down to dinner and enjoyed both a physical and an intellectual feast. President Charles F. Wishart, of the College of Wooster, and Director Edmund Seerest, of the Ohio Agricultural Experiment Station, extended a cordial welcome to the visiting scientists, to which Dr. Dayton C. Miller, of the Case School of Applied Science, responded most appropriately. Then followed the real event of the evening, namely, the presidential address by President Charles G. Shatzer on "When Are We Scientific?" He gave the listening scientists much to think about from a rather new and novel point of view. After the presidential address Dr. George B. Barbour, of the University of Cincinnati, showed "Vesuvius in Eruption" by the use of colored films.

The eight sectional programs were replete with interesting papers and the sectional meetings well attended. Space will not permit even the listing of these papers. Several excursions were provided to points of interest, notably to the arboretum at the Experiment Station.

The following officers were elected for the coming year:

*President:* Claude E. O'Neal; *Vice-Presidents:* Zoology, Robert A. Hefner; Botany, Paul B. Sears; Geology, W. Storrs Cole; Medical sciences, Dr. L. F. Edwards; Psychology, James R. Patrick; Physics and astronomy, Leon E. Smith; Geography, Fred A. Carlson; Chemistry, Harvey V. Moyer; *Secretary:* William H. Alexander; *Treasurer:* Eugene Van Cleef.

WILLIAM H. ALEXANDER,  
Secretary

## THE NEW HAMPSHIRE ACADEMY OF SCIENCE

THE twentieth annual meeting of the New Hampshire Academy of Science was held on May 27 and 28 at The Tavern, Laconia. Dr. E. J. Roberts was chairman of the local committee. At the Friday evening session Professor J. W. Goldthwait summarized his studies on the glacial geology of central New Hampshire in a paper "Was there a Glacial Lake Winnepesaukee?", and Professor G. W. White, who was the academy's representative at the seventeenth International Geological Congress in the U. S. S. R., gave an illustrated report on the Geological Congress excursions to Caucasia and Armenia and to Novaya Zemlya.

Papers by members were read at the Saturday morning session. At the Saturday afternoon session following the business meeting, the presidential address, "The Advantages of a Long Hard Winter," was given by Professor Karl W. Woodward, of the University of New Hampshire.

At the business meeting it was voted to award the grant-in-aid for the current year from the American Association for the Advancement of Science to Mr. Clayton E. Fisher, of Colby Junior College, for assistance in continuing "An Investigation of Some Derivatives of Phenanthrene." The committee on conservation reported that its studies of "natural association areas" show that a fair number of such areas are already in public ownership, and the committee was authorized to confer with public ownership bodies on the proper setting aside of "association" and "type" areas. The publications committee, Mr. Frank Foster, Claremont, chairman, was authorized to recommend further publications and oversee their issuance, subject to approval by the executive council.

The following officers were elected for 1938-39: *President*, Dr. Henry I. Baldwin, State Forestry Department; *Vice-President*, Professor George W. White, University of New Hampshire; *Secretary-Treasurer*, Professor W. W. Ballard, Dartmouth College; *Member Executive Council*, Professor Karl W. Woodward, University of New Hampshire; *Councilor to the American Association for the Advancement of Science*, Professor George F. Potter, University of New Hampshire.

GEORGE W. WHITE,  
Retiring Secretary

## SPECIAL ARTICLES

### ELECTROPHORESIS EXPERIMENTS WITH EGG ALBUMINS AND HEMOGLOBINS

THE species specificity of proteins, readily demonstrable serologically and indeed detected by this means,

has been investigated only to a small extent by chemical and physicochemical methods, with the exception of studies on hemoglobin, *e.g.*, by crystallographic measurements. The possibility of distinguishing be-

tween proteins with the aid of the very valuable electrophoretic technique of Tiselius<sup>1</sup> suggested the application of this method for the study of species differences in proteins. In this way some information might possibly be obtained on the chemical basis of these differences, since the electrochemical properties of proteins depend upon the number and nature of

their acidic and basic amino acids. In particular, the question arises whether variations in isoelectric points and mobility parallel the serological relationships in the two cases, possibly not equivalent in this respect, namely, proteins of a certain type derived from various species (as serum albumins) and, on the other hand, various types of proteins occurring in a single species.

Some preliminary experiments undertaken along this line were carried out with egg albumins of chicken, turkey, guinea hen, duck and goose, the first three having been thrice recrystallized and the remaining two separated by ammonium sulfate with the addition of acid. The mobilities,  $u$ , are recorded in Table I. The schlieren photographs, taken at 30-minute intervals

TABLE I  
ELECTROPHORETIC MOBILITIES OF 0.5 PER CENT. EGG ALBUMINS IN 0.02 NORMAL SODIUM ACETATE BUFFER AT pH 5.20. TEMPERATURE 0.0° C.

Egg albumins	$u \times 10^{-5}$
Chicken	-3.53
"	-3.56
Guinea hen	-3.76
Turkey	-3.53
Duck	-4.27
Goose	-4.60

TABLE II  
ELECTROPHORETIC MOBILITIES OF THE COMPONENTS OF MIXTURES OF EGG ALBUMINS AT DIFFERENT pH VALUES AND CONSTANT IONIC STRENGTH 0.02. TEMPERATURE 0.0° C.

Protein 1	Protein 2	Buffer	pH	$u_1 \times 10^{-5}$	$u_2 \times 10^{-5}$
0.5 per cent. duck	0.5 per cent. guinea hen	Acetate	5.21	-4.34	-3.77
" " " turkey	" " " "	"	5.21	-3.49	
" " " "	" " " "	Phosphate	7.18	-9.81	
" " " chicken	" " " "	Acetate	3.48	+5.57	
" " " "	" " " "	"	5.16	-3.73	
" " " "	" " " "	Phosphate	7.19	-9.86	

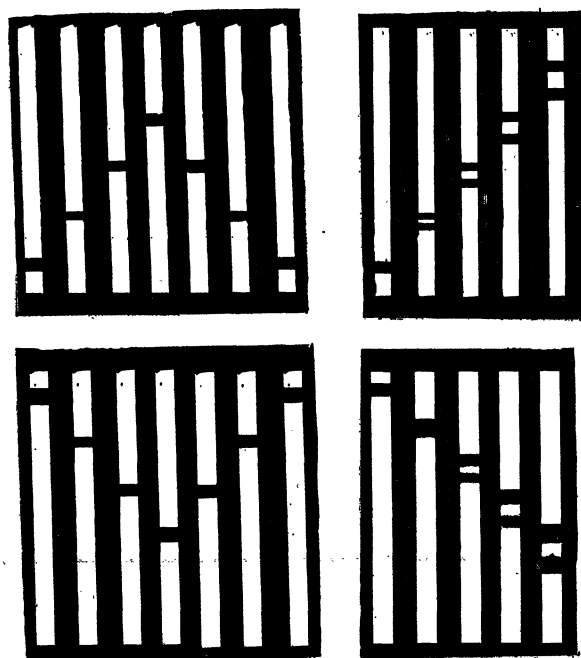


FIG. 1

FIG. 1. Schlieren photographs of the anode (upper) and cathode (lower) sides taken at 30 minute intervals during the electrophoresis of egg albumin (guinea hen). The three last exposures (right) were made after reversal of the current.

FIG. 2

FIG. 2. Schlieren photographs of the electrophoresis of a mixture of guinea hen and duck egg albumins.

<sup>1</sup> Arne Tiselius, *Trans. Faraday Soc.*, 33: 524, 1937.

both before and after the reversal of the current, of a typical experiment with guinea hen egg albumin are shown in Fig. 1.

Confirmation and extension of the data of Table I were obtained from measurements on the mixtures reported in Table II.

In only the mixture of duck and guinea hen egg albumins were the mobilities of the components sufficiently different to permit resolution into two bands, as shown in Fig. 2, which is the schlieren photograph of this mixture. In all the other mixtures, however, the boundaries were more diffuse than for the pure components and gave indications that prolonged electrolysis or increased resolving power of the schlieren apparatus would make visible the very slight separation of the components to be expected from the data of Table I. Considering the differences in total protein concentration the data of Table II at pH 5.2 are in satisfactory agreement with those of Table I.

It appears that several ovalbumins—chicken, guinea hen, turkey, belonging to three families, but to the same order (Galliformes)—which, although related, are easily distinguishable by serological precipitin reactions, exhibit close resemblance in the electrophoresis experiment. Consequently, there must exist a great number of proteins of a given type which are very similar electrophoretically. The albumins from duck and goose eggs, both birds belonging to the Order Anseriformes, are seen to form a second group with mobilities definitely different from the others, which

is in agreement with the zoological (and serological) relationships of these birds.

Another type of protein examined was hemoglobin from dog, rabbit, guinea pig, sheep and horse used as solutions obtained by laking washed blood cells. The measurements of these solutions are recorded in Table III. The hemoglobin boundaries were, in general, more diffuse than those of egg albumin.

TABLE III

ELECTROPHORETIC MOBILITIES OF 0.5 PER CENT. HEMOGLOBIN SOLUTIONS IN A PHOSPHATE BUFFER OF pH 7.95 AND IONIC STRENGTH 0.1. TEMPERATURE 0.0° C.

Hemoglobins .....	$u \times 10^{-5}$
Rabbit .....	-2.15
Sheep .....	-2.3
Dog .....	-2.4
Horse .....	-3.0
Guinea pig .....	-3.0
Chicken* .....	-1.6

\* The boundaries with both laked blood and crystallized hemoglobin were very diffuse. A second and slower band of unknown significance appeared in the cathode side of the U-tube. This band was sharp and distorted.

Some of the hemoglobins behaved very similarly when subjected to cataphoresis (sheep, dog, rabbit and horse, guinea pig) despite the fact that the animals represent different orders and are, serologically, almost unrelated. The close agreement of the mobilities of dog and sheep hemoglobins is in accord with values by Michaelis<sup>2</sup> for the isoelectric points of these proteins from dog and sheep. It would be desirable to extend the determinations of mobilities and isoelectric points systematically to hemoglobins and other proteins from a large number of species.

The mobility differences of the hemoglobins of Table III, although not inappreciable, are not as striking as is the rather wide disparity in solubilities of various hemoglobins. It may be presumed that the observed likenesses in electrochemical behavior of serologically distinct hemoglobins of distant species point to a similarity in their content of acid and basic groupings, and the same question is to be considered for the species variations of other types of proteins. The very pronounced serological differences would then depend on structural features of the molecule other than those which determine the charge. Here, reference may be made to the analytical results of Block,<sup>3</sup> and Vickery and White,<sup>4</sup> discussed by Bergmann and Niemann<sup>5</sup> in the light of their theory of protein structure, from which the hemoglobins of the horse, sheep, cattle and dog all contain the same amounts of the basic amino acids, arginine, histidine and lysine, whereas there are differences in the cysteine and total sulfur content.

<sup>2</sup> L. Michaelis and Z. Bien, *Biochem. Zschr.*, 67: 198, 1914.

<sup>3</sup> R. J. Block, *Jour. Biol. Chem.*, 105: 663, 1934.

<sup>4</sup> H. B. Vickery and A. White, *Proc. Soc. Exp. Biol. and Med.*, 31: 6, 1933.

<sup>5</sup> M. Bergmann and C. Niemann, *Jour. Biol. Chem.*, 118: 301, 1937.

Further accurate figures on other "neutral" (mono-amino monocarboxylic) and the dicarboxylic amino acids will be of particular interest. In connection with the foregoing mention should be made that hemocyanins of various species, even of two species of snails, appear to have distinctly different mobility slopes, according to measurements reported by Svedberg.<sup>6</sup>

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### THE DETERMINATION OF SULFANILAMIDE

THE method recently proposed for the determination of sulfanilamide in blood and urine<sup>1,2,3</sup> has been widely used both in experimental work and in controlling the administration of the drug to patients. The purpose of the present note is to call attention to certain improvements in the method which have been in use for the past two months in this laboratory. By destroying the excess nitrous acid after diazotization and buffering the solution before coupling with dimethyl- $\alpha$ -naphthylamine more rapid color development and more permanent colors are obtained. In addition, it has been found that in the presence of sodium chloride and certain other substances, the excess nitrous acid destroys some of the azo dye formed. This is entirely avoided by destruction of the excess nitrous acid after diazotization is complete.

The revised procedure is as follows. To 10 cc of the blood filtrate or acidified standard solution, 1 cc of a 0.1 per cent. freshly prepared solution of sodium nitrite is added. After 3 minutes standing, 1 cc of a 1 M sodium dihydrogen phosphate containing 0.5 per cent. of ammonium sulfamate<sup>4</sup> ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ , 13.8 gms; ammonium sulfamate, 0.5 gm; water 100 cc) is added and after 2 minutes standing, 5 cc of the alcoholic solution of dimethyl- $\alpha$ -naphthylamine are added. After 10 minutes, the solutions are compared in a colorimeter. By buffering, the pH of the final colored solution is maintained at 1.7 to 1.8. It has been found that pH changes from 1.0 to 2.0 do not influence the diazotization but do affect the rate of coupling.

<sup>6</sup> T. Svedberg, *Ind. and Eng. Chem.*, Analytical Ed. 10: 113, 1938.

<sup>1</sup> E. K. Marshall, Jr., Kendall Emerson, Jr., and W. C. Cutting, *Jour. Am. Med. Assn.*, 108: 953, 1937.

<sup>2</sup> E. K. Marshall, Jr., *Proceedings, Soc. Exp. Biol. and Med.*, 36: 422, 1937.

<sup>3</sup> E. K. Marshall, Jr., *Jour. Biol. Chem.*, 122: 263, 1937.

<sup>4</sup> We are indebted to Dr. H. A. Lubs, of E. I. du Pont de Nemours and Company, for suggesting the use of sulfamic acid to destroy nitrous acid, and for furnishing us with this substance. Ammonium sulfamate can be obtained from LaMotte Chemical Products Company of Baltimore.