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[2] 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,[3] and Vickery and White,[4] discussed by Bergmann and Niemann[5] 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.

2 L. Michaelis and Z. Bien, *Biochem. Zschr.*, 67: 198, 1914.

3 R. J. Block, *Jour. Biol. Chem.*, 105: 663, 1934.

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

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

Further accurate figures on other "neutral" (monoamino 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.[6]

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THE DETERMINATION OF SULFANILAMIDE

THE method recently proposed for the determination of sulfanilamide in blood and urine[1,2,3] 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-α-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[4] ($NaH_2PO_4 \cdot H_2O$, 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-α-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.

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

1 E. K. Marshall, Jr., Kendall Emerson, Jr., and W. C. Cutting, *Jour. Am. Med. Asn.*, 108: 953, 1937.

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

3 E. K. Marshall, Jr., *Jour. Biol. Chem.*, 122: 263, 1937.

4 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.

With the removal of excess nitrous acid, sodium chloride does not appear to influence the determination. Hence, acetyl-sulfanilamide can be just as readily determined in trichloroacetic acid filtrates as in those obtained by the use of p-toluenesulfonic acid by the following procedure.

One volume of a 15 per cent. solution of trichloroacetic acid is used for each 4 volumes of diluted blood. The free sulfanilamide is determined in the filtrate as described above. To determine the total, 10 cc of filtrate are treated with 1 cc of 2 N hydrochloric acid, heated in a boiling water bath for one hour, cooled and the volume adjusted to 10 cc. The subsequent procedure is as above, except that in place of the 1 M phosphate buffer, a 2 M phosphate buffer containing 0.5 per cent. of ammonium sulfamate is used. The standard solution of sulfanilamide (containing 18 cc of 15 per cent. trichloroacetic acid per 100 cc) is treated as the standard used for determining free sulfanilamide.

For determination of both free or conjugated sulfanilamide in blood, we now employ a 1:20 dilution instead of a 1:10 when an ordinary type of colorimeter is used. With a photoelectric colorimeter greater dilutions of blood can be advantageously used.

Since the disturbing effect of sodium chloride is avoided by the destruction of excess nitrous acid with sulfamate, satisfactory determinations can be made in lower dilutions of urine than previously.

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INDUCING "DORMANCY" IN POTATO TUBERS WITH POTASSIUM NAPHTHALENEACETATE AND BREAKING IT WITH ETHYLENE CHLOROHYDRIN[1]

POTASSIUM naphthaleneacetate inhibits the growth of the buds of non-dormant potato tubers (*Solanum tuberosum* L.) and the pieces treated with it behave like pieces of dormant or freshly-harvested potato tubers inasmuch as they do not grow for a month or more after planting. The inhibiting action of auxin-like substances on the growth of axillary buds is well known.[2] If the potato pieces are treated with ethylene chlorohydrin after treatment with potassium naphthaleneacetate they are stimulated to grow much before similar pieces not treated with ethylene chlorohydrin. The results are like those obtained by treating dormant tubers with ethylene chlorohydrin.

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 167.

[2] K. V. Thimann and F. Skoog, *Proc. Roy. Soc. B*, 114: 317–339, 1934.

Pieces from tubers of the Green Mountain variety were used. These had been stored all winter and untreated pieces showed emergence of sprouts about 12 days after planting. The tubers were cut into approximately cubical pieces weighing about 20 g each, with the skin at the top and one bud in the center of the upper side. They were washed, dried with cheesecloth and placed, bud up, in open petri dishes containing a solution of potassium naphthaleneacetate, 100 mg per liter, so that they were about two thirds immersed in the solution. After standing in the solution four days at 10° C., the pieces were planted in soil for eight days. They were then dug up, washed and the callus cut off in a thin layer. Some of the pieces were treated with ethylene chlorohydrin by the dip method of Denny.[3] They were dipped into a solution of 25 cc of 40 per cent. ethylene chlorohydrin per liter of water and after draining off the excess solution, were stored in a closed container for 24 hours. Control pieces were dipped in water. The pieces were then planted. Ten days later 20 out of 24 treated pieces showed sprouts above ground, while no sprouts had started on the 24 control pieces treated originally with potassium naphthaleneacetate but not subsequently treated with ethylene chlorohydrin.

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[3] F. E. Denny, *Contrib. Boyce Thompson Inst.*, 1: 59–66, 1926.

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MITCHELL, PHILIP H. *A Text Book of General Physiology for Colleges.* Third edition. Pp. xviii + 853. 200 figures. McGraw-Hill. $6.00.

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