

glected, the values become 5.96 for serine and 3.41 for threonine as g per 100 g protein.

The estimated number of amino acid residues per molecule of protein appears in the second set of figures for each method. A comparison of these numbers reveals the 3 determinations to agree on histidine only; although aspartic acid, cystine, isoleucine, leucine, lysine, phenylalanine, tyrosine, and valine show agreement in 2 out of 3 of the analyses. In the latter 8 instances, 7 of the identical sets of numbers include the chromatographic data, whereas one set includes the 2 microbiological data. If the uncorrected chromatographic data for serine were used, it would agree with one of the microbiological figures for this amino acid. The remaining numbers do not show agreement although the differences are not large, except for the chromatographic values for methionine and proline.

The preponderance of acidic over basic amino acids is in keeping with the isoelectric point of the protein (11) and also points to a site of action for the cationic exchange properties which this protein quite possibly displays (12).

References

1. LI, C. H., EVANS, H. M., and SIMPSON, M. E. *J. Biol. Chem.*, **149**, 413 (1943).
2. STEIN, W. H., and MOORE, S. *Ibid.*, **176**, 337 (1948).
3. MOORE, S., and STEIN, W. H. *Ibid.*, 367.
4. STEIN, W. H., and MOORE, S. *Ibid.*, **173**, 53 (1949).
5. MOORE, S., and STEIN, W. H. *Ibid.*, 79.
6. Shankman Laboratories, Los Angeles, California.
7. LI, C. H. *J. Am. Chem. Soc.*, **73**, 4146 (1951).
8. ———, *Federation Proc.*, **5**, 144 (1946).
9. HIER, S. W. The Wilson Laboratories, Chicago, Illinois. Western Avenue Boulevard, Chicago 9, Illinois.
10. REES, M. W. *Biochem. J.*, **40**, 632 (1946).
11. LI, C. H., EVANS, H. M., and SIMPSON, M. E. *Science*, **96**, 450 (1942).
12. ASTWOOD, E. B. *Adrenal Cortex*. New York: Josiah Macy Jr. Fdn. (1950).

Manuscript received December 1, 1952.

P^{32} Distribution in the Serum Proteins of the Chicken¹

R. E. Clegg and R. E. Hein

Kansas State College, Manhattan

The source of the phosphorus found in the egg has been the subject of numerous investigations and, in general, these studies have shown that extra phosphorus appears in the blood of birds during egg formation. In this respect, the relationship between the phospholipids and the site and rate of formation of these substances has been discussed previously (1, 2). Recently, differences in the number and amount of the electrophoretic components of the serum proteins of laying and nonlaying birds have been observed (3, 4); however, except for the possible lipoprotein nature of certain of these components (4), no other characteristics have been reported. According to Chargaff (5), phosphorus plays an important role in binding the lipid to the protein, and the amount of phospho-

¹ Contribution No. 480, Department of Chemistry.

phorus found in various lipoproteins tends to confirm this statement. A method for measuring the P^{32} distribution in the various components of protein mixtures is now available (6), and by using this technique the P^{32} in the electrophoretic components of the sera of birds has been under investigation. Certain of the results, and the relationship of these results to the distribution of the lipoprotein fractions reported by Moore (4), are discussed in this communication.

The sera were prepared from the blood of 8-week-old chickens, from 8-week-old chickens injected with diethylstilbestrol, and from laying hens. The 8-week-

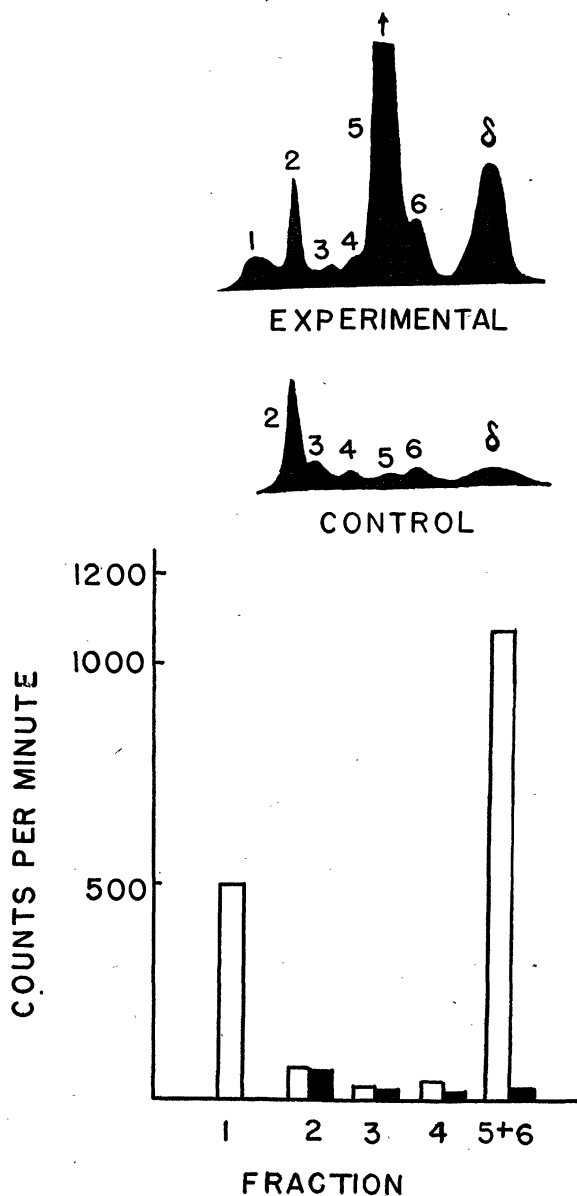


FIG. 1. P^{32} activity of the electrophoretic components of the blood serums of normal and diethylstilbestrol-treated chickens. The solid bars represent the activity of the components of the control; the open bars represent the activity of the hormone-treated birds.

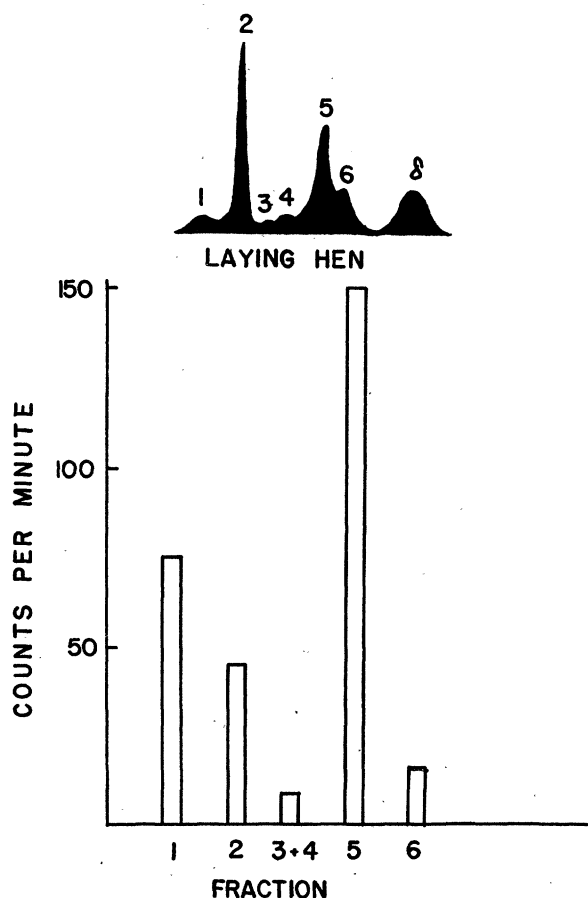


FIG. 2. P^{32} activity of the electrophoretic components of the blood serum of laying hens.

old chickens were fed 1 mc of P^{32} daily for 3 or 4 days, and the laying hens were fed in a similar fashion for 7 days. The electrophoretic (3) and radiochemical (6) techniques have been described elsewhere. For the purpose of this discussion the plateaus of the P^{32} activity curve have been subtracted one from the other so that the activities shown in Figs. 1 and 2 represent the relative activities of the various electrophoretic components.

The chief difference in the radioactivity associated with the sera of the normal nonlaying birds and those injected with the diethylstilbestrol was the high P^{32} content of 2 of the electrophoretic components. As illustrated in Fig. 1, component 1 and the greatly enlarged component 5, which are present only in the hormone-treated birds, contained relatively high concentrations of P^{32} . In the case of the laying hens (Fig. 2), the same phenomena were evident. The lower total amount of activity in the laying hens was probably caused by the loss of P^{32} in the eggs during the experimental period, however, inasmuch as the same pattern was present in both the laying and hormone-treated birds.

Moore (4) has shown that the ether extraction method of McFarlane removed the extra or enlarged

components found in the sera of laying and hormone-treated birds. The fact that repeated freezing at -25°C and thawing was needed to remove the fat-soluble substances is indicative of the presence of rather stable phospho-lipo-protein complexes and demonstrates the absence of mixtures or loosely adsorbed systems. With this in mind the fractionation and properties of these components are under investigation.

References

1. HAHN, L., and HEVESY, G. *Nature*, **140**, 1059 (1937).
2. ENTENMAN, C., et al. *J. Biol. Chem.*, **124**, 795 (1938).
3. CLEGG, R. E., et al. *Science*, **114**, 437 (1951).
4. MOORE, D. H. *Endocrinology*, **42**, 38 (1948).
5. CHARGAFF, E. *Advances in Protein Chemistry*, Vol. 1. New York: Academic Press, 3-6 (1944).
6. HEIN, R. E., and CLEGG, R. E. *Nucleonics*, **10**, 70 (1952).

Manuscript received November 19, 1952.

Degradation of Glucose¹

C. T. Bishop²

*Division of Applied Biology,
National Research Laboratories, Ottawa, Canada*

The original method of glucose degradation (1), based on bacterial conversion of glucose to lactic acid and subsequent chemical degradation, was laborious and time-consuming. Aronoff and Vernon (2) suggested a chemical method that appeared more satisfactory. Glucose was converted to its osazone which was then oxidized by periodic acid. The products of oxidation were: formaldehyde from C-6, formic acid from C-4 and C-5, and the osazone of mesoxalaldehyde from C-1 + C-2 + C-3. Further oxidation of the osazone of mesoxalaldehyde with 1% alcoholic potassium hydroxide should yield glyoxalosazone derived from C-1 + C-2. However, Vittorio, Krotkov, and Reed (3) reported difficulty in isolating glyoxalosazone from this oxidation and were forced to adopt a modified technique involving precipitation of acetaldehyde as aldome-done.

Osazones are unstable compounds and have generally been discarded in favor of the phenylosotriazoles as characteristic derivatives of sugars. It was, therefore, decided to try the Aronoff and Vernon procedure, with slight modifications, on the stable phenylosotriazole derivative of glucose. The scheme of this degradation is shown in Fig. 1.

Glucose (I) was converted to glucosazone (II) (4) which was changed to glucose phenylosotriazole (III) (m.p. $194-195.5^{\circ}\text{C}$, $[\alpha]_{\text{D}^{27}} = 79.3$, $C = 1.01$ in pyridine) by the method of Hann and Hudson (5). Periodate oxidation of III, as reported by the same authors (5), yielded 1 mole of formaldehyde from C-6, 2 moles of formic acid from C-4 and C-5, and 2-phenyl-4-formyl osotriazole (IV) from C-1 + C-2 + C-3. Com-

¹ N.R.C. No. 3014.

² The author wishes to thank G. A. Adams for calling attention to this problem. The technical assistance of J. E. Fraser is gratefully acknowledged.