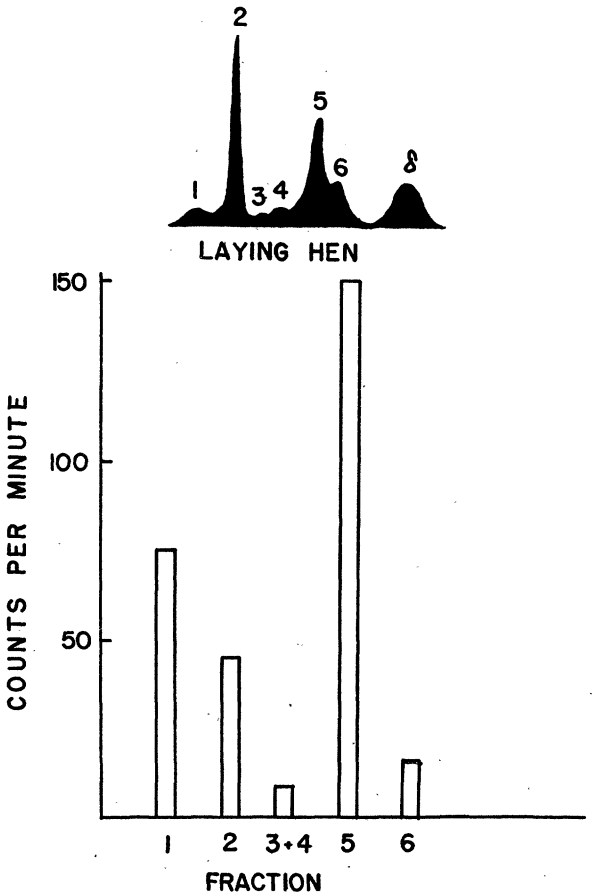

FIG. 2. P^{32} activity of the electrophoretic components of the blood serum of laying hens.

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).

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Degradation of Glucose[1]

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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 aldomedone.

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° C, $[\alpha]\ D^{27} = 79.3$, 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.

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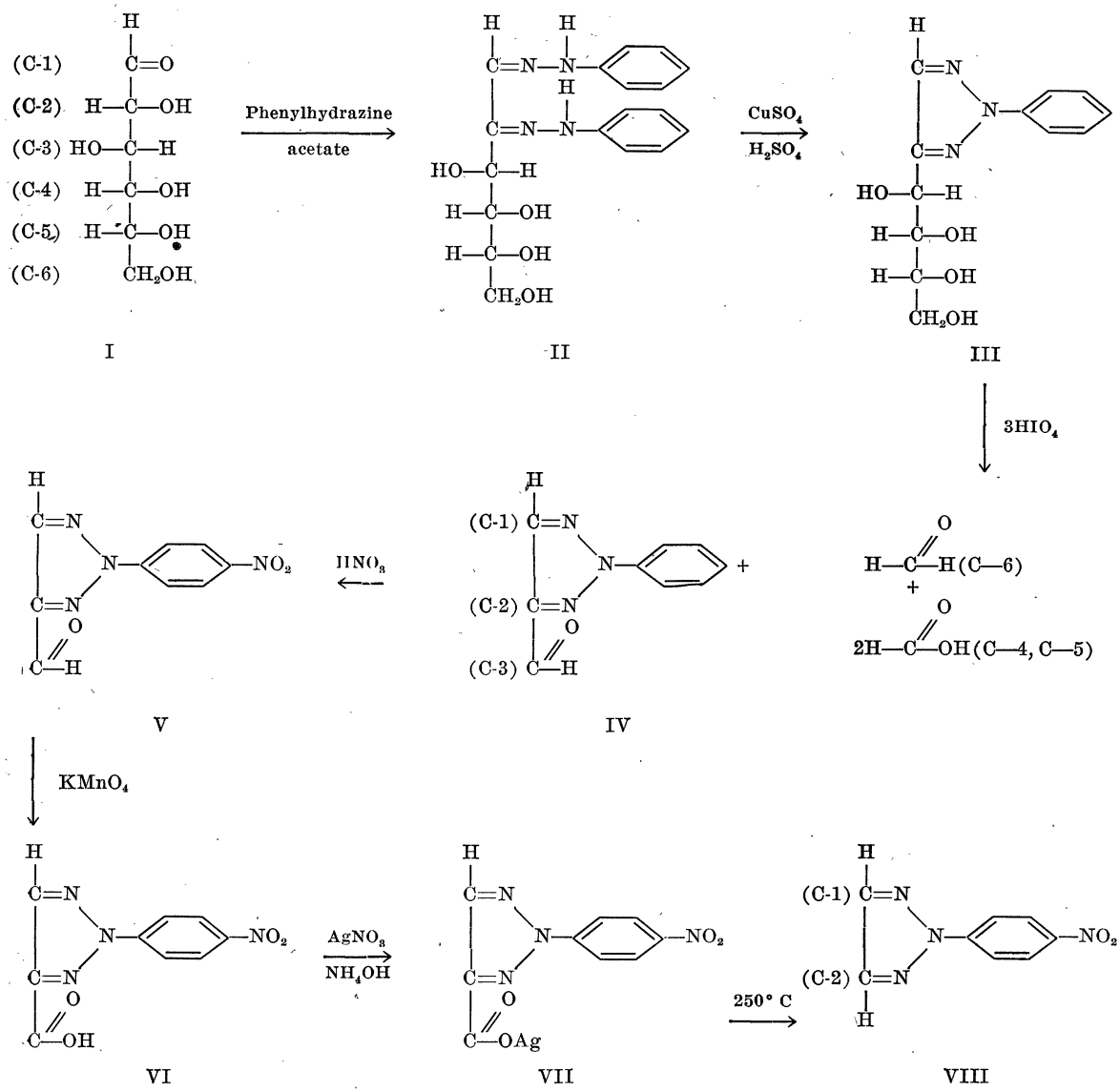

FIG. 1. Scheme for glucose degradation.

pound IV was filtered from the periodate oxidation as colorless crystals having the reported m.p. 69–70° C (*5, 6*). Nitration of IV with fuming nitric acid at room temperature (*6*) yielded 2-(*p*-nitrophenyl)-4-formyl osotriazole (V), m.p. 136–137° C, reported here for the first time. Infrared absorption spectra supported the proposed structure and the results of analysis agreed with the calculated values.

Calculated for $C_9H_6O_3N_4$: C = 49.54%, H = 2.77%, N = 25.68%. Found: C = 49.41%, H = 3.03%, N = 25.51%. Further confirmation of the structure of V was given by the next step in the degradation: Alkaline permanganate oxidation (*7*) produced the acid, 2-(*p*-nitrophenyl)-4-carboxy osotriazole (VI) which was recrystallized from water to a constant m.p. 236–237° C, in agreement with the reported value (*6*). The silver salt of VI was formed by heating the acid in ammoniacal silver nitrate until solution was complete. Three volumes of a mixture of acetone : methanol (1 : 1 by volume) were added and the solution was boiled gently to remove ammonia. The removal of ammonia caused the precipitation of the silver salt of 2-(*p*-nitrophenyl)-4-carboxy osotriazole (VIII) which was isolated by centrifuging. After washing with acetone to remove any ammonium nitrate, the precipitate was dried at 0.1 mm over phosphorus pentoxide. The acid VI was then decarboxylated by heating its silver salt (VII) in a test tube, at 250° C in an oil bath (*6*). The product, 2-(*p*-nitrophenyl) osotriazole (VIII), derived from C-1 + C-2, distilled and crystallized out on the upper part of the tube. Its m. p. was in agreement with the reported value of 183–184° C (*6*).

This degradation has been carried out using nonradioactive glucose, but no difficulties are anticipated in applying it to the radioactive compound. The nitration is necessary because compound VIII without the nitro group is an oil, not as easily isolated or characterized as the crystalline derivative. In using radioactive glucose a barium hydroxide trap should be attached to the dry distillation of compound VII, the final step in degradation. Silver carbonate, formed in this reaction, decomposes at 218° C with liberation of carbon dioxide. The trapping and counting of this gas would confirm the radioactivity on C-3 of the glucose. Heating aqueous solutions in recrystallizations should be done under reflux because some osotriazole derivatives are volatile in steam. All compounds formed in this degradation are well characterized crystalline compounds, soluble in organic solvents such as chloroform, ethanol, pyridine and acetone.

References

1. WOOD, H. G., LIFSON, N., and LORBER, U. *J. Biol. Chem.*, **159**, 474 (1945).
2. ARONOFF, S., and VERNON, L. *Arch. Biochem.*, **28**, (3), 424 (1950).
3. VITTORIO, P. V., KROTKOV, G., and REED, G. B. *Science*, **115**, 567 (1952).
4. BROWNE, C. A., and ZERBAN, F. W. *Physical and Chemical Methods of Sugar Analysis*. New York: Wiley, 670 (1941).
5. HANN, R. M., and HUDSON, C. S. *J. Am. Chem. Soc.*, **66**, 735 (1944).
6. v. PECHMANN, H. *Ann.*, **262**, 265 (1891).
7. SHRINER, R. L., and FUSON, R. C. *The Systematic Identification of Organic Compounds*. New York: Wiley, 164 (1945).

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The Adaptation of the Voges-Proskauer Reaction for the Quantitative Assay of Streptomycin

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At the present time there are several chemical methods for the assay of streptomycin. The principal chemical methods are the tests of Sullivan and Hilmer (*1*), Boxer, Jelinek, and Leghorn (*2*), and Marshall *et al.* (*3*). The test of Sullivan and Hilmer (*1*), is not sensitive with concentrations of the antibiotic lower than 1000 units/ml and values are high due to breakdown products formed during the test. The methods of Boxer, Jelinek, and Leghorn (*2*), and Marshall (*3*) are excellent chemical assay methods for streptomycin, but neither is adaptable to the assay of dihydrostreptomycin. All 3 of these assay methods require considerable time to perform.

The Voges-Proskauer reaction depends on the production of acetylmethylcarbinol or acetoin from glucose metabolism. In the presence of potassium hydroxide, acetylmethylcarbinol is oxidized to diacetyl which reacts with substances containing a guanidine residue to give a red-colored compound. Streptomycin and dihydrostreptomycin both contain 2 free guanido groups, which might be expected to complete this reaction. The purpose of this paper is to report a modification of the Voges-Proskauer reaction that permits a rapid quantitative assay for either form of the antibiotic.

Modifications of the Voges-Proskauer reaction such as the methods of Harden and Norris (*4*), Barritt (*5*), O'Meara (*6*), and Coblentz (*7*) were investigated to determine their desirability for use in the assay of either form of the antibiotic. Acetylmethylcarbinol (Matheson Co.) was used for preliminary work but, because of its unstable nature, diacetyl (Eastman Distillation Products Industries) was later adopted as the reagent of choice. These various modifications of the Voges-Proskauer reaction were repeated many times, using varying amounts of reagents, different concentrations of reagents, and different sequences of addition of reagents. After optimum conditions had been determined, tests were carried out with different concentrations of streptomycin and dihydrostreptomycin to determine the sensitivity of the assay. Color readings were made with a Klett-Summerson photoelectric colorimeter using filters #42, 54, and 66.

Except for the modifications of Harden and Norris (*4*) and Barritt (*5*), all the procedures of the Voges-Proskauer reaction proved to be unsatisfactory because of lack of sensitivity. After modification of these 2 methods the following procedure was developed. The amounts and concentrations of reagents were placed in Klett-Summerson tubes in the following order.

Streptomycin (varying concentrations)	1.0 ml
Alpha naphthol (5% in 95% ethanol)	0.5 ml
Potassium hydroxide (40%)	0.1 ml
Distilled water	2.9 ml
Diacetyl (1-1000 dilution)	0.5 ml

The function of potassium hydroxide in the Voges-Proskauer reaction is thought to be that of oxidation of acetylmethylcarbinol to diacetyl. Therefore, it would seem that if diacetyl were used, potassium hydroxide would not be needed. It was found that no color developed if potassium hydroxide was omitted. This indicates a new and unexplained function of potassium hydroxide in the reaction. It was found that the addition of streptomycin first and diacetyl last will give positive results whereas any other order of addition will give negative results. The tubes were shaken for 10 min to develop maximum color and then read for transmittency percentage in the colorimeter, using the #54 green filter. Maximum color was found to be stable for 15 min and tubes must be read within this time. In order to determine concentrations of streptomycin or dihydrostreptomycin greater than 1000 units/ml, 1.0-ml samples must be diluted to a total volume of 20.0 ml with distilled water, and sample amounts may then be assayed. Concentrations as low as 25 units/ml may be determined for either form of the antibiotic. This procedure gives a reproducible quantitative color test which fulfills Beer's law for any concentration from 25 units/ml to 1000 units/ml.