were given intramuscular injections of penicillin oil suspensions remained bacteriostatic for a longer period of time than when penicillin was administered in aqueous solution. In these experiments all the rabbits received intramuscularly 4,000 Oxford units of penicillin per kilo. When the penicillin was administered in aqueous solution, the blood became bacteriostatic for only one hour.[5] This was evidenced by the absence of growth of *Staphylococcus aureus* in broth culture mixed with rabbit blood. But bloods obtained one and one half, two, three, four and five hours later did show growth. On the other hand, when the blood of other rabbits which received penicillin in oil suspension was tested, the results proved that it was bacteriostatic one, one and a half and two hours after intramuscular injection, as determined by the absence of growth. Three, four, five and six hours afterwards, growth was observed—thus indicating the cessation of bacteriostatic effect.

The next experiment involved the treatment of rabbit syphilis by intramuscular injections of penicillin sodium suspended in oil. Rabbits were infected intratesticularly with a suspension of *Spirocheta pallida,* according to the method previously described by us.[6] One syphilitic rabbit was given intramuscularly 2,500 Oxford units per kilo body weight of penicillin sodium in aqueous solution twice a day consecutively for 8 days, totaling 40,000 units. The testicles were not free of spirochetes until the 14th day, and the complete healing of the lesions occurred at the end of 42 days. Two other rabbits were each administered intramuscularly 5,000 Oxford units per kilo of penicillin in oil. In this case, however, each animal was given penicillin only once a day for a period of 8 days, comprising again a total of 40,000 units. In both rabbits, the testicular lesions became negative for spirochetes in less than 10 days and the testicles appeared normal at the end of 35 days.

From the above it may be concluded in a preliminary way that penicillin in oil suspension is therapeutically somewhat more active in experimental rabbit syphilis than penicillin in aqueous solution. The superiority of the oil suspension, however, lies chiefly in the fact that the administration of the drug can be reduced to only one treatment a day. This experimental study opens the way to a method by which syphilis patients can be treated with penicillin in oil by a greatly simplified procedure. One daily intramuscular injection may thus suffice. This study further suggests a possibility of replacing the intravenous drip method and similar procedures by a few intramuscular injections of penicillin in oil to be used in the treatment also of various bacterial infections.

GEORGE W. RAIZISS

[5] Rabbit's blood was diluted with broth culture in the proportion of 1 to 20. This culture was seeded with a resistant strain of *Staphylococcus aureus* using one 4 mm loop of a 1–1,000 dilution.

[6] George W. Raiziss, *et al., Am. Jour. Syph. and Neurol.,* 19: 473, October, 1935.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD FOR THE COLORIMETRIC DETERMINATION OF PHOSPHORUS[1]

IN 1920 Bell and Doisy[2] devised a method for the colorimetric determination of phosphorus. This consisted in adding sulfuric and molybdic acids to the unknown ortho phosphate solution and reducing the phosphomolybdic acid thus formed by means of hydroquinone in a carbonate-sulfite medium. The resulting blue solution was read in a colorimeter against a standard. Briggs[3] improved the method by changing the order of adding the reagents. Fiske and Subbarow[4] considerably improved the method by substituting aminonapthol sulfonic acid for hydroquinone. Since the reduction of phosphomolybdic acid by aminonapthol sulfonic acid proceeds relatively slowly at room temperature, Lohmann and Jendrassick[5] have advocated heating the reactants to 37° C. for from 5 to 7 minutes. Allen[6] has employed amidol (2,4 diaminophenol dihydrochloride) in place of aminonapthol sulfonic acid, since this is a very powerful reducing agent. Kuttner *et al.*[7, 8] have used stannous chloride as the reducing agent, and this compound has been employed successfully by Youngburg and Youngburg.[9] Bodansky[10] found that trichloracetic acid caused deviations from Beer's law when the stannous chloride procedure was employed. He published a table of corrections.

While the Allen method has a number of minor dis-

[1] From the Biochemistry Laboratory, Cornell University, Ithaca, N. Y.

[2] R. D. Bell and E. A. Doisy, *Jour. Biol. Chem.,* 44: 55, 1920.

[3] A. P. Briggs, *Jour. Biol. Chem.,* 53: 13, 1922; 59: 255, 1924.

[4] C. H. Fiske and Y. Subbarow, *Jour. Biol. Chem.,* 66: 375, 1925.

[5] K. Lohmann and L. Jendrassick, *Biochem. Zeits.,* 178: 419, 1926.

[6] R. J. L. Allen, *Biochem. Jour.,* 34: 858, 1940.

[7] T. Kuttner and H. R. Cohen, *Jour. Biol. Chem.,* 75: 517, 1927.

[8] T. Kuttner and L. Lichtenstein, *Jour. Biol. Chem.,* 86: 671, 1930.

[9] G. E. Youngburg and M. V. Youngburg, *Jour. Lab. Clin. Med.,* 16: 158, 1930.

[10] A. Bodansky, *Jour. Biol. Chem.,* 99: 197, 1932.

advantages, in my opinion it is to be preferred to all other methods, if one is estimating inorganic phosphate in the presence of some easily hydrolysable phosphorus compound, such as glucose-I-phosphate. When, however, there is no likelihood of decomposing some such labile phosphorus compound I have found it preferable to employ reduction by stannous chloride, as in the method of Kuttner *et al.*, or Youngburg and Youngburg. The chief disadvantage of stannous chloride is that it will give a blue reduction compound with molybdic acid itself unless a relatively high concentration of sulfuric acid is first added, and this high concentration of sulfuric acid causes a rather rapid hydrolysis of such esters as glucose-I-phosphate, thus making determination of the preformed phosphate impossible.

I have found ferrous sulfate to be a more satisfactory reducing agent than either amidol or stannous chloride, all things considered. When added in slight excess it reduces phosphomolybdic acid to the end-point within a few seconds. The blue color thus produced has been found to increase in intensity by about 2 per cent. after standing for 2 hours. Change in room temperature from 18 to 25° C. is practically without effect. As much as 10 cc of 10 per cent. trichloracetic acid does not interfere with the test. With ferrous sulfate, as with stannous chloride, it is necessary to add sulfuric acid in order to prevent color production through a reduction of the molybdic acid. But with ferrous sulfate the quantity of sulfuric acid that must be added is so much less that a rapid hydrolysis of labile esters of phosphoric acid does not occur. Solutions of ferrous sulfate have a slight color, but this can be ruled out by using a solution containing only the reagents to set the photoelectric colorimeter at the null point. This blank solution remains usable for several hours.

The only drawback with ferrous sulfate is that its solutions undergo spontaneous oxidation. I have found it necessary to make up new solutions every 2 hours. This may sound disadvantageous; actually it is not, since only a few seconds are needed to weigh out granular ferrous sulfate roughly, add water from a graduated cylinder, add sulfuric acid and mix.

The Method

Pipette into a 24×200 mm tube, graduated at 50 cc, a known volume of the orthophosphate solution which is to be analyzed. The amount of phosphorus present should lie between 0.01 and 2.2 mg. Add 5 cc of 6.6 per cent. ammonium molybdate, $(MH_4)_6Mo_7O_{24} \cdot 4H_2O$, and distilled water to about 40 cc. Add 5 cc of 7.5 N sulfuric acid and mix gently by rotating the tube. Now add 4 cc of the ferrous sulfate (5 gm $FeSO_4 \cdot 7H_2O$ in 50 cc of water and 1 cc of 7.5 N H_2SO_4). Dilute to the 50 cc mark, stopper with a clean rubber stopper and invert the tube 4 or 5 times. Prepare a blank in a second tube, using the same amount of molybdate sulfuric acid and ferrous sulfate. Now employ the blank solution to set the photoelectric colorimeter at the null point using the red glass filter. Next read the unknown. The mg of phosphorus present are obtained from a graph. This is prepared by running determinations of various quantities of a standard solution of pure KH_2PO_4 and plotting the scale A readings against mg of phosphorus. This will give a perfectly straight line. Table 1 gives some values which I have employed to construct a graph for our Fisher photoelectric colorimeter. These values will not apply to other instruments.

TABLE 1

Mg. phosphorus	Scale A reading
0.0263	10.7
0.0527	21.6
0.105	42.5
0.153	64
0.211	84

Summary

A method is described for the colorimetric determination of phosphorus, using ferrous sulfate as a reducing agent.

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James B. Sumner

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