Spectrophotometric Investigation on the Orcein Derivative of Elastin

IRREVERSIBILITY of the reaction between orcein and elastin¹ and the strength of the bond, which varies with different dyestuffs and proteins, supports the hypothesis of purely chemical bond rather than that of a hydrogen bond or an adsorption complex.

The existence of a chemical bond may be demonstrated spectrophotometrically, employing the principle of conjugation bathochromy: it is known that conjugation of two chromophore groups produces a shift of the absorption maximum towards longer wavelengths.

Orcein and its reaction product with elastin (orceinelastin) have been spectrophotometrically investigated. Orcein was dissolved in a pH 8.9 buffer. Purified orceinelastin, obtained by complete removal of the free colour from the reacted elastin, was brought into solution by digestion with elastase (the elastolytic enzyme of Balò and Banga) at pH 8.9.

The absorption spectrum of the coloured digestion products of orceinelastin was found to be the same as orcein except for a bathochromic shift of the absorption maximum from 574 to 589 m μ . Since a bathochromic shift is a property of the conjugation link only, the existence of a sharing bond between orcein and elastin is indicated.

By comparing extinction values of pure orcein with digested orceinelastin ones, the stoichiometry of the compound was found to be 1.265 gm. (2.53×10^3 moles) orcein/100 gm. elastin. This quantity was found not to depend, within large limits, on the reaction temperature and orcein concentration. This provides further evidence that there is a chemical bond in orceinelastin. The only value reported in the literature is by Weiss² (no methods specified) of 2×10^2 moles/100 gm. elastin.

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¹ Scarselli, V., *Giorn. Biochim.*, **7**, 20 (1958).

² Weiss, J., *J. Histochem. Cytochem.*, **2**, 21 (1954).

Ultra-violet Fluorescence of Quinine Sulphate for Detection of Phosphate Ester Spots on Paper

A WIDE variety of phosphate esters have great importance as intermediates in biological reactions. Development of adequate procedures for the chromatography and detection of these phosphate esters has, in many respects, lagged behind. With the exception of the method of Runeckles and Krotkov¹, which was developed as a modification of the procedure of Wade and Morgan², the detection of phosphate esters on paper chromatograms has been very laborious and relatively insensitive. Most of the molybdate procedures, in addition to requiring many manipulations, have the disadvantage of employing perchloric acid, which weakens the paper and causes some charring during heating.

This communication reports a simple dip procedure by which phosphate spots containing as little as 0.007 μ mole of phosphorus can be detected. The phosphate spots are located by immersing the developed and dried paper in a solution of 0.5 per cent quinine sulphate $\cdot 2H_2O$ in absolute ethanol. After

drying the paper at room temperature for a few minutes, the phosphate spots can be detected by examining the paper under ultra-violet light in a darkened room. The spots show up as light areas against an intense grey-blue fluorescent background.

The best results were obtained with a low-pressure mercury source filtered to exclude visible light and producing predominantly ultra-violet radiation in the 2537-A. region. A long-wave lamp with ultra-violet radiation in the 3600-A. region and also filtered to exclude visible light was, in general, found to be only a little less satisfactory than the 2537-A. source.

In only one case was a qualitative difference noted between the two ultra-violet sources; that was with adenosine triphosphate, guanosine triphosphate and uridine triphosphate on a paper developed with a butanol, acetic acid and water solvent. With the 3600 A. source all three compounds appeared as light spots against a fluorescent background, but with the 2537 A. source, the first two appeared as dark blue spots while the uridine triphosphate appeared as a light spot against the fluorescent background.

The quinine sulphate dip, in addition to its ease of use, offers the great advantage of employing an alcohol-soluble and apparently non-reacting spot-detecting compound. After marking the spots under ultra-violet light as rapidly as possible, one can wash the bulk of the quinine sulphate off the paper with absolute ethanol, thus leaving the phosphate ester spots unmoved and free for elution and further determinations. The spots may also be left on the paper, following the alcohol wash, for study by other spot-detection procedures such as specific ketose reactions.

The sensitivity of this dip procedure for detecting various phosphate compounds which were developed on paper chromatograms is shown in Table 1.

Table 1. DETECTION OF COMPOUNDS CONTAINING PHOSPHORUS WITH QUININE SULPHATE ON DEVELOPED PAPER CHROMATOGRAMS

Substance tested	Smallest amount of test material showing a strong spot (μ gm. free acid)
Orthophosphate	0.7
Pyrophosphate	0.7
Phosphoglyceric acid	3
Dihydroxyacetone phosphate	3
β -Glycerophosphoric acid	3
Fructose-6-phosphate	4
Fructose-1,6-diphosphate	3
Galactose-1-phosphate	4
Glucose-1-phosphate	4
Uridine monophosphate	2
Uridine diphosphate	1
Uridine diphosphoglucose	2
Uridine triphosphate	1
Adenosine triphosphate	2
Guanosine triphosphate	2

Quinine sulphate is not selective for phosphate esters, but other organic compounds which might be present in chromatograms of this type, such as free sugars and amino-acids, must be present in relatively high concentrations before they are detected under ultra-violet light. Free sugars are not detected in amounts smaller than 40 μ gm. per spot and only a few of the amino-acids tested could be detected in amounts as small as 15 μ gm. per spot. A confirmatory ninhydrin dip or spray to detect any amino-acids which might be present can be run on the same paper following the quinine sulphate dip and subsequent ultra-violet examination. The quinine sulphate left on the paper does not interfere with the ninhydrin test. Purines, pyrimidines and nucleosides show up strongly. This was expected, since fluorescent procedures such as that of Pfennig³ have been used