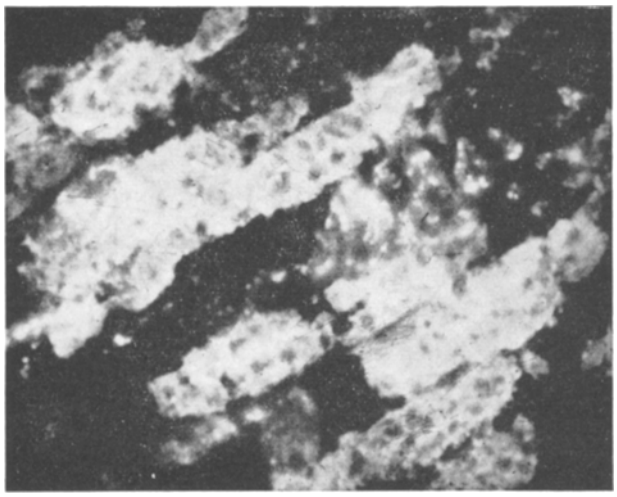
escing tubule and was not limited to specialized cell types, such as the so-called intercalated cells.

The function of TAMM-HORSFALL mucoprotein is not known, although its physical properties would imply usefulness as a lubricating agent. In addition, its ability to act as a substrate for myxovirus neuraminidase[16] and its presence in and possible production by renal tubular cells suggest that this mucoprotein may constitute part of a defense system against viral infection of the urinary tract[17].



Photomicrograph of fluorescent tubules, magnification × 440.

Zusammenfassung. Das Vorkommen von TAMM-HORSFALL Mucoprotein in Distalkanälchen und Sammelkanälchen nichtfixierter Nierenschnitte kann durch Immunofluoreszenzmethoden gezeigt werden. Die fluoreszierenden Kanälchen in Schnitten verschiedenen Ursprungs sind einheitlich mit Antikörper markiert.

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Semiquantitative Estimation of Phosphatase Activity in the Sephadex Layer

The necessity of fast estimation of the inorganic pyrophosphatase activity in bull semen led to elaboration of a method, which (1) requires minute amounts of biological material for examination; (2) is fast and simple; (3) can be used not only in a laboratory but also as a routine test.

STANSLY and RAMSEY[1] have drawn attention to the possibility of estimation of enzyme activity after diffusion in an agar gel. We have used this principle for the evaluation of inorganic pyrophosphatase activity and for its detection after agar gel electrophoresis (HRUŠKA[2]). Further improvement of that method was reached by using Sephadex as a medium, in which the diffusion is more constant. The layer of Sephadex, which can be prepared in advance so that before use only its swelling is necessary, serves as a carrier for a buffer and substrate, the splitting of which is proved by a colour reaction.

Preparation of a layer for the estimation of inorganic pyrophosphatase activity. After swelling, Sephadex G-75 Medium (Pharmacia, Uppsala) was suspended in deionized water (1 g of swelled gel/ml water) and this suspension was dried on a Petri dish (12 ml on a dish 9 cm in diameter) under filter paper (Niederschlag 388 W). 24 h before use, the layer is swelled again with 8 ml of $0.02M$ sodium pyrophosphate in glycine buffer pH 8.45. Accurate moisture of a layer is ascertained by compression with a glass rod. Raised liquid must soak into the layer immediately after interrupting the compression. The dry layer can be stored indefinitely.

The estimation of enzyme activity. As many as 12 samples can be applied on the outer part of a layer after its cooling to 0 °C. 20 μl of the samples are applied either separately or all together by means of a system of 12 constriction micropipettes. After 30 min of incubation at 37 °C, 6 standard solutions are applied to the centre part of a layer. The solutions of orthophosphate in concentrations 100, 50, 25, 12.5, 6.25 and $3.1 \cdot 10^{-3}M$ represent 200 to $6.25 \cdot 10^{-8}M$ of orthophosphate in 20 μl. Immediately after application of standard solutions the layer is poured on by a detection gel, prepared from 16 ml of suspension of Sephadex G-75 in acetate buffer pH 4.0 (1 g of swelled gel/ml buffer) and from 2 ml of 2% ascorbic acid and 2 ml of 1.5% ammonium molybdate. The conditions for the reaction are similar to the conditions for the estimation of phosphate according to LOWRY and LOPEZ[3] and orthophosphate gives distinct blue spots on white background. The intensity of colour is the highest after 30 min and does not change in the next 30 min. During this time the spots which have appeared at the sites of applied samples are compared visually with the spots of standard solutions. The results are expressed by the amount of liberated phosphate. The material used (bull and rabbit seminal plasma and bull seminal vesicle secretions) gave no reaction before incubation or after incubation in a layer without substrate.

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Discussion. During the use of this method in routine examination, it was established that the method fulfils the above-mentioned demands. The high sensitivity in the detection of orthophosphate ($6.25 \cdot 10^{-8} M$) allows this method to be used in the estimation of activity of other specific phosphatases. The splitting of the α-glycerolphosphate and glucose-1-phosphate in acetate buffer pH 5.0 and of adenosine-5'-triphosphate in veronal-acetate buffer pH 8.6 were demonstrated. Moreover, a reaction of phenol (15.6 *ng*) with ferric chloride could be used for demonstration of non-specific phosphatase with phenylphosphate as a substrate. The principle could also be used for evidence of a reduction of tetrazolium salts, of the liberation of acids from esters (in a layer containing bromthymol blue), and for evidence of amylase in a layer containing starch. As standards, ammonium sulfide was used for reduction of triphenyltetrazolium chloride and acetic acid for bromthymol blue and the reactions were demonstrable with 125 *ng* and $7.8 \cdot 10^{-8} M$ of standards, respectively. The principle that has been described allows also fast semiquantitative estimation of hemoglobin in minute amounts (8 *ng* and more) in a layer containing benzidine reagent.

Zusammenfassung. Rasche und einfache semiquantitative Methode zur Bestimmung der Aktivität von spezifischen Phosphatasen bei Reihenuntersuchungen: Detektion des Orthophosphats nach Diffusion des Enzyms durch eine Sephadex G-75-Schicht. Auf die Möglichkeiten ihrer Anwendung zur Bestimmung anderer Enzyme wird hingewiesen.

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[4] The author is indebted to Mrs. J. KORYTÁROVÁ for technical assistance during this work.

Purification of Hemocyanin from Hemolymph by Adsorption to Calcium Phosphate

An expedient way of purifying hemocyanin from hemolymph is available if advantage is taken of the adsorption of hemocynin to calcium phosphate. We have used this property to purify hemocyanin from the hemolymph of the whelk, *Murex trunculus*.

Hemolymph was obtained from *M. trunculus* by breaking off the apex of the shell and squeezing the snail inside. 25 ml of this material obtained from 36 snails were made up to 50 ml with $0.01 M$ acetate buffer, pH 5.0, and centrifuged to remove tissue debris. 10 ml of a suspension of hydrated tricalcium phosphate[1] in distilled water (0.1 g/ml) were added to the supernatant. The mixture was stirred and centrifuged to remove the calcium phosphate, which was subsequently eluted with 25 ml $0.1 M$ phosphate buffer, pH 7.0. The pH of the eluate was adjusted to 4.5 with $6 M$ acetic acid, and the solution was half-saturated with ammonium sulphate. The precipitated hemocyanin was separated by centrifuging at 3000 *g* at 4 °C for 30 min, and dissolved in a small quantity of $0.1 M$ phosphate buffer, pH 7.0.

The purification of the hemocyanin during the various stages is given in the Table. A fraction of the material available at each stage was diluted until it had an absorbance of approximately 1.00 at 280 nm in a 1 cm cell. The copper concentration was then determined by the method of PETERSON and BOLLIER[2]. The ratio of copper concentration to absorbance was used as a measure of the purity of the hemocyanin.

It is seen from the Table that 30% purification of the hemocyanin in the starting material was achieved by adsorption to calcium phosphate and elution; a further small degree of purification was obtained by precipitation with ammonium sulphate. This step also served to concentrate the hemocyanin.

The hemocyanin which was obtained migrated towards the anode as one component in agar-gel electrophoresis at pH 7.0. The protein concentration of two preparations was calculated from their nitrogen content, which was determined by a micro-Kjeldahl method[3] after removal of ammonium sulphate by gel filtration through a column of Sephadex G-25 equilibrated with $0.1 M$ phosphate buffer, pH 7.0. It was assumed that the hemocyanin contained 16% nitrogen. These preparations were found to contain 0.26% and 0.27% copper respectively. These values compare with the value of 0.260% reported by GHIRETTI-MAGALDI et al.[4] for hemocyanin of *M. trunculus* prepared by ultracentrifugation of hemolymph.

The adsorption of hemocyanin to hydroxylapatite has been described by other workers[5], but this convenient property has not been used to purify hemocyanins. The

Stage	Copper concentration (μg/ml)	Absorbance (280 nm/ 1 cm cell)	Cu concentration Absorbance	Cu concentration Purification
Diluted hemolymph	1.09	0.94	1.16	1.0
Calcium phosphate eluate	1.45	1.00	1.45	1.3
Ammonium sulphate precipitate	1.66	0.97	1.58	1.4

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