Our instrument has the disadvantage that, without cuvette, the readings of the photometer at the 2 positions of the polarizer are not exactly the same, although they should be theoretically. We do not wholly understand this phenomenon, but partial polarization of the light in the monochromator and inhomogeneity of the polarizer may be two of the causes. Without cuvette we find a small difference ($E_L - E_R$) which varies with wavelength but is independent of slit width and hence of light energy. The values are not altered by placing a cuvette with solvent in the beam. Therefore a blank curve should be taken with a cuvette with solvent, and the values measured for the solution are corrected by subtraction of the blank values. The quality of the cuvettes used for the measurements is very important. Some of our cuvettes appeared to be birefringent (owing to strain) and therefore useless, and in some cases we found high blank values for a particular cuvette which could be influenced by inverting the cuvette. Nevertheless some commercial cuvettes were selected that showed none of these disadvantages.

To demonstrate the utility of the apparatus, the CD curves of testosterone and 3β-hydroxy-(5α)-androstan-17-one obtained with the instrument are given in Figures 4 and 5. These curves agree well with the published values[5]. Probably, the transmission of the apparatus in the UV-region can be improved by using a quartz polarizer instead of a calcite one. (We used the calcite polarizer because it happened to be available[11]).

Zusammenfassung. Es wird ein einfacher Apparat beschrieben, der als Zusatzgerät zum Zeiss-Spektralphotometer PMQ II zur Messung von Zirkulardichroismus zu verwenden ist.

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Immunofluorescent Localization of TAMM-HORSFALL Mucoprotein

TAMM-HORSFALL mucoprotein[1] exists normally in human urine as a highly aggregated, double stranded filamentous molecule[2,3] having a molecular weight of approximately 7 million[1]. It has recently been shown that the large, native form of this protein may be dissociated or disaggregated by various means[4–8], and some evidence has become available to indicate that the basic monomeric subunit, retaining hemagglutination inhibition activity and all the immunologic determinants of the parent molecule, has a molecular weight in the region of 95,000–112,000[9]. The cellular origin of the mucoprotein has been assumed to be rather high in the nephron, since attempts to demonstrate serum precursors have been unsuccessful[10,11], and since cast material from patients with acute renal failure or with the nephrotic syndrome has been found to consist almost entirely of TAMM-HORSFALL mucoprotein[11,12]. An immunofluorescent technique has been used in a study of the localization of this mucoprotein in sections of human kidney.

Native TAMM-HORSFALL mucoprotein was prepared from human urine by precipitation in 0.58 molar NaCl, according to the method described by TAMM and HORSFALL[1]. This procedure has been shown to yield a product which behaves homogeneously in starch gel electrophoresis, ultracentrifugation, gel filtration and immunodiffusion[1,8]. Antiserum was prepared in rabbits by 2 intramuscular injections of purified mucoprotein with complete Freund's adjuvant at 3 week intervals, and the initial harvest was at 6 weeks. Antibody was conjugated with fluorescein isothiocyanate[13,14] and adsorbed with mouse liver powder[15] after dialysis and filtration on G-25 Sephadex.

Kidney sections were obtained at the time of autopsy and were cut at a thickness of 4 or 5 μ and transferred to glass slides. The sections were washed with phosphate-buffered saline, covered with conjugated antiserum and kept for 45–60 min in a moist chamber at room temperature. After 3 subsequent washings with the buffered saline, the slides were mounted in buffered glycerol and examined with a Zeiss UV-microscope.

Bright cytoplasmic fluorescence was found in kidney sections from patients with congenital heart disease, acute lymphocytic leukemia, idiopathic thrombocytopenic purpura and fibrocystic disease of the pancreas. Glomeruli and proximal tubules remained uniformly nonfluorescent in all sections. Cells of the distal tubules and collecting tubules and perhaps the loops of Henle showed specific fluorescence which was blocked by pre-incubation with unconjugated antiserum as well as by mixing antigen with conjugated antiserum (Figure). The binding of antibody was evenly distributed among all cells in a fluor-

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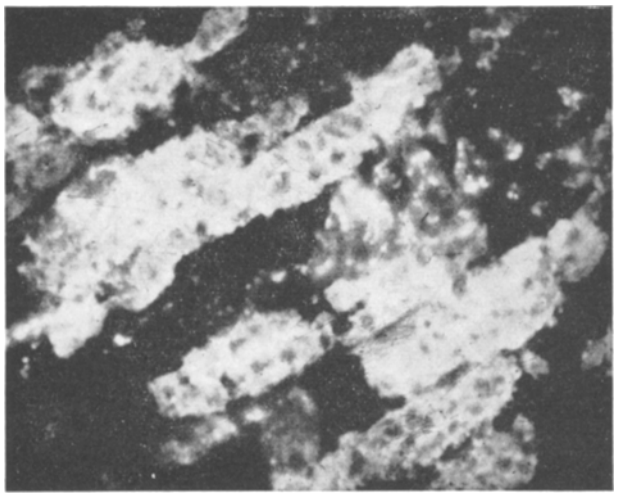
escing tubule and was not limited to specialized cell types, such as the so-called intercalated cells.

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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