wet strip using a pair of dividers and a suitable millimetre scale. The antigen concentrations in the unknowns can then be read off from a graph correlating the reference standards with peak heights.

Comment

Figure 1 shows typical electroimmunodiffusion quantitations of immunoglobulins on Cellogel. The technique is also satisfactory for estimating other serum proteins, eg, β-lipoproteins. It has the advantages of speed and economy of antiserum: the whole procedure from preparing the immunostrip to estimating the antigen can be carried out within five hours and it consumes only very small measured amounts of antiserum. It appears to be especially applicable to measurement of 'minor' serum components and immunoglobulins present at low dilutions in other biological fluids.

Cellogel also achieves economy in the Mancini technique, since a single immunostrip will accommodate up to 59 determinations.

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A simple capillary tube method for the determination of the specific gravity of 25 and 50 μl quantities of urine

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When large amounts of urine are available in the laboratory, measuring specific gravity is one of the simplest procedures. Methods for small quantities, however, usually entail more elaborate techniques such as placing a drop of urine in a mixture of two solvents, one of high and the other of low specific gravity, and adjusting the proportions by titration (Bowler, 1951) or suspending a drop of urine in a specially calibrated gradient column (Brown, 1958). The titration method is laborious and time consuming. The gradient column method is quicker, but troublesome to assemble and the column reagent has a life limited to a few months.

The weighed specific gravity bottle principle is much simpler, and a constant volume capillary tube of small capacity can be substituted for the larger volume pycnometer. The method was employed in the investigation of a strain of mice suffering from an inherited kidney disease, the determining gene for which has been given the name and genetic symbol, *kidney disease, kd* (Lyon and Hulse, 1971). It is suggested that the method may be applicable clinically, eg, to the small urine samples derived from infants or from ureteric catheterization in man.

Method

Disposable microcap tubes of 25 or 50 μl capacity (Drummond Scientific Company) are selected according to the available volume of fluid. A small number of trials made with plain microhaematocrit tubes (Hawksley) having a volume of around 45 μl indicated that any other small capillary tube of uniform bore and standard size can be used. Urine samples are permitted to reach room temperature (22°C) before weighing. Weighings are made to 0·1 mg on an automatic analytical balance immediately after the introduction of the liquid into a tube.

A dry microcap tube filled by capillary attraction with glass-distilled water is dried on the outside by

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wiping with a paper tissue, weighed, removed from the balance, drained of water by holding the end of the tube against the paper tissue, and weighed again. The weight of the wet tube deducted from that of the filled tube gives the weight of the distilled water. Immediately the same wet tube is filled with urine, weighed, and the weight of the urine noted. Whenever air bubbles enter, the liquid is returned to the urine pool and the tube is refilled. Since specific gravity is the number of times a liquid is as heavy as an equal volume of water, it follows that the specific gravity of the urine is the ratio of the weight of the urine to the weight of the water.

Accuracy and precision were determined with human urine, all the measurements being made at a reasonably constant room temperature of 22°C. The mean of 10 measurements with a 25 ml pycnometer was 1·01527 ± 0·00002. The means of 10 estimations with the 25 and 50 μl microcap tubes were 1·01628 ± 0·0014 and 1·01576 ± 0·0005 respectively, neither differing significantly from the pycnometer value. The standard deviation of the measurements was 0·004 and 0·002 respectively so that precision was good. A single tube weighed twice therefore suffices for routine work, and only about four minutes are then required for a determination.

Illustrative Example of the Use of the Method in a Genetically Determined Kidney Disease of Mice

The urines of mice homozygous for the kd gene (ie *kdkd*) and of mice of the parent strain (CBA/CaH) were examined, the ages of the mice ranging from 20-day-old weanlings to adults. A limit of around 280 days was imposed because few *kdkd* mice survive beyond that time as compared with 1000 days for CBA mice.

To obtain the urine the mouse was held firmly over a Petri dish and encouraged to micturate (Silverstein, 1961). One micturition from a mouse of either strain produced approximately 30 to 100 μl of urine which was collected in a disposable plastic Petri dish. To minimize evaporation of the urine, the Petri dishes were housed in a closed box with a moistened atmosphere.

Urine protein levels were assessed with Hema-Combistix reagent strips (Ames). Blood urea estimations were made by a modified Urastrat technique (Baron and Hughes, 1965) on a different but similar batch of *kdkd* mice (Lyon and Hulse, 1971).

In both strains of mice the mean specific gravity of the urine increased from 20 to 80 days of age (Fig. 1). Thereafter the values for healthy CBA mice were in the range 1·06 to 1·08 and none died. From

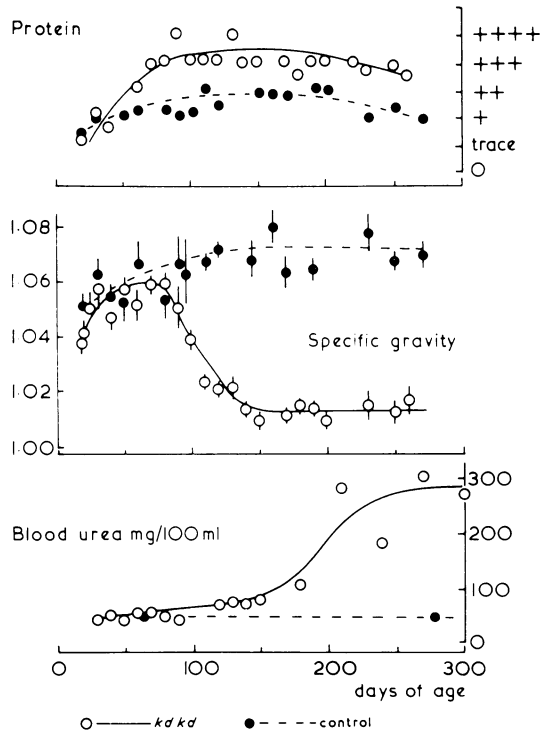

Fig. 1 *Urine protein, urine specific gravity, and blood urea in mice with an inherited kidney disease* (kdkd) *and in normal controls at ages from 20 to 300 days. Means with standard errors are given for specific gravity (3-19 animals per point).*

80 days of age onwards there was a progressive and rapid drop in the urine specific gravity of the diseased *kdkd* mice from 1·06 to 1·012 at about 150 days preceded by a progressive increase in proteinuria. (Normal mouse urine commonly gives + or ++ protein reaction.) Blood urea started to rise after the specific gravity had fallen considerably, and increasing numbers of *kdkd* mice died between 150 and 300 days with severe uraemia and gross kidney damage (Lyon and Hulse, 1971).

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Demonstration of basement membrane in renal biopsies by silver-methenamine on thin epoxy-resin sections

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In the recent paper on an improved technique for renal biopsies, Meadows and Schoemaker (1970) describe the use of a special wax to give fine sections at 1-2μ. They illustrate their excellent results with Jones's silver technique. In this laboratory, however, difficulty was experienced with their use of ammoniacal ethanol and as we, in agreement with Eastham and Essex (1969), believe that the thinner sections possible with polymer-embedding are of greater diagnostic value, the present note describes the successful application of a methenamine-silver method on sections from Araldite.

Fixation

Buffered formol saline is perfectly satisfactory and no differences in results have been observed with material fixed from six hours to two weeks.

Attempts to impregnate the reticulin with Foot's method have not been satisfactory with epoxy-resin sections. The silver-methenamine method of Gomori and Grocott, already successfully used on acrylic-resin sections, is now suggested as a simple way of demonstrating reticulin on epoxy-resin sections. A real advantage of epoxy resin (Araldite) is that thin sections (0·5μ) adhere to glass and this allows 'staining' on the slide and a flat, clean, mounted section.

Methods

After fixation in formol saline, a portion of the renal biopsy, not longer than 5 mm, is selected for embedding in Araldite. The remainder of the needle biopsy may be processed for paraffin. The selected material is dehydrated through:

30% acetone	15 minutes
70% acetone	30 minutes (overnight)
100% acetone	3 hours
30% Araldite in dry acetone	15 minutes
70% Araldite in dry acetone	15 minutes
70% Araldite in dry acetone at 60°C	30 minutes
100% Araldite at 60°C	30 minutes

Embed in a previously warmed, size O, gelatine capsule mould and allow to polymerize at 60°C for two days.

Araldite, as used in the method, refers to the following formula (modified from Luft, 1961): 20·0 ml Ciba Araldite epoxy (Araldite M) CY212, 20·0 ml Ciba Epoxy hardener HY964, 1·5 ml Ciba accelerator, and 1·0 ml di-butyl phthalate.

Sections are cut at 0·5-1·0μ on a Reichert OMU2 ultramicrotome using a glass knife. The sections are floated onto a clean glass slide and affixed without the use of adhesives (Eastwood *et al*, 1969), dried flat on a hotplate at 60°C for half an hour, allowed to stand at room temperature for a further 30 minutes, immersed in saturated alcoholic NaOH (Lane and Europa, 1965) for a minimum of one hour, and washed in running tap water for two to three minutes.

Impregnation Method

1 Rinse thoroughly in distilled water.
2 Treat with 1·0% aqueous periodic acid for 30 minutes. (This solution lasts for several months.)
3 Rinse briefly in four changes of distilled water (each four to eight seconds).
4 Place slides in Grocott's silver methenamine solution at room temperature then transfer the slides and silver solution, in the coplin jar, to a 60°C water bath.
5 After approximately 30 minutes the section, macroscopically, appears a light autumn brown colour. Check microscopically at intervals of two to three minutes after this time until the glomerular capsule is a deep black and the membrane of the capillary loops is dark brown.
6 Rinse thoroughly in distilled water.
7 Tone in 0·1% gold chloride until the capillary loops of the glomerulus have changed from dark brown to black and the background is almost colourless.
8 Wash well in distilled water.
9 Treat with 2·0% aqueous sodium thiosulphate (three to five minutes).
10. Wash thoroughly in running tap water, dehydrate through ethanol, clear in xylol, and mount in D.P.X.

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A simple capillary tube method for the determination of the specific gravity of 25 and 50 μl quantities of urine—continued

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