of endogenous permeability factors affecting the response(6).

Summary. A method of quantitative extraction of pontamine sky blue from the skin was described, allowing a quantitative measurement of increased capillary permeability to plasma proteins in the skin.

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Determination of Chloride in Serum and Urine by a Modified Mercuric Thiocyanate Method. (28315)

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Determination of chloride ion by reaction with mercuric thiocyanate and complexing liberated thiocyanate with ferric ions has been reported by Iwasaki *et al.*(1). These workers applied this method to determination of chloride in water. Bergmann and Sanik(2) extended the method to determination of chloride in naphthas. The precision and simplicity of the procedure make it attractive for use in clinical chemistry. The present paper extends the use of the method to the determination of chloride in serum and urine specimens.

Methods. Reagents. 1. Mercuric thiocyanate ¾ saturated solution. Shake about 400 mg mercuric thiocyanate with 100 ml ethanol (ethanol denatured with methanol is satisfactory) for about 10 minutes at 25°C. Filter and dilute 90 ml of the filtrate with 30 ml of ethanol. 2. Acetic acid, 0.5 M. 0.3 ml glacial acetic acid diluted to 100 ml with distilled water. 3. Nitric acid, 0.8 M. 5 ml concentrated nitric acid diluted to 100 ml with distilled water. 4. Ferric nitrate. 5 g $Fe,(NO_3)_3 \cdot 9H_2O$ is dissolved in 100 ml of reagent 3. 5. Stock standard, 0.1 M NaCl. Working standard is prepared by diluting 2.0 ml stock to 20 ml with distilled water. *Procedure.* To 0.5 ml serum or urine in a test tube are added, with mixing, 4.0 ml distilled water and 0.5 ml reagent 2. The tube is stoppered and placed in a boiling water bath for 4 - 5 minutes, cooled in tap water, the condensate on the walls mixed with the contents of the tube and the tube then centrifuged. Glass-stoppered tubes or cork stoppers covered with Saran wrap may be used. Blank, standard and unknown are then set up by diluting 1.0-ml aliquots of water, working standard and supernate from above to 10 ml with reagent 3, respectively. After mixing, 1.0-ml aliquots are transferred to suitable test tubes or photometer tubes, 4.0 ml reagent 4 added and mixed, and 1 ml reagent 1 added and mixed. Absorbances are measured against a water blank at 460 mμ or with a filter with nominal wavelength in this region, except that with the Klett photometer the no. 42 filter should be used. Calculation:

$$\text{meq } Cl^-/1 = \frac{A_{Unknown} - A_{Blank}}{A_{Standard} - A_{Blank}} \times 100.$$

Results. Beer's Law. Several instruments were used. Table I shows the absorbance for each instrument tested below which a linear relation between chloride concentration and absorbance was observed. The color obtained showed a maximum at 460 mμ and was stable for at least 5 hours.

Recoveries. Mean recovery for chloride added to 3 urine and 3 serum specimens at a level of 50 meq/l was 103% and 98%, re-

TABLE I.

Instrument	Reading below which Beer's law was followed
Beckman B	1.300
Coleman	.700
Leitz	.800
Klett	500

spectively. When the level of added chloride was 100 meq/l, in a similar experiment, mean recovery for urine was 100% and for serum 97%.

Precision. Twenty-eight urine specimens were analyzed in duplicate. The standard deviation calculated from these duplicate determinations was 1.86 meq/l and the 95% confidence limits were ± 2.1%. A similar experiment using 28 serum specimens yielded a standard deviation of 2.32 meq/l and 95% confidence limits of ± 4.6%.

Comparison with other methods. The same urine specimens were analyzed by the open Carius method of Van Slyke and Sendroy(3) and the serum specimens by the method of Schales and Schales(4). Values for urine averaged 2.3 meq/l lower than the reference method and values for serum averaged 1.25 meq/l higher than the reference method. By the use of the t test, these differences were not significant at the 5% level (t = 1.51 for urine; t = 1.71 for serum).

Summary. A simple procedure for determination of chloride in biological fluids, based on the mercuric thiocyanate method, is described. It is shown that results obtained by this procedure do not differ significantly from those yielded by the Van Slyke and Sendroy method for urine chloride and the Schales and Schales method for serum chloride.

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Circulating Antibodies to Egg Albumin in Man.* (28316)

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This study presents an estimate of the incidence of circulating antibodies to egg albumin (EA) in a general hospital population. Egg albumin was chosen as the antigen since it a) is a ubiquitous dietary item, b) is available in reasonably pure form, c) is a small protein (MW 40,000) and perhaps more readily absorbed, d) is well adapted for use in the hemagglutination test system, and e) is an effective antigen in rabbits so that positive control sera can be obtained.

Methods and materials. Sera were obtained from 1086 unselected patients admitted to a 325 bed general hospital and from 38 second year medical students. The bloods were stored and centrifuged at 4°C and the sera frozen at −70°C. The usual elapsed time from collection of blood until freezing was about 4 hours although approximately 20% were held overnight at 4°C before freezing.

Egg Albumin, 1x crystallized and bovine-γ-globulin (BGG) were obtained from the Mann Research Laboratories and stored at −20°C.

Antibody assays employed both hemagglutination and microprecipitation technics. The hemagglutination method used was that of Ingraham(1) with slight modifications. Instead of storing the formalinized erythrocytes in concentrated form at 4°C, the cells were diluted to final concentration and kept frozen at −70°C until use. For titrations 12 x 75

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