anions are strongly attracted to the highly charged aluminum polynuclear cation. As the charge is neutralized, the complex disassociates and aluminum hydroxide forms. Because the solution pH was raised to 8 by the addition of sodium hydroxide, bayerite, the aluminum hydroxide polymorph that forms under basic conditions, was produced.

Aluminum chlorohydrate also is unstable if it is diluted with water (5, 6). Figure 2 shows that when aluminum chlorohydrate was diluted to 2.5×10^{-2} *M* in aluminum, the pH first increased and then decreased. Earlier reports only noted a decrease in pH following dilution. However, the initial increase in pH is important and is consistent with the recently proposed structure of aluminum chlorohydrate (1). The central tetrahedral aluminum in the I complex is surrounded by 12 aluminum atoms in octahedral configuration. In dilute aqueous solution, the highly charged aluminum polycations are widely separated, and the stabilizing effect of the chloride counterions and other aluminum chlorohydrate complexes is reduced. As the I complex disassociates, the tetrahedral aluminum is exposed to the aqueous environment. Since the octahedral configuration is the stable form of aluminum below pH 8 (7–10), the tetrahedral aluminum converts to an octahedral configuration. Exposure of the four negatively charged oxygens, which form the outer shell of the aluminum tetrahedral, to the aqueous environment results in the attraction of protons and water to complete the octahedral configuration. The adsorption of protons is reflected in the initial increase in pH. However, the overall trend is a decrease in pH, which is consistent with the formation of octahedral aluminum into aluminum hydroxy polymers by the deprotonation–dehydroxylation mechanism (3).

The stability of aluminum chlorohydrate is concentration dependent since very little change in pH was observed unless the solution was <0.1 *M* in aluminum. At high concentration, the positively charged I spherical complexes are tightly packed and surrounded by stabilizing chloride counterions. Thus, aluminum chlorohydrate complex is self-stabilizing at high concentration.

Turbidity measurements of the diluted aluminum chlorohydrate solutions also showed that the stability of the complex was concentration dependent. Turbidity was not observed during this study unless the concentration was ~0.1 *M* or lower in terms of aluminum. Figure 2 also shows the development of turbidity following dilution to 2.5×10^{-2} *M* in aluminum.

The insoluble degradation product resulting from the dilution of aluminum chlorohydrate was collected by filtration and examined by IR spectroscopy. The hydroxyl-stretching bands coincide with the hydroxyl-stretching bands of gibbsite (Fig. 3). The IR spectrum of the lyophilized filtrate was identical to the reference spectrum for aluminum chlorohydrate (11). Thus, it is believed that the I complex disassociates in dilute solution. The tetrahedral aluminum converts to an octahedral configuration due to the pH, and the octahedral aluminum species form aluminum hydroxide by deprotonation–dehydration. Gibbsite is the end-product of dilution since it is the stable polymorph of aluminum hydroxide in the acidic region. Therefore, the conversion of aluminum chlorohydrate to aluminum hydroxide upon complete neutralization or following dilution with water is consistent with the structure of the I complex that was proposed recently to be aluminum chlorohydrate.

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Modified Colorimetric Method for Plasma Prednisolone

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Abstract □ A simple, precise, rapid, and sensitive colorimetric method was adapted for the quantitative analysis of prednisolone in dog plasma. A paper chromatographic procedure was modified by the use of thin-layer plates coated with microcrystalline cellulose. Heparinized blood samples were separated from the cellular elements and extracted with methylene chloride. After washing with acid and alkali, the extract was evaporated to dryness. The residue was dissolved in methanol and streaked on the TLC plate. After development, the band that was detected by UV light was scraped off and extracted with methanol. The methanol extract was treated with the Porter–Silber reagent (phenylhydrazine). Absorbance was measured at 410 nm. Replicate assays indicated a mean recovery of 97.5% and a coefficient of variation of 5.13%.

Keyphrases □ Prednisolone—modified method for colorimetric determination in dog plasma □ Colorimetry—modified method for determination of plasma prednisolone □ Glucocorticoids—prednisolone, colorimetric determination in plasma

The various methods used for the determination of prednisolone in plasma all have one or more shortcomings. Jenkins and Sampson (1) used a paper chromatographic procedure for the isolation of prednisolone after preliminary extraction with methylene chloride. Their procedure was modified by replacing the paper chromatographic procedure with thin-layer plates coated with microcrystalline cellulose. Microcrystalline cellulose was tried after preliminary screening revealed that inefficient separation occurred on the silica gel-coated plates recommended in the USP XX procedure (2).

The modified procedure decreased the time required for separation and also increased assay sensitivity. As a consequence, a simple, direct, and rapid analytical method for prednisolone in plasma was developed independently.

EXPERIMENTAL

Materials—Anhydrous prednisolone USP[1], methanol[2] (HPLC grade),

[1] Merck & Co., Rahway, N.J.
[2] Fisher Scientific Co., Fair Lawn, N.J.

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toluene[3], methylene chloride[4], sodium hydroxide pellets[2] (analytical reagent grade), acetic acid[2], nitrogen[4], sulfuric acid[2] (analytical reagent grade), and phenylhydrazine hydrochloride[5] were obtained commercially.

Equipment—A spectrophotometer[6], a centrifuge[7], an analytical balance[8], a multiple developing tank[9], TLC glass plates[10] precoated with Avicel F (250-μm thickness), a micropipet[11], a camera with photo-UV system[12], a minerlight lamp, a multiband UV detector[13] (254/366 nm), and a water bath[14] were used.

Preparation of Prednisolone Standard Solution in Methanol—A stock solution of prednisolone was prepared by dissolving 100 mg of anhydrous prednisolone USP in 1000 ml of methanol. The stock solution then was diluted serially with methanol to give standard solutions containing 0.5, 1.0, 5.0, 10.0, 20.0, 25.0, and 50.0 μg of prednisolone/ml.

Preparation of Reagents—Dilute sulfuric acid solution (1.63:1) was prepared by adding 190 ml of water to 310 ml of concentrated sulfuric acid. Phenylhydrazine hydrochloride solution (Porter–Silber reagent) was prepared by dissolving 65 mg of phenylhydrazine hydrochloride in 100 ml of the dilute sulfuric acid (3).

Quantitative Color Reaction for Prednisolone—Eight milliliters of the phenylhydrazine reagent solution was added to a prednisolone sample in 1 ml of methanol (*i.e.*, various standard solutions of prednisolone in methanol), and the solution was heated to 60 ± 1° for 20 min. The resultant yellow solution was cooled to 25° in a running cold water bath. The methanol–phenylhydrazine–sulfuric acid blank was treated similarly. With a 5-cm cell, the absorbance of the standard solution was measured against a methanol–sulfuric acid–phenylhydrazine reagent blank at 410 nm. Spectrophotometric readings usually were taken within 5 min after color formation. The standard curve then was constructed by plotting the absorbance values *versus* the corresponding prednisolone concentrations.

Preparation of Plasma Samples—Blood samples collected from fasted beagle dogs were placed in heparinized tubes and centrifuged at 2300 rpm for 15 min to harvest the clear plasma.

Preparation of Plasma Standards—A series of standards was prepared by spiking 4 ml of clear plasma samples with 5.0, 10.0, 15.0, and 20.0 μg of prednisolone.

Extraction—Four milliliters of the plasma standard was extracted with 25 ml of methylene chloride, and the extract was washed with 2 ml of 0.1 *N* NaOH followed by 2 ml of 0.1 *N* acetic acid and then with 2 ml of distilled water. The extract was evaporated to dryness and redissolved in 200 μl of methanol by agitating on a vortex mixer.

TLC—Glass plates (20 × 20 cm) precoated with microcrystalline cellulose to a thickness of 250 μm were used. The solvent system was toluene–methanol–distilled water (84.8:14.6:0.6).

The chromatographic plate was divided into three equal sections. The left section was used for spotting the prednisolone standard solution in methanol, the right section was used for spotting prednisolone solution extracted from plasma, and the center section was used for the plasma blank. The solutions were applied as streaks 2.5 cm from the bottom of the plate. The solution was dried during application with the aid of a nitrogen stream.

With the described solvent system, the chromatogram was developed in a multiple development tank, previously equilibrated and lined with absorbent paper. The solvent then was allowed to move until it reached 15 cm above the baseline. The plate was removed, dried at room temperature, and visualized under shortwave UV light. The prednisolone standard band and the corresponding bands in the spiked plasma samples and the blank solutions were marked. The R_f value for prednisolone was 0.3. The bands corresponding to this R_f value were removed by scraping the microcrystalline cellulose onto a glazed weighing paper, and the scrapings were pulverized and transferred to 25-ml centrifuge tubes. Methanol (10 ml) was added, and the samples were agitated on a vortex mixer for 5 min and centrifuged at 2300 rpm for 5 min.

The clear methanol layers were decanted, and the residue was treated similarly with 5 ml of methanol three times. The pooled methanolic extracts then were evaporated to a 1-ml volume. These methanolic extracts of prednisolone from plasma were analyzed according to the method described previously. The standard curve of prednisolone in plasma then was constructed by plotting the absorbance values *versus* various prednisolone concentrations.

Table I—Estimation of Prednisolone (20 μg/4 ml) in Plasma [a]

Sample Replicate Number	Estimated, μg/4 ml	Recovery, %
1	19.25	96.25
2	19.50	97.50
3	20.25	101.25
4	21.00	105.00
5	18.25	91.25
6	18.75	93.75
Mean		97.50

[a] Coefficient of variation = (standard deviation × 100)/mean = (1 × 100)/19.50 = 5.13%.

RESULTS AND DISCUSSION

Variation of Absorbance with Prednisolone in Methanolic Phenylhydrazine Acid Solution—With 0.5–50 μg of prednisolone, the absorbance varied linearly with the amount of prednisolone. Under the experimental conditions, the Porter–Silber colorimetric method was sensitive to 1 μg of prednisolone. The regression equation obtained was $y = 0.023x + 0.0062$, and the correlation coefficient was 0.9998.

Standard Curve of Prednisolone in Plasma—Plots of absorbance *versus* 5–20 μg of prednisolone/4 ml of plasma were linear, passing through the origin with a slope of 0.023. The extraction caused a prednisolone loss of ~10%. The regression equation obtained was $y = 0.023x + 0.0068$, and the correlation coefficient was 0.9997.

Method Accuracy and Precision—Table I lists the results obtained from the analysis of a number of spiked plasma samples (20 μg/4 ml). These data reflect the accuracy and precision of the method. The mean recovery and the coefficient of variation were 97.5 and 5.13%, respectively.

In summary, TLC separation of microgram quantities of prednisolone extracted from dog plasma were assayed efficiently by a simple colorimetric procedure. This procedure should be considered when more sophisticated equipment or procedures are not available.

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