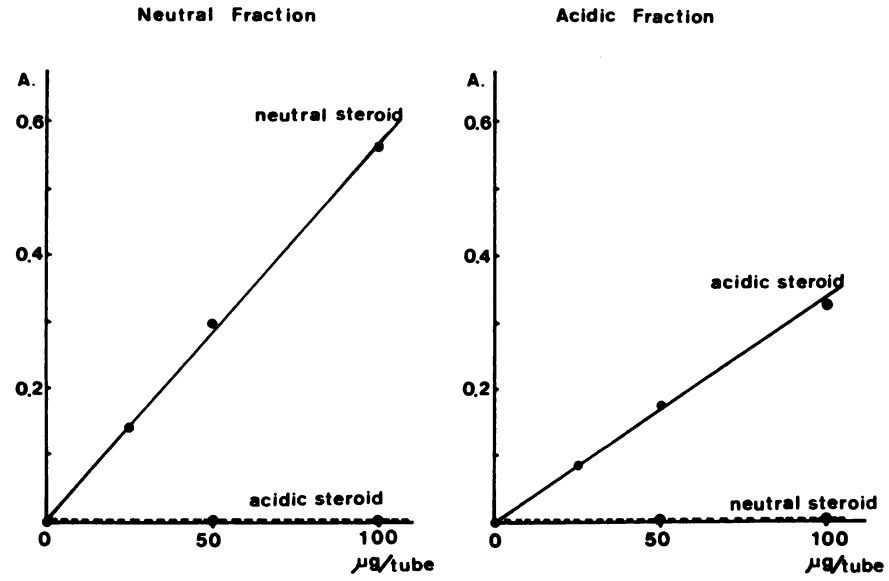

Fig. 1. Standard curves for acidic fraction and neutral fraction
Cholic acid was used as the acidic steroid, cortol as the neutral steroid. Absorbance (*A*) was measured at 500 nm

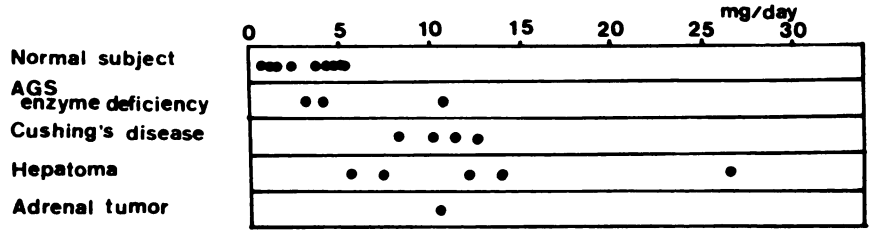

Fig. 2. Excretion of urinary acidic 3α-hydroxysteroids by normal subjects and some patients
Values (mg/day) are expressed in terms of cholic acid (standard)

fuging, discard the urine layer and keep 10 mL of the ethyl acetate layer for another 24 h at 37 °C to assure complete solvolysis. Wash the ethyl acetate with 5 mL of concentrated sodium carbonate. After centrifugal separation, wash 4 mL of the alkaline layer with 10 mL of ethyl acetate and discard the ethyl acetate layer. Acidify the alkali layer to pH 1 and saturate with sodium chloride. Shake the solution with 15 mL of ethyl acetate for 5 min, centrifuge, transfer 10 mL of the ethyl acetate layer to a tube, and evaporate the ethyl acetate. Develop the color of the acidic fraction as previously described (*1*).

Add 2 mL of enzyme solution to a tube of extract residue and incubate for 20 min at 37 °C. After incubation, measure the absorbance of the solution at 450, 500, and 550 nm.

Add 50 μL of the standard to 10 mL of water and submit this to the whole procedure as for the sample.

The absorption curve of the dye formed has a maximum at 500 nm. Standard curves for the acidic fraction and neutral fraction are shown in Figure 1. For preparation of standard curves for acidic and neutral steroids, we used cholic acid as the acidic steroid, cortol as the neutral steroid. We saw no effect of neutral steroid on calibration of acidic steroids, or vice versa.

Analytical recovery from water and urine was 95.8% and 93.5%, respectively, for quintuplicate assays of 50-μg additions.

Excretion values for 10 normal subjects were 0 (i.e., not detectable) to 5.5 mg/day. Values for some patients are shown in Figure 2. Acidic 3α-hydroxysteroids can be simply determined by using the enzyme specific for the 3α-hydroxy group. The specificity of the enzyme for neutral steroids has been shown in a previous paper (*1*), but not for acidic steroids. We could not obtain an acidic steroid such as cortolic acid for testing, so we only tested for 5β-androstan-3α-ol-17β-carboxylic acid and cholic acid. For these, the absorbance at 500 nm was 0.480 and 0.310, respectively, with 20 μg per tube. We have tested few patients but are encouraged to believe that such results would be of diagnostic value. This enzymic method for urinary acidic 3α-hydroxysteroids is suitable for use as a clinical test.

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Bromide Interferes with Determination of Chloride by Each of Four Methods

To the Editor:

Positive interference by bromide with determination of chloride is well documented for different analytical systems (*1–7*). For serum from several patients receiving medication containing bromide we noted a spurious increase in chloride concentration as determined with the PVA-4 (Photovolt Corp.). We then, with four different instruments, evaluated chloride in serum specimens containing increasing concentrations of added bromide. The four instruments are based on three different principles for chloride determination: ion-selective electrode, PVA-4; continuous flow mercuric thiocyanate colorimetry, SMAC (Technicon) (*8*, *9*); and coulometry, Chloride/Carbon Dioxide Analyzer (Beckman) (*10*) and the CMT 10 Chloride Titrator (Radiometer).

Sodium bromide (AR) was dissolved in 100 mL of triply-distilled water to give a 200 mmol/L solution. We added 0.1 mL of this solution to 1.9 mL of an aliquot of a human serum pool to yield a final bromide concentration of 10 mmol/L. Additional dilutions of the sodium bromide solution with water were used to yield final concentrations of 8 and 6 mmol/L added to serum. Aliquots of these were assayed three to six times for Cl, in ascending and descending order. The entire study was repeated twice. The results were analyzed by linear regression, the regression line being forced through the origin.

The PVA-4 showed the largest positive interference ($p < 0.0001$) by bro-

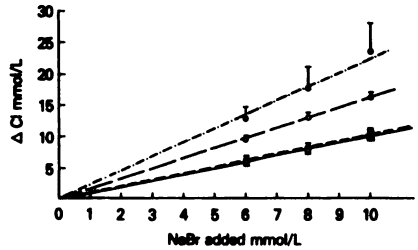

Fig. 1. Linear regression for the recovery of bromide as chloride with four different systems

Lines (*top to bottom*) are for PVA-4, SMAC, CMT10 Chloride Titrator, and Cl/CO_2 Analyzer. Each point represents the mean of three experiments and the bar represents the standard error of the mean

mide in the determination of chloride, with each bromide ion being determined as 2.25 chloride ions (Figure 1). Bromide also caused a significantly greater than one-for-one positive interference (p <0.0001) when determined with the SMAC; each bromide being determined as 1.61 chloride ions (Figure 1). The response of the two coulometric instruments (measured chloride/added bromide) did not differ significantly from 1 (p >0.6).

This is the first report to document a positive bias for chloride in the presence of bromide for the SMAC. The SMAC instrument manual states: "a study has indicated that for each meq/liter of bromide present in the serum sample, an absorbance equal to approximately 1.00 meq/liter of chloride occurs." The method in the reference is different than that used on the SMAC. In the current study, the linear regression slope for the SMAC results was greater than 1 (p <0.0001), indicating a positive bias significantly greater than the one-for-one interference claimed by Technicon.

On the basis of this study, a coulometric method would be the method of choice for determination of total halide in the presence of bromide. The ion-selective chloride electrode of PVA-4 and the colorimetric thiocyanate method of the SMAC both give an apparent chloride concentration that is significantly greater than the actual total halide concentration in sera containing bromide. If a significantly increased chloride concentration is determined with either of these systems, the chloride concentration should be confirmed with a coulometric system. If the repeat measurement with a coulometric system gives a significantly lower chloride result, interference by bromide should be suspected.

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Immunochemical Determination of LD-1 and LD-2 in CK-MB Fraction of Column Eluates

To the Editor:

Previously, I described a column-chromatographic procedure for simultaneous separation of CK-MB, LD-1, and LD-2 isoenzymes (*1*). CK-MB was measured free from CK isoenzymes MM and BB, but LD isoenzymes 1 and 2, although free from LD-3, -4, and -5, were measured in combination (LD-1 and LD-2). A modified LD column system (*2*) was developed to specifically separate LD-1 from LD-2 for the purpose of calculating LD-1/2 ratios. LD-1/2 ratios are a more specific indicator of myocardial infarction than total LD-1 and LD-2 measurements (*2*, *3*). Unfortunately, the LD-1/2 column procedure was not optimal for MB measurement, and two separate column systems were required to provide both CK-MB and LD-1/2 ratios for monitoring suspected cases of myocardial infarction. Here, I describe a single-column system, which, when used in combination with an immunochemical LD assay, results in simultaneous CK-MB and LD-1/2 measurement.

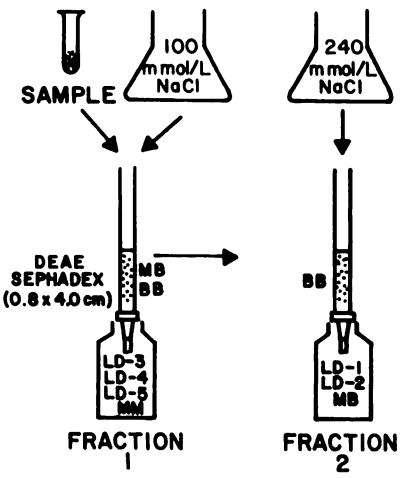

Fig. 1. Diagrammatic representation of the ion-exchange chromatographic procedure for separation of CK-MB, LD-1, and LD-2 isoenzymes

The column-chromatographic process is diagrammatically represented in Figure 1. The procedure: Apply 0.50 mL of serum to an 8 × 150 mm glass column (Roche Diagnostics, Hoffmann-La Roche, Nutley, NJ 07110) filled to a height of 4 cm with DEAE-Sephadex A-50, prepared as previously described (*1*). Collect the effluent in the first vial (Fraction 1), then elute stepwise with tris(hydroxymethyl)methylamine hydrochloride (50 mmol/L) containing, successively, 100 and 240 mmol of sodium chloride per liter (pH 8.0 at 25 °C). Collect two 4-mL fractions of the elute from the 100 mmol/L sodium chloride buffer in the first vial (Fraction 1). Electrophoretic analysis of Fraction 1 will show the presence of CK-MM, LD-5, LD-4, and LD-3 isoenzymes. Collect one 4-mL fraction of eluate from the 240 mmol/L sodium chloride buffer in the second vial (Fraction 2). This fraction contains CK-MB, LD-1, and LD-2 isoenzymes.

Separate LD-1 from LD-2 in Fraction 2 eluate with immunochemical reagents from the Isomune-LD kit (Roche Diagnostics). Add 50 μL of goat antiserum to the LD-M subunit to 200 μL of Fraction 2 and incubate for 5 min at room temperature. Immunochemical reaction occurs between LD-M subunit antibodies and the M-subunit of LD-2 but not with LD-1, which lacks M-subunit (*4*). Now add insoluble second antibody, 200 μL of donkey anti-goat gamma globulin conjugated to polyvinylidene fluoride floccules, and incubate at room temperature for 5 min. Centrifuge (1000 × g, 5 min) and remove the insoluble complex between polymer second antibody and LD-2 first antibody. Electrophoretic analysis of Fraction 2 supernate