lytical error and chemical variation (for example, some of the calcium may be precipitated as oxalate).

Discussion

The Ca/P ratio of the sediment is surprisingly inconsistent. The wide range makes the average (1.48) almost meaningless, although it is intriguingly close to the corresponding figure, 1.5, for synthetic tricalcium phosphate [as Cifuentes and Esteban (7) discuss].

It would be interesting to have such data to compare for urines from hyperparathyroid patients, idiopathic hypercalciurics, and patients who are chronic phosphatic stone-formers.

Such information might be useful in helping to estimate the chances for stone formation. In a certain subgroup of calculous-disease patients (those who have frequent and severe incidents of crystalluria) one may conjecture that stone formation is a "statistical" process in the following sense: Every incident of crystalluria may involve a proportion of crystals large enough, or aggregated enough, to be retained in a tubule. Of these, a certain proportion is not washed out when intra-tubular back-pressure builds up and (or) a more dilute urine is produced. Of these "microcalculi," a certain proportion occurs in persons whose urine composition (concentration) is such that these rudimentary stones are exposed to a milieu that facilitates their enlargement to clinically important size.

Reference


Murayama Test for Hemoglobin S: Simplification in Technique


An improved technique is reported for the Murayama test, which is a simple and specific test for S hemoglobin [CLIN. CHEM. 16, 945 (1970)]; moisturized N₂ is used optimally and several steps are eliminated in the part of the test in which concentrated hemolysates are prepared by use of a dense sucrose solution. Each test, which took 1 h, can now be done in 20 min, with equal reliability and accuracy.

The Murayama test, a new specific test for hemoglobin S based on the molecular mechanism of sickling for S hemoglobin, as proposed by Murayama, has been previously reported in this journal (1). The test is a simple visual method for the detection of implicated hydrophobic bonds within deoxygenated S hemoglobin hemolysates. Murayama has published his original conception of the molecular events involved in sickling of S hemoglobin (2, 3), but has recently provided a modified hypothesis (1, 4).

This note reports a significant simplification of the technique of the Murayama test, which makes it possible to perform the test in one-third the time with equal reliability and accuracy.

The reliability of the method has been tested by each of the above three reporting institutions with use of blood specimens proven to be hemoglobin S by (a) the 2% sodium metabisulfite test; (b) hemoglobin electrophoresis at pH 8.4; and (c) improved Murayama test. The 13 control specimens (hemoglobin A) were uniformly negative in all tests. Test specimens (hemoglobin S) numbered 45.

This modification of the technique of Murayama test has two advantageous results: the method is simplified because several steps are eliminated and the performance time per test is decreased from 1 h to 20 min. This technical improvement is achieved principally by preparing the concentrated hemolysates by a variation of the method of Scott (5). Another improvement is related to the optional use of moistened nitrogen instead of solid carbon dioxide for deoxygenation of the hemolysate as a matter of increased convenience to some laboratories.

Method

A. Preparation of the Hemolysate

2. Dilute the blood with an equal volume of saline solution (10 g/liter). Mix.

CLINICAL CHEMISTRY, Vol. 17, No. 10, 1971 1059
3. Place 5 ml of cold (3° to 5°C) sucrose solution (300 g diluted to 1 liter with Tris buffer, 0.01 mol/liter) in a 12-ml centrifuge tube.

4. Layer 5 ml of the diluted blood on top of the sucrose solution. Centrifuge for 10 min at 2,300 g.

5. Decant the sucrose–plasma layers. Rinse the sides of the tube with a small amount of distilled water to remove all the sucrose, and decant the water.

6. Add a quantity of distilled water equivalent to one-fourth the volume of packed erythrocytes. Mix well by inversion by hand.

7. Add one drop of toluene (to dissolve the erythrocyte stromata) and mix gently by inversion by hand.

8. Centrifuge at 2,300 g for 10 min. Decant the supernatant fluid. The concentrated hemolysate must now be deoxygenated, by using solid CO₂ as published (I), or by the following technique.

B. Deoxygenation

1. Pierce a rubber stopper (to fit a 10 × 75 mm tube) with a 20-gauge and with a 22-gauge needle (do not use a larger tube).

2. Prepare a gas moisturizing bottle. Connect the inlet of the bottle to a tank of pure N₂ (with gauges to reduce the flow to 2 or 2.5 liters per min). Connect the outlet to the 20-gauge needle. The N₂ must be moisturized (bubble through water with a gas deflector) to prevent drying of the hemolysate.

3. Insert the rubber stopper, with its needles, into a 10 × 75 mm test tube containing 0.5 ml of the hemolysate from the last step of the hemolysate preparation.

4. Place the tube in a water bath at 37°C. Gently tilt the tube from time to time. (Do not allow bubbles to form; the N₂ needs only to pass over the surface of the hemolysate, not through it.) Observe the hemolysate for 10 min before concluding that the test is negative. Most S hemoglobin systems give a gel within 5 min. When a gel forms, the tube should be inverted to demonstrate the fact (I).

5. If a gel forms, place the tube into an ice water bath. Tilt the tube gently to observe if the gel becomes a liquid at 0°C. Avoid bubble formation. The gel–sol transformation at 0°C and 37°C should be repeated several times to demonstrate the negative temperature coefficient of gelation. Do not disconnect the N₂ or CO₂ tubing in these deoxygenating steps at any time.

The hemoglobin concentration of the hemolysate must be 17 g/100 ml or greater. This is important. It is advisable to do a hemoglobin determination on the hemolysate.

If a larger volume of hemolysate is desired, the erythrocyte and water mixture (step A-6) may be frozen to obtain complete lysis. Such freezing does not affect the results of the Murayama test.

The concentration of the sucrose solution must be as described, and it must be used while still cold to achieve good separation of plasma and erythrocytes.

The interpretation of the Murayama test, discussed previously (I, 4), remains unchanged.

Results

Data are given in Table 1.

Supported in part by the International Sickle Cell Anemia Foundation and the Michigan Heart Association.

References


3. Murayama, M., Structure of sickle cell hemoglobin and molecular mechanism of the sickling phenomenon. CLIN. CHEM. 13, 578 (1967).
