easy adjust the decision limits for each laboratory procedure, or to only provide a report with flagged protein abnormalities, the present program would be more useful. It should also be able to provide further information, such as "possible secondary clinical causes for the protein abnormality."

Such prompting through computer reports has aided physicians in the screening for other possible causative diseases (3). With the present software available as an accessory for the Hele-nan Densitometer to interpret serum protein electrophoretic patterns, the small advantage gained in the reduction of human interpretative errors is offset by the limitations of the software program.

References

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Interference by Intravenous Fluorescein with Drug Assays in the Abbott TDx

To the Editor:

A 64-year-old black woman with a chronic tachyrhythmia being treated with digoxin was admitted to our hospital complaining of abdominal pain. On the second hospital day she underwent a bowel resection. About 5 h postoperatively, blood was sampled for digoxin assay. The specimen was analyzed in the "TDx" (Abbott Laboratories, Diagnostics Division, Irving, TX 75061) according to the recommended protocol. The message "Net I Small" was printed in lieu of a concentration value on repeated analysis. Further investigation revealed that the specimen had a strong green-yellow fluorescence under long-wavelength ultraviolet light. Subsequent clinical follow-up revealed that the patient had received 1 g of sodium fluorescein intravenously during the operation, to allow blood flow to the bowel to be made visible. Because fluorescein is used as the tracer in this fluorescence polarization method, we attempted to confirm the presence of fluorescein in the patient's serum and the duration of interference with the digoxin and other TDx assays. Interference with assay of digoxin could be expected to be greatest, because it involves the largest sample of any of the tests run on the TDx. Scanning fluorometry of the patient's diluted serum gave excitation and emission maxima identical to a standard solution of fluorescein.

From results for a timed series of specimens already available in the laboratory we calculated an elimination half-life of 3.8 h for this patient. Sodium fluorescein is primarily excreted unchanged in the urine. This patient was demonstrated to have uncompromised renal function. We attempted to determine from the same serial specimens when a test specimen could validly be collected, and found it to be about 64 h (17 half-lives after the injection) for digoxin (sample size, 160 μL). Tests with smaller sample sizes would be expected to experience proportionately less interference. This was confirmed for theophylline, 16 h or four half-lives (sample size, 1.8 μL), and gentamicin, 10 h or 2.5 half-lives (sample size, 1.0 μL).

Accordingly, we recommend that laboratories using the Abbott TDx be aware, and advise their surgical staffs, of the consequences to therapeutic drug monitoring tests of the use of intravenous sodium fluorescein in operative procedures. For example, it may be practicable to minimize the dosage of fluorescein, so as to shorten the time during which specimens are unsuitable for analysis by TDx and to provide for alternative methods of analysis during this period.

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Enzymic Determination of Total Cholesterol in Skin Tissue

To the Editor:

Various methods have been described for the determination of total cholesterol in tissues, including colorimetry (1, 2), fluorometry (3), gas chromatography (4), and enzymic techniques (5), the most rapid and least expensive. In recent years, the enzymic method has been often modified (6–9). Our purpose is to describe an enzymic technique for measuring cholesterol in skin tissue. This technique is rapid, specific, and quite simple and can be performed without any expensive instrument or corrosive reagents. It can be used routinely to determine cholesterol even in very small skin samples (20 mg) obtained from punch biopsy, thereby presenting a very efficient way to evaluate the risk of vascular disease (9).

This enzymic technique was applied to skin biopsies from 130 corpses (ages at death, 24 to 87 years). Three punch biopsies from the same area were taken from each body, not later than 12 h after death. After dissection with alcohol (90%) and contact anesthesia with dichlorotetrafluoroethane (to simulate the condition of sampling in patients), 4-mm-diameter samples of skin were obtained with an Arouette's trocar in the deltoid area. With the trocar driven perpendicular to the skin surface, epidermis, dermis, and hypodermis were included in the biopsy produkt. After removal of adipose tissue, the samples were immediately processed or frozen at −80 °C.

The lipids were extracted from the skin tissues as follows: wash each punch biopsy in isotonic saline solution, dry, and weigh (fresh weight); put the skin biopsy in a test tube with 3 mL of chloroform/methanol (7/11 by vol) (10), stirrings with a magnetic stirrer for 3 h. Evaporate the extract in a rotary evaporator, then re dissolve the residue in 3 mL of chloroform and 1 mL of petroleum spirit (this second extraction lasted 1 h). Filter, wash the skin tissue with pure chloroform, and weigh the skin fragment (dry weight). Add 10 μL of Triton X-100 to 100 μL of the extract (7) and let the solution react for 10 min. After evaporating the solvent under nitrogen, determine the cholesterol in the extract.

For enzymic determination of cholesterol, mix each sample with 2 mL of cholesterol reagent (cholesterol esterase, cholesterol oxidase, peroxidase, and aminopyrine, from Boehringer Mannheim, F.R.G.). Incubate the samples and appropriate blanks for 15 min at 37 °C; read the absorbance at 546 nm vs a reagent blank.

Three 20-μL serum samples used as controls to study the precision, linearity, and accuracy of the procedure did not contain any preservatives, which could interfere in the chemical process of the analysis. They were processed the same way as the skin tissues and demonstrated that the organic solvents did not inhibit the enzymic reactions.

For 120 corpuses included in our study, the coefficient of variation (CV) of the three biopsy samples was always less than 10% (mean, 6.79%). The results of nine assays of skin extracts by both the enzymic (x) and the colorimetric (Y) methods (y) correlated well: y = 1.663 x − 1.009 μmol/g (r = 0.992, p < 0.0001).