Hospital, the mean urinary excretions (μg/24 h) as measured by this method were 34 for norepinephrine (range 9–84), 4 for epinephrine (range 0–24), and 252 for dopamine (range 74–500), in good agreement with the results of Moyer et al. (1) for normal and hypertensive subjects.

Isolation of the amines with the ion-exchange columns takes about an hour; the chromatography requires 6 min per sample. All components of the assay procedure are commercially available without any special preparation, which makes it particularly suitable for use as a routine screening procedure.

References

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Blood/Plasma Distribution Ratios of Psychotropic Drugs

To the Editor:
Many studies evaluating the relationship between concentrations in plasma and the therapeutic effect of the tricyclic antidepressants in affective illness have been done during the past seven years. Not all have led to the same conclusions; in some a curvilinear or linear relationship is described, in others no relationship at all (1). One explanation that has been put forward for the lack of a strong correlation between clinical effect and the concurrent concentration in plasma has been interindividual variations in tissue binding of drug, and it has been proposed that drug concentrations in a tissue compartment might more accurately reflect drug concentrations in the central nervous system than those in plasma (2). Using the erythrocyte, a readily available tissue, Linnoila et al. (2) have shown marked interindividual variations in steady-state concentrations of tricyclic antidepressants. They have also found that some of the drugs (e.g., amitriptyline) had higher concentrations in plasma than in erythrocytes, while for others (e.g., nortriptyline) the reverse was true.

To investigate this matter further, we have looked at the “in vitro” distribution between erythrocytes and plasma of six psychotropic drugs: amitriptyline, nortriptyline, imipramine, maprotiline, nomifensine, and diazepam.

Blood (20 mL) was taken by venipuncture from six drug-free volunteers into polystyrene blood-sampling tubes containing either lithium heparin (150 int. units/10 mL) or di-potassium ethylenediaminetetraacetate (EDTA, 15 mg/10 mL).

One-milliliter aliquots of blood were then incubated for 30 min at 37 °C with 100 µL of drug solution in an air/CO2 atmosphere, in capped tubes. Previous experiments had shown that equilibrium between drug in plasma and erythrocytes was attained under these conditions, and that the pH did not change during the experiment.

The drugs in the solutions were radioactively labeled drug—[3H]nortriptyline, [3H]imipramine, [14C]amitriptyline, [14C]maprotiline, [3H]nomifensine, and [3H]diazepam—plus unlabeled drug where necessary to obtain concentrations similar to those observed in patients on therapeutic doses of the drugs. All radiolabeled drugs were purified before use by thin-layer chromatography, then dissolved in isotonic saline, pH 7.4.

After incubation, the plasma was separated by centrifugation and a 100-µL aliquot taken for determination of radioactivity by liquid scintillation spectrometry. Neither the small volume of plasma nor the antidepressants caused any quenching of the samples. Binding of the radiolabeled drugs to the incubation tubes was negligible.

The amount of radioactivity found was corrected to give the amount per milliliter of plasma and divided into the amount originally added per milliliter of whole blood to give the blood/plasma distribution ratio. The precision (CV) of the assay, calculated by using blood from one volunteer and one drug solution, was 7%.

Table 1 shows the ratios obtained for each of the six drugs.

The first conclusion to be drawn from this study is that the two antidepressants resulted in a significantly different distribution of the tricyclic antidepressants—amitriptyline, imipramine, and maprotiline—between plasma and erythrocytes. Nortriptyline values were also altered, but insignificantly (p > 0.06). Differences attributable to the two antidepressants were small but could be important if low concentrations are to be measured—for example, in pharmacokinetic studies involving single oral doses. Use of only one antidepressant within a study would lessen within-individual variation in values for plasma.

The second point of interest is that some of the drugs are concentrated into erythrocytes, as shown by a blood/plasma ratio exceeding 1; nortriptyline and maprotiline show this, and nomifensine to a lesser extent. Amitriptyline and imipramine are more or less equally distributed between erythrocytes and plasma, while diazepam seems to be largely excluded by the erythrocytes.

Inter-individual variations in the blood/plasma ratio for all these drugs were observed, but smaller than those seen by Linnoila et al. (2).

The present study, carried out “in vitro,” may not reflect quantitatively the situation “in vivo.” However, it serves to point out that common practices such as the use of different antidepressants should not be taken for granted. The decision as to whether concentrations in whole blood, erythrocytes, or plasma should be measured also requires much more investigation and should be evaluated for individual drugs.

References
Immunoglobulin G in Cerebrospinal Fluid

To the Editor:

In a recent paper, Bouloukos et al. (1) dispute the frequently confirmed observation that the relative IgG content is significantly increased in many patients with multiple sclerosis (MS).

Interpretation of results of immunoglobulin determinations in cerebrospinal fluid (CSF) should be based on knowledge of CSF physiology and the pathology of neurological diseases. Tourtelotte and colleagues (2) extensively reviewed the relevant insights into CSF physiology and the pathology of MS.

Briefly: (a) It is generally accepted that an above-normal protein concentration of the CSF is an expression of an impaired blood-CSF barrier for proteins. The more the function of this barrier is impaired, the more extensive will be the transudation of the various plasma proteins, including IgG. (b) There are many indications that in some neurological diseases, including MS, IgG may be synthesized extravascularly in the central nervous system.

Reporting results for IgG in CSF as the fraction of the total protein concentration that is IgG is logically justified in the light of the foregoing. The quotient will normalize increases of CSF IgG owing to abnormal transudation and signal an extravascular source of IgG.

Contrary to point b, Bouloukos et al. believe that the increase of IgG in CSF in multiple sclerosis and presumably in other diseases is exclusively connected with increases in CSF total protein concentration. If they were right, the logical conclusion would be that the measurement of IgG in CSF should be abandoned because it cannot offer more information than determination of total protein concentration alone—a conclusion they fail to draw.

Moreover, the data they present can hardly be seen as supporting their view.

The standard deviations of the means they give in their Table 1 are in most instances greater than half of the mean values, and are nearly equal to the mean in some cases. This signifies that those mean values cannot be used as central values for the distributions and that the standard deviations of those means are meaningless figures. Statistical calculations based on these figures are insufficient evidence to change the accepted views on the interpretation of the results of IgG determinations in the CSF.

References

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Manual Ferrozine Method for Serum Iron and Total Iron-Binding Capacity Adapted to a Computer-Directed Analyzer, the Gilford 3500

To the Editor:

Recently we adapted the Ferro-Chek II (Hyland Diagnostics, Division of Travenol Laboratories, Deerfield, IL 60015) manual colorimetric kit for serum iron and total iron-binding capacity (TIBC) for use with a computer-directed analyzer (Gilford 3500; Gilford Instrument Laboratories, Oberlin, OH 44074). This communication describes its main features.

We used Hyland Diagnostics kit reagents without modification. This kit is based on the modification by Nakamura et al. (1) of Webster's (2) procedure. An anionic detergent prevents precipitation of serum proteins, and ferrozine is the colorimetric complexing reagent (3). Serum and reagent volumes were reduced by 70% so that the final volume was 0.55 mL as compared to 3.1 mL for the manual method. Serum, standards, controls, and the color reagent were pipetted with Oxford pipets (Oxford Laboratories, Foster City, CA 94404) and all other reagents were pipetted with an automatic Pipettor/Diluter (Beckman Instruments, Fullerton, CA 92634) in the continuous mode.

A calibration curve was made by using 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 mg/L iron standards (New England Research Laboratories, East Providence, RI 02914). After the color developed the reaction mixtures for these standards were poured onto the reaction strips and their absorbances were measured with the Gilford 3500 at 560 nm with use of an End Point 1, Mode 3 computer card (Gilford Instrument Laboratories).

All the standards were assayed in triplicate for five consecutive days. The calibration curve was linear and a factor was calculated by dividing the concentration of the standard by its average absorbance for 15 readings. The average of these calibration factors for different standards, 557, was used in the assay of iron and TIBC with the Gilford 3500 and another computer card. The flow chart for the computer-directed analysis and calculations can be obtained from one of us (Z.U.). The computer card is made by Gilford Instrument Laboratories. Serum and controls were pipetted into iron-free plastic tubes labeled as iron blank, iron test, TIBC blank, and TIBC test, followed by the appropriate reagents. TIBC reagents were pipetted first, to save time, because saturation of transferrin takes 20 min. Into all the tubes labeled as iron and TIBC blank, 0.05 mL of de-ionized water was pipetted, to correct for the 3% dilution caused by the addition of the ferrozine color reagent. This dilution correction is significant when the TIBC is low and the serum is turbid. The first rack on the analyzer was assigned for water blanks in duplicate (cups 1 and 2) and iron standards (cups 3 and 4), to verify the factor used in the calculations. All other racks represent a separate serum or control. After the color developed the solutions were poured onto the reaction strips, which were then placed on the racks. The computer reads the rack number and the absorbance of the solution in cups 1–4 (iron blank, iron test, TIBC blank, and TIBC test, respectively) and does all the calculations. The printout gives the absorbance of the solution in cups 1–4, iron and TIBC concentration, and percent saturation for each specimen.

Reproducibility data on the Great Lakes Quality Assurance Program Level I and II pooled sera (Michigan, Ohio, and Indiana Societies of Pathologists) and control sera (Validate and Validate-A; General Diagnostics, Morris Plains, NJ 07980) indicated 0.3–1.5% lower CVs for the iron and 0.7–2.2% lower CVs for the TIBC for the manual method for these controls. The reference interval for serum iron, as estimated by the manual method at our hospital (137 apparently healthy individuals, 52% male, ages 19–68 years) was

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