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Note

Determination of free and bound plasma tryptophan

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In a preceding paper[1], it was demonstrated that the treatment of a plasma sample with sodium dodecyl sulphate (SDS) solution before deproteinization with sulphosalicylic acid (SSA) is essential for complete recovery of plasma tryptophan.

More recently, it was also observed in our laboratories that the tryptophan molecule can be adsorbed by a dextran-coated charcoal (DCC) suspension, which is routinely used in radioimmunoassay techniques. Consequently, the determination of the tryptophan fraction bound to albumin (and hence the free tryptophan) can be achieved as follows. To the plasma sample is added a DCC suspension, which adsorbs the free fraction, and then the remaining bound tryptophan is determined after treatment of the centrifuge supernatant solution with SDS solution, followed by deproteinization with SSA. Concurrently, on another aliquot of the same sample, the total tryptophan is determined as previously described[1]. By subtracting from this value the amount of the bound fraction, the concentration of the free tryptophan, which previously has usually been measured by dialysis[2,3] or ultrafiltration[4], can be obtained.

EXPERIMENTAL AND RESULTS

Apparatus and materials

An apparatus for automatic amino acid analysis described by Mondino[5], and manufactured by Optica (Milan, Italy) was used. The flow-rate settings, columns and resin used, photometer and recorder setting, lithium buffer composition, ninhydrin colour reagent and amino acid calibrating solution were as described previously[6].

Tryptophan solutions. L-Tryptophan (Fluka, Buchs, Switzerland) solutions were prepared in pH 7.4 (0.05 *M*) phosphate buffer, containing 100, 150, 200 and 250 μmoles/l of amino acid.

SDS solution. A freshly prepared 1% solution of SDS in distilled water was employed.

SAA solution. A 5% solution of SAA in distilled water was used.

DCC suspension. Amounts of 0.5 g of charcoal (Norit A; Sigma, St. Louis, Mo., U.S.A.) and 0.05 g of Dextran 20 (Pharmacia, Uppsala, Sweden) were suspended in 100 ml of pH 7.4 Tris (10 m*M*)–EDTA (1 m*M*) buffer. This suspension was stirred for 18 h at 4° with a magnetic agitator before use. The suspension was kept at 4°.

Plasma samples. Human, rat and dog (beagle) blood samples were drawn into test-tubes containing EDTA. After centrifuging at 2000 *g* for 10 min, the plasma was separated and immediately analyzed.

Separation of free from bound tryptophan

Directly in a centrifuge test-tube, 1 ml of plasma or tryptophan solution was treated in an ice–water bath with 0.5 ml of the DCC suspension and shaken for 15 min. The mixture was then centrifuged at 3000 *g* for 5 min. A 1.2-ml portion of the supernatant solution was treated with 0.1 ml of 1% SDS solution at room temperature for 10 min and then with 0.4 ml of 5% sulphosalicylic acid solution. The mixture was centrifuged at 3000 *g* for 10 min and a 0.5-ml portion of the supernatant solution was loaded on to the column.

Determination of total tryptophan in plasma

Directly in a centrifuge test-tube, 1 ml of plasma was treated with 0.5 ml of the above buffer solution. After shaking, a 1.2-ml portion of the mixture was treated with 0.1 ml of 1% SDS solution and shaken for 10 min at room temperature. Then, 0.4 ml of 5% SSA solution was added; after shaking, the mixture was centrifuged at 3000 *g* for 10 min and a 0.5-ml portion of the supernatant solution was loaded on to the column.

Calculation

As previously reported[7], the tryptophan concentration was calculated by measuring the height of the peaks in millimetres above the base-line on the chromatograms. The following equation gives the amount of free tryptophan in micromoles per litre of plasma:

$$\text{Free tryptophan } (\mu\text{moles/l}) = \frac{C\,(H_t - H_b)}{H_c}$$

where H_t is the height of the total tryptophan peak, H_b is the height of the bound fraction peak, H_c is the height of the peak obtained by loading 0.5 ml of calibrating solution and C is the concentration of the calibrating solution expressed in μmoles/l.

By treating 1 ml of the 100 μmole/l tryptophan solution with 0.5 ml of DCC suspension, as indicated above, for different times (5, 10, 15, 20 and 30 min), it was found that after 15 min the tryptophan has been completely adsorbed. The same trial was performed on 150, 200 and 250 μmole/l tryptophan solutions. Only in the 250 μmole/l solution was there found to be a residue of the amino acid, to the extent of about 5%; in the others, no tryptophan was found. Consequently, it can be stated that the adsorptive capacity of the DCC suspension, when employed as indicated, is completely adequate for the separation of the free tryptophan fraction present in plasma.

Similarly, human, dog (beagle) and rat plasma samples were treated for different times (5, 10, 15, 20, 25, 30 and 40 min). After treatment for 15 min, the adsorption of free tryptophan was virtually constant. It can be assumed that under these conditions the DCC suspension is incapable of displacing bound tryptophan from albumin.

The results of both the above experiments are shown in Fig. 1. Ten determi-

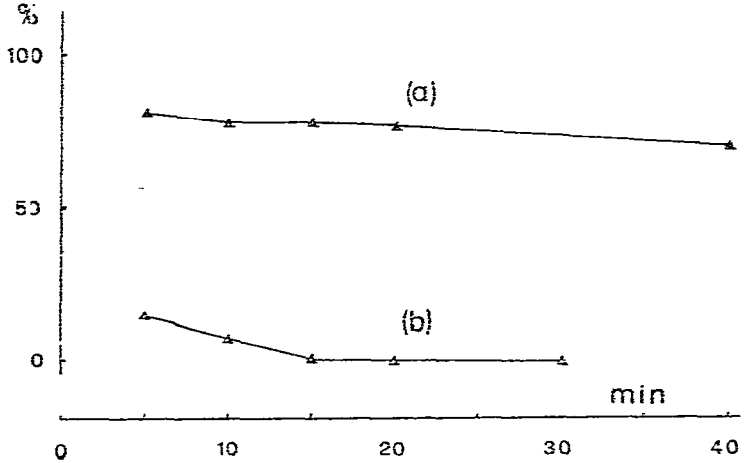

Fig. 1. Effect of time of treatment with DCC suspension on (a) a human plasma sample and (b) a tryptophan solution containing 100 μmol/l. The tryptophan values (ordinate) are expressed as a percentage of the total tryptophan. The values reported for the plasma sample represent the albumin-bound fraction.

nations were performed on the same dog(beagle) plasma sample. The mean result obtained for the bound fraction, which is the one that is actually determined, was 76.4 $\pm$ 1.81 μmole/l (standard deviation).

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