# A modified competitive protein binding method for measuring plasma progesterone levels

Neill, Johansson & others (1967) described a technique for measuring progesterone in plasma based on the competitive protein binding analysis of Murphy (1964). Tritiated corticosterone is bound to corticosteroid binding globulin (CBG) of dog plasma and subsequently displaced by the addition of progesterone extracted from plasma and separated by thin-layer chromatography. The corticosterone remaining bound to the CBG is counted and the reaction quantitated by comparing these counts with those on a standard curve produced by adding known amounts of progesterone to the CBG-tritiated corticosterone system. The values obtained were corrected for procedural losses by measuring the recovery of tritiated progesterone from plasma subjected to identical extraction and chromatographic procedures—a "parallel standard".

Neill & others (1967) obtained a mean recovery of 75·3%. However, we have found that in some instances there is variation in recovery, even between aliquots of the same plasma sample run on one chromatoplate. An internal standard of tritiated progesterone has therefore been incorporated in each plasma sample and the procedural losses subsequently measured for each sample rather than using a mean value for all the samples on one chromatoplate. The amount of tritiated progesterone added is insufficient to interfere significantly with the subsequent counts of the tritiated corticosterone.

Aliquots (0·1 ml) of a solution containing 2000 d/min tritiated progesterone in ethanol is added to each of 1·5 × 15 cm test tubes and evaporated to dryness under nitrogen on a water bath at 45°. Similar aliquots are added to each of two counting bottles to measure the counts added. Plasma is added to the tubes and after 10 min at room temperature, the progesterone is extracted and isolated as described by Neill & others (1967). Progesterone is eluted from the chromatoplate with methanol and the volume made up to 3·0 ml. One ml is transferred to a counting bottle and hence the recovery of progesterone after extraction and chromatography is measured. The remaining 2 ml is evaporated to dryness and thereafter the procedure of Neill & others (1967) followed.

Table 1 shows plasma progesterone levels, measured in duplicate, in 6 goats at various stages of pregnancy. On one chromatoplate the recoveries are fairly uniform,

Table 1. *Plasma progesterone levels (duplicate samples) before and after correction for procedural losses.*

| | Sample | | Recovery % | Progesterone (ng) Before correction | After correction |
|---|---|---|---|---|---|
| Chromatoplate 1 | G | A | 70·9 | 0·6 | 0·8 |
| | | B | 78·1 | 0·8 | 1·0 |
| | F | A | 78·2 | 3·3 | 4·2 |
| | | B | 83·6 | 3·2 | 3·8 |
| | M | A | 79·1 | 7·3 | 9·2 |
| | | B | 73·7 | 6·9 | 9·4 |
| Chromatoplate 2 | 138 | A | 69·5 | 2·4 | 3·5 |
| | | B | 21·6 | 0·7 | 3·2 |
| | 114 | A | 64·8 | 1·1 | 1·7 |
| | | B | 65·1 | 1·3 | 2·0 |
| | 940 | A | 39·9 | 2·4 | 6·0 |
| | | B | 73·1 | 5·7 | 5·2 |

which is the assumption made in the original method. In the second case however, the recoveries are variable, but the use of individual internal standards to correct for procedural losses brings the corrected duplicate values closer.

Progesterone was added to male goat plasma, devoid of any measurable endogenous activity, to give concentrations of 5·0 ng/0·5 ml and 2·4 ng/0·5 ml. When these were assayed the results obtained were 5·3 $\pm$ 0·44 (s.d.) with a coefficient of variation of 7·8% (n = 25). In the second case the mean was 2·4 $\pm$ 0·18 (s.d.) with a coefficient of variation of 7·6% (n = 15).

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# The effect of anti-inflammatory drugs on the protein-binding of [1,2-$^3$H] cortisol in human plasma *in vitro*

We have shown that protein-binding of endogenous 11-hydroxysteroids measured by a fluorimetric technique in the plasma of rheumatoid arthritic patients is unaffected by the administration of aspirin, indomethacin and phenylbutazone (Stenlake, Davidson & others, 1968). Similar results were obtained in *in vitro* studies for indomethacin, ibufenac and phenylbutazone at plasma concentrations four times the therapeutic level and with supra-normal levels of 11-hydroxysteroids. Under the same conditions, however, aspirin increased and oxyphenbutazone decreased the concentration of unbound 11-hydroxysteroids (Stenlake, Williams & others, 1969). Recent work, however, has shown that in a group of normal subjects non-specific fluorogens consisting of free and esterified cholesterol average 3·4 $\mu$g of apparent cortistol/100 ml, equivalent to 22·4% of the total fluorogen present (Stenlake, Davidson & others, 1970). In order, therefore, to confirm our earlier findings, we have studied the effect of anti-inflammatory drugs on the protein-binding of [1,2-$^3$H]cortisol in human plasma.

Plasma pooled from groups of three normal or three rheumatoid arthritic patients, untreated with anti-inflammatory drugs for at least seven days, was added to the dried residue from radiocortisol solutions (1 ml) containing purified [1,2-$^3$H]cortisol (9·7 ng/ml; 2 $\times$ $10^6$ d/min $ml^{-1}$) and non-radioactive cortisol (0·0 or 20 $\mu$g/ml), so that the concentration of added cortisol was equivalent to 0 or 50 $\mu$g/100 ml of plasma. The mixtures were incubated at 37° (30 min), and aspirin (1·25 or 5·0 mg), ibufenac (0·1 or 0·4 mg), indomethacin (25 or 100 $\mu$g), oxyphenbutazone (0·25 or 1·0 mg) or phenylbutazone (0·25 or 1·0 mg) representing therapeutic or four times therapeutic plasma concentrations, was dissolved in separate aliquots (5 ml) of the incubated plasma. The solutions and plasma controls without added drugs were ultra-filtered at 37° for 30 min (Stenlake & others, 1968), and the percentage of unbound radiocortisol determined in each experiment as the mean of three duplicate results.