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 μg 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 μg/ml), so that the concentration of added cortisol was equivalent to 0 or 50 μg/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 μg), 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.

Table 1. *The effect of anti-inflammatory drugs at therapeutic and supra-therapeutic concentrations on the protein-binding of* [1,2-^{3}H]*cortisol in the plasma of rheumatoid arthritic and normal subjects with endogenous and raised cortisol levels.*

Subject[1]	Plasma cortisol[2]	Drug concentration in plasma[3]	Unbound [1,2-^{3}H]cortisol[4] %					
			Control	Aspirin	Ibufenac	Indomethacin	Oxyphenbutazone	Phenylbutazone
RA	E	T	10·9	11·3	10·8	10·8	10·9	11·0
N	E	T	11·9	12·4	12·1	12·0	12·0	11·8
RA	E	4T	12·2	12·5	11·8	12·3	12·5	12·3
N	E	4T	10·9	11·2	11·0	11·1	10·7	11·0
RA	E + 50	4T	24·2	30·6	23·9	23·9	20·0	23·7
N	E + 50	4T	24·3	31·0	24·1	24·1	18·9	24·3

[1] Normal (N); rheumatoid arthritic (RA).
[2] Endogenous (E); endogenous + 50 μg/100 ml (E + 50).
[3] Therapeutic (T); four times therapeutic (4T).
[4] Mean of three duplicate results in each experiment.

The results (Table 1) show that neither therapeutic nor four times therapeutic drug concentrations have a discernible effect on the protein-binding of [1,2-^{3}H]cortisol at normal endogenous levels of 11-hydroxysteroids. However, at cortisol concentrations raised 50 μg/100 ml above endogenous levels, aspirin at four times therapeutic concentrations increased the unbound radiocortisol by 6·4 and 6·8% in rheumatoid arthritic and normal plasma samples respectively. In contrast, oxyphenbutazone at four times therapeutic concentrations caused decreases of 4·2 and 5·3%, whilst the remaining drugs had no effect. These results agree with those obtained by our previous fluorimetric method.

A second similar series of experiments in which endogenous steroids were removed by the method of Heyns, van Baelen & de Moor (1967) and the drugs dissolved in the plasma before the addition of radiocortisol, was designed to test the postulate (Miller, 1965) that anti-inflammatory drugs do not displace cortisol, but instead occupy vacant cortisol-binding sites and so render them unavailable to newly-available cortisol. The results were essentially the same as those in Table 1, showing that protein-bound anti-inflammatory drugs do not interfere with protein-binding of subsequently added [1,2-^{3}H]cortisol to any greater extent than when they are added to plasma already containing [1,2-^{3}H]cortisol.

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