HYDROXYAPATITE AS A SEPARATING AGENT IN THE RADIOIMMUNOASSAY OF STEROIDS

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Dextran-coated charcoal (DCC) has been used extensively to separate free from bound ligands in many radioimmunoassay systems (Abraham, Hopper, Tulchinsky, Swerdloff & Odell, 1971). However, when a large number of samples are assayed, inconsistent results may occur because of stripping of antiserum-bound steroids (Saba, 1976). The present study was undertaken to assess pentacalcium hydroxytriphosphate (hydroxyapatite) as an alternative separating agent for the radioimmunoassay of steroids.

The chemicals used were of reagent grade. Activated charcoal was purchased from Sigma Chemical Company, St Louis, U.S.A., Dextran T40 from Pharmacia Fine Chemicals, Uppsala, Sweden, hydroxyapatite (HAP) from E. Merck, Darmstadt and [^{3}H]oestradiol-17β (sp.act. = 109 Ci/mmol) from The Radiochemical Centre, Amersham, U.K. The anti-oestradiol-17β used was raised in rabbits against 17β-oestradiol-6-(*O*-carboxymethyl)-oxime conjugated to bovine serum albumin. The radioimmunoassay of oestradiol-17β used in this study was previously described by Chew & Ratnam (1976). Only the total binding (Bo) and the non-specific binding (NSB) were evaluated. The assay was carried out in duplicate. Antiserum (100 μl, 1:1500 dilution) and [^{3}H]oestradiol-17β (100 μl, approximately 30000 d.p.m.) were mixed with phosphate-buffered saline (200 μl, pH 7·4) and incubated at 4 °C for 18–24 h. The amounts of HAP and DCC required to separate the free from bound ligands were 35 and 1 mg/tube respectively. After the addition of HAP or DCC, the tubes were vortex mixed. One set was then incubated at 29 °C and another at 4 °C for various times between 0 and 60 min. The tubes were immediately centrifuged. The supernatant liquid was decanted into a scintillation vial containing 10 ml toluene-based scintillation fluid with Triton X-100 and the radioactivity was determined in a Nuclear Chicago Mark II liquid scintillation counter. The total and non-specific binding were calculated as percentages of the total bound radioactivity.

Table 1 shows that at 4 and 29 °C, HAP had no effect on either the total or the non-specific binding as the incubation time increased, but there was a 10% decrease in the total binding at 29 °C, compared with the values at 4 °C. Table 1 shows that with DCC, the total binding decreased with increasing time of incubation, and at 29 °C the decrease was more drastic than at 4 °C.

It appears from our study that it may be preferable to use HAP, rather than DCC, as a separating agent. There is no stripping problem and the binding is not greatly affected by temperature. The advantage of using HAP was further seen when it was used in the radioimmunoassay of free oestra-1,3,5(10)-triene-3,16α,17β-triol (oestriol). The intra-assay coefficient of variation for 40 replicates of a control sample of plasma was 4·3%, compared with 21·8% when DCC was the separating agent (H. H. Goh, unpublished data, 1977). It is known that HAP absorbs not only gamma-globulin but also other proteins such as albumin (Glueckauf & Patterson, 1974). Therefore the presence of other proteins will interfere with the binding of the antibody-antigen complexes to HAP, and hence it can only be used when the proteins present in the biological fluids are separated from the steroids.

Table 1. *Effects of incubation time and temperature on the total (Bo) and non-specific (NSB) binding of steroids to pentacalcium hydroxytriphosphate (hydroxyapatite) and dextran-coated charcoal.*

Temp. (°C)	Binding (%)*	Time of incubation with separating agent (min)									Mean ± S.D.
		0	5	10	15	20	30	40	50	60	
						Hydroxyapatite					
4	Bo	85·8	86·3	87·4	88·3	86·5	86·6	86·2	85·1	85·4	86·4 ± 1·0
	NSB	8·1	8·8	8·8	9·5	7·5	10·0	7·3	6·5	8·8	8·4 ± 1·1
29	Bo	75·7	76·5	75·2	76·1	75·9	76·3	77·5	77·2	76·9	76·3 ± 0·7
	NSB	10·8	10·1	7·9	6·5	8·7	8·7	10·1	10·1	8·0	8·9 ± 1·4
						Dextran-coated charcoal					
4	Bo	79·0	73·0	73·8	69·4	64·7	64·0	59·9	57·7	55·4	
	NSB	1·7	1·4	1·6	1·4	1·2	1·4	1·2	1·3	1·4	1·4 ± 0·2
29	Bo	45·9	32·5	24·8	22·1	19·8	16·7	15·2	14·3	13·9	
	NSB	1·3	1·2	1·3	1·5	1·6	1·8	2·0	2·2	2·5	1·7 ± 0·4

* Expressed as a percentage of the total bound radioactivity.

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