aggregates increase, whilst the number remains about the same. If the number of particles had increased much larger values for viscosity would have been found since there would now be a much larger contact area and the solutions would not have remained Newtonian. In addition a greater lowering of vapour pressure would be expected at high concentrations. It thus seems that as concentration increases the PVP aggregates grow in size, so as to still maintain a relatively small interparticulate contact area.

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Aqueous solubility of steroid hormones: an explanation for the discrepancy in the published data

Available data on the aqueous solubility of a number of steroids are scattered, to say the least (see Madan & Cadwallder, 1973 for references). Reliable data on aqueous solubility of steroid hormones is important in many aspects of biochemical and physiological studies besides their obvious value to the chemical and pharmaceutical industry.

With the availability of radiolabelled steroids, the measurement of the steroid concentration in a given sample (of known specific activity) has been rendered simpler and highly reliable. In more recent reports, therefore, radiolabelled steroids have been used for measuring the aqueous solubility. However, in many cases, membrane filters have been employed to separate the steroid in solution from the undissolved solute (see Madan & Cadwallder, 1973 for references). But these filters adsorb large quantities of the steroids from their aqueous solutions during filtration (Batra, 1974). The amounts adsorbed varied with different steroids. For example, over 95% of progesterone and only 10% of hydrocortisone from the respective aqueous solutions was taken up by the filter (Batra, 1974). These observations led to the re-examination of the aqueous solubilities of some steroid hormones.

Aqueous solubility was determined by the use of tritium labelled steroids. [1,2-^{3}H]-progesterone (48 Ci mmol^{-1}) and [6,7-^{3}H]oestradiol-17β (46 Ci mmol^{-1}) were purchased from New England Nuclear Corporation. [1,2,6,7-^{3}H] Testosterone (102 Ci mmol^{-1}), was given by the Radiochemical Centre, Amersham. Radiochemical purity was checked by thin-layer chromatography. After equilibration for a predetermined time (see Fig. 1) under continuous shaking at room temperature (23–24°), the undissolved solute was separated from the solution by filtration through glassfibre (Whatman) filters which did not adsorb any significant amount of the steroid from its aqueous solution. To compare data, samples were also filtered through membrane filters simultaneously. Since only a fraction of the labelled steroid is in solution when relatively high concentration of steroids are used in the assay (Fig. 1), it is necessary to run blanks, containing all of the radioactivity in solution, simultaneously. Blanks containing 1 μg ml^{-1} of the respective steroid and the same amount of radioactivity

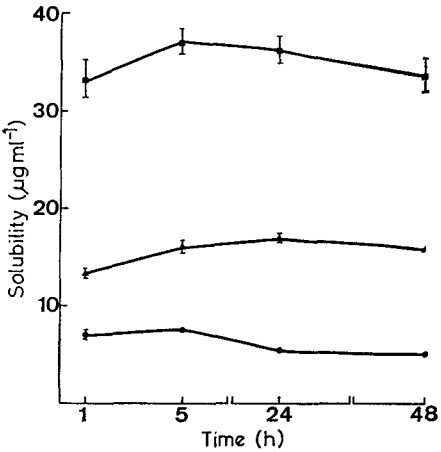

FIG. 1. The aqueous solubilities of some steroid hormones. Solubility was determined by equilibrating 100 μg ml^{-1} of each ^{3}H-labelled steroid and the undissolved solute was separated by filtration through a glassfibre filter (Whatman, GF/C) at the times shown. Each point is the mean of five experiments. Vertical bars represent $\pm$ s.e.m., and in the absence of a bar s.e.m. is included within the point. ■ Testosterone. ▲ Progesterone. ● Oestradiol.

as in the samples were therefore used for the determination of total radioactivity in the sample. Solubility was determined by the ratio of the radioactivity in the filtrate to that of the total. The radioactivity in an aliquot (0·5 ml) of the filtrate and the blank was counted with a liquid scintillation spectrophotometer (Packard Tri-Carb, Model 3320).

Data in Table 1 show the extent of adsorption of the three steroids to the membrane (Millipore) and glassfibre (Whatman) filters. Whereas more than 96% of progesterone was adsorbed onto the membrane filters by the passage of its aqueous solution only 11% was adsorbed by the glassfibre filters. This property of virtually complete adsorption of progesterone from its aqueous solution by the membrane filters was exploited in a previous study to separate free from protein-bound progesterone (Batra, 1974). The adsorption of oestrogen (oestradiol-17β) and testosterone onto the membrane filter was lower but considerable. The glassfibre did not take up either of these two steroids (Table 1). The adsorption of the steroids onto the membrane filter expressed as % of total, generally decreased with an increase in concentration.

It is fortuitous that in previous investigations where membrane filters were used, very high concentrations of steroids, 10 to 20 times their aqueous solubility, were equilibrated with the aqueous medium (see Madan & Cadwallder, 1973). The problem of adsorption onto membrane filters is not as serious at these high concentra-

Table 1. *The degree of adsorption of steroids onto membrane and glassfibre filters on passage of their aqueous solution.* Each steroid in concentration of 1 μg ml^{-1} was equilibrated for 5 h before filtering through a membrane (Millipore HAWP 02500) or glassfibre (Whatman, GF/C) filter. The concentration used (1 μg ml^{-1}) is well below the aqueous solubility of each steroid (Fig. 1). Each value is the mean $\pm$ s.e.m. of ten experiments.

Steroid	Adsorption %	
	Membrane filter	Glassfibre filter
Progesterone	96·19 $\pm$ 0·17	10·94 $\pm$ 1·25
Oestradiol	24·01 $\pm$ 2·05	3·25 $\pm$ 0·98
Testosterone	38·56 $\pm$ 2·03	1·28 $\pm$ 0·66

Table 2. *Mutual effect of oestradiol and progesterone on their aqueous solubilities.* Steroids, 100 μg ml^{-1} of each, were equilibrated for 5 h and filtered thereafter through a glassfibre (Whatman, GF/C) filter. Each value is the mean $\pm$ s.e.m. of four experiments.

Steroids	Solubility μg ml^{-1}	% change in solubility
Oestradiol alone	6·99 $\pm$ 0·34	
Oestradiol with progesterone ..	14·54 $\pm$ 0·22	+108·01
Progesterone alone	15·21 $\pm$ 0·25	
Progesterone with oestradiol ..	17·50 $\pm$ 0·38	+ 15·06

tions (unpublished observation) as at low concentrations due to the limited adsorbing capacity of these filters.

Fig. 1 shows that equilibrium is reached between 5 and 24 h for the steroids assayed (Bischoff & Stauffer, 1954; Madan & Cadwallder, 1973). The aqueous solubility values for the steroids assayed (Fig. 1) are higher than those reported in the literature which would be expected since glassfibre filters used in the present study, in contrast to the membrane filters, did not adsorb the dissolved steroid.

It was recently reported by Heap, Symon & Watkins (1971), who used a dialysis bag to separate the dissolved and undissolved steroid, that progesterone increased the aqueous solubility of oestrogen (oestradiol-17β). Although the solubility of the steroids in the present results was much higher than those reported by Heap & others (1971), their interesting observation on the increment of oestrogen solubility in the presence of progesterone has been confirmed. There was, however, a quantitative difference between the present results and those of Heap & others (1971) in this respect also. In the present results (Table 2) the solubility of oestrogen was more than doubled when present in combination with progesterone whereas the corresponding increment in oestrogen solubility in the study by Heap & others was 57%. There was only a small increase in progesterone solubility in the presence of oestrogen (Table 2), which is at variance with the results of Heap & others who reported a slight decrease. This difference may be due to the difference in assay method.

The physicochemical interaction of oestrogen and progesterone in aqueous medium resulting in significant change in their solubility behaviour could be of importance in consideration of their mode of biological action.

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