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The Polarographic Determination of Ketosteroids Solubilized in Aqueous Solutions of Association Colloids

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In earlier publications [1,2] it has been shown that clear and stable aqueous solutions of water-insoluble steroid hormones can be prepared with the aid of association colloids. In view of its practical importance we have investigated whether analytical methods can be developed for the determination of the hormone contents of these solutions. The analytical procedures previously developed for the quantitative determination of the hormones cannot be employed without modification in the presence of an association colloid and in many cases it was found necessary to separate the hormone from the colloid. The present paper deals with methods for the separation and polarographic determination of testosterone propionate, desoxycorticosterone acetate and progesterone solubilized in aqueous association colloid solutions. The procedure has been used to determine the solubilities of these hormones in a number of colloid solutions of different concentrations.

Eisenbrand and Picher [3] developed a method for the polarographic determination of Δ^4-ketosteroids containing the system $O=\overset{|}{C}-\overset{|}{C}=C$, such as testosterone, desoxycorticosterone and progesterone. The hormones were reduced in an alcoholic lithium chloride solution at the dropping mercury electrode. The half wave potentials were about — 1.8 volts and the heights of the polarographic waves were under certain conditions proportional to the hormone concentration. Sartori and Bianco [4] later used this method to determine methyl testosterone and pregnenin -3-one-17-ol. The same method was applied in the present investigations.

The association colloids have a disturbing effect on the depolarization process. It is not sufficient to break up the micelles present in their aqueous solutions by adding alcohol. The hormone must be freed from the colloid substance and also from traces of sodium and potassium ions before a polaro-

graphic determination can be carried out. The most direct method to accomplish this would be to extract the hormone from the aqueous solution with a solvent that does not dissolve the association colloid. From cholate solutions it was possible to extract the hormone quantitatively directly with petroleum ether or benzene. The procedure can also be used in the case of certain nonionic association colloids. With other colloids, however, the attempts to carry out such direct extractions were unsuccessful since emulsions were formed. Experiments were therefore made to precipitate the anionic association colloids with heavy metal ions (Ag, Pb, Hg) and to extract the hormone from the precipitate but this procedure was also found to be impractical. Satisfactory results were obtained in many cases if the hormone solution was evaporated to dryness and the hormone was extracted from the solid residue with petroleum ether or benzene. The evaporation of the petroleum ether or benzene gave the pure crystalline hormone. The latter could then be dissolved in an alcoholic lithium chloride solution and determined polarographically by the method of Eisenbrand and Picher.

EXPERIMENTAL

Experiments were conducted to isolate testosterone propionate (TP), desoxycorticosterone acetate (DOCA) and progesterone (PRG) * from aqueous solutions of potassium myristate, sodium oleate, sodium lauryl sulphate, sodium myristyl sulphate, sodium cholate, polyoxyethylene sorbitan monooleate ("Tween 80", Atlas Powder Co., Wilmington, Del.) and the di-isobutyl phenyl polyethylene glycol derivative, "Triton N100", (Rohm and Haas Co., Philadelphia, Pa.). The first five colloids were purified in the same manner as in our previous study [2]; the last two were commercial products and were used as such.

Extraction. From the aqueous solutions of the fatty acid salts and the alkyl sulphates the hormones were isolated by the following procedure. A known amount of the hormone solution (containing 1–5 mg hormone) in a beaker was placed in a vacuum desiccator and the solution evaporated to dryness under reduced pressure at room temperature (about 24 hours). The hormone was extracted from the dry residue with petroleum ether (B.P. 40–60° C, May & Baker, Dagenham). It was found advantageous to allow the residue to stand covered with a layer of petroleum ether at a temperature just below the boiling point of the petroleum ether. The solution was hastened by mixing the solid colloid with a glass rod to obtain a fine suspension. A quantitative extraction was usually effected with five 20 ml portions of petroleum ether. With higher colloid concentrations and hormone contents it was necessary to employ twice this number of washings. The undissolved colloid was separated from the petroleum ether solution by filtering through an ordinary filter. The filtration was followed by a thorough washing with petroleum ether. The filtrate was transferred to a beaker and the petroleum ether was slowly evaporated. The hormone which precipitated in crystalline form was then determined polaro-

* The hormone substances were products of the American Roland Corporation and were kindly set at our disposal by Oy. Medica Ab., Helsingfors.

graphically as described below. In order to be certain that the washings were quantitative the last wash solution was analyzed separately.

This extraction procedure cannot be used when the hormone is dissolved in a sodium cholate solution because the hormone is more strongly bound in the residue obtained on evaporating such a solution than in the case of other association colloids. From the cholate the hormone can be extracted with petroleum ether only by boiling for a long time under reflux. Thus in one case the residue of a 20 per cent sodium cholate solution containing 3.32 mg progesterone per millilitre was extracted in a Soxhlet apparatus with petroleum ether. The amount of hormone extracted was only about 30 per cent after 10 hours, 86 per cent after 30 hours and about 95 per cent after 48 hours. Even poorer results were obtained in other experiments. The reason for this is apparently the known tendency of bile acids to form complex compounds with various substances.

As already mentioned, it is possible, however, to extract the hormone directly from aqueous cholate solutions. These solutions do not readily form emulsions when petroleum ether or benzene is added as the other association colloid solutions do. In the case of cholate solutions the volume of petroleum ether used in each extraction must be twice the volume of the aqueous solution. On extracting a 20 per cent cholate solution containing 3.32 mg progesterone per ml, the first extraction gave a 95 per cent yield, and after the second extraction the yield was 98.2 per cent. Three extractions with 20 ml petroleum ether were generally sufficient to effect a quantitative separation of the hormone.

The latter extraction procedure was also found to be suitable for hormones dissolved in solutions of "Tween 80". In this case the volume of the aqueous phase must be relatively small compared with the volume of petroleum ether to avoid emulsification. Six to seven extractions are required and the residue from the extracts must be redissolved in water and extracted again six or seven times to obtain the hormone uncontaminated with colloid substance.

Up to the present we have not succeeded in isolating the hormone quantitatively from aqueous solutions of "Triton N".

The polarographic measurements. The crystallized hormones obtained as described above were dissolved in a small volume of 96 per cent ethanol and the solution was transferred to a 12 ml volumetric flask. 1 ml 1 *M* aqueous lithium chloride solution was added and the flask then filled to the mark with ethanol. (The resulting solution thus contained 0.083 moles of lithium chloride per litre of 83 per cent ethanol.)

The hormone content of the alcoholic hormone solution was determined using a Leybold m-35 polarograph and a mirror galvanometer of moving coil type with a maximum sensitivity of about 3.10^{-9} amp. per mm.*

The cell used was constructed so that gas could be bubbled through the solution. The nitrogen gas used passed through an alkaline pyrogallol solution and through 96 per cent ethanol before entering the cell. From the cell the gas bubbled through an alcohol layer. Before each measurement nitrogen was passed through the solution until the galvanometer deflection was constant with a suitable constant voltage, *e. g.* 1.5 volts.

All polarograms were taken with a total voltage of 3 volts applied across the potentiometer. On the horizontal scale of the polarograms the voltage increase was 150 millivolts per cm. The galvanometer sensitivity was maintained at 1 : 20.

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* This apparatus is described, *e. g.*, in H. Hohn *Chemische Analysen mit dem Polarographen.* Berlin (1937).

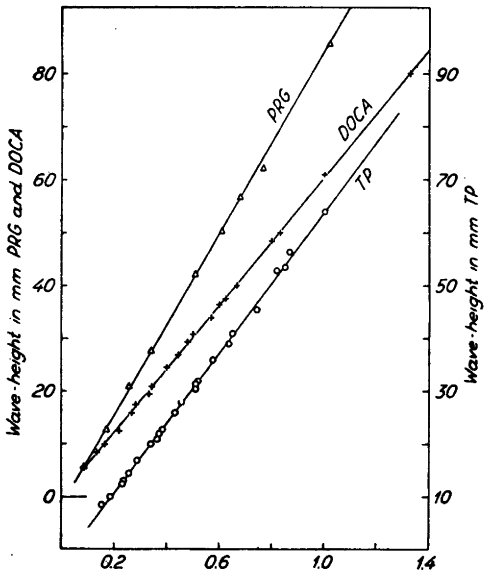

Fig. 1. Waveheight — concentration curves for progesterone, desoxycorticosterone acetate, and testosterone propionate.

The polarogram was recorded for the 1.5 — 2.25 volts range. The mercury drop rate was kept relatively high; on the average it was 0.6 seconds per drop with an applied voltage of — 1.5 volts.

RESULTS AND DISCUSSION

Our experimental results for the pure hormones dissolved in alcoholic lithium chloride solution confirmed those of Eisenbrand and Picher. The half-wave potentials of the polarographic waves are situated as given by these authors and the relation between wave height and hormone concentration was linear in all cases (Fig. 1).

The polarograms obtained with the hormones isolated from the colloid solutions had the same form as the polarograms for the pure hormones (Fig. 2). Experiments conducted with known amounts of hormone solubilized in the colloid solutions showed that the extractions were complete (Table 1). These observations prove that the hormones do not undergo any chemical change during the solubilization process.

The accuracy of the analytical method is seen from the values given in column 5 of Table 1. In the majority of cases the error is less than $\pm$ 5 per cent. A hormone concentration of 0.1 mg per ml is suitable for the recording of the polarogram. The lowest hormone concentrations in the colloid solutions were about 0.01 mg per ml. By extracting from large volumes of colloid solution, even very small hormone contents can, however, be determined. The largest

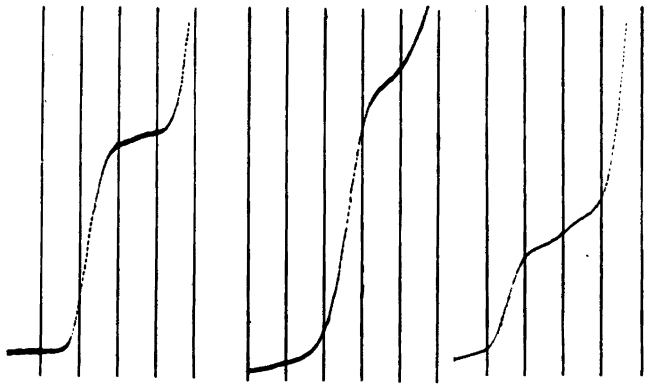

Fig. 2. Polarograms obtained with ketosteroids isolated from aqueous association colloid solutions:

a) 0.833 mg TP per ml in 12 per cent sodium oleate.
b) 1.30 mg DOCA per ml in 20 per cent potassium myristate.
c) 0.343 mg PRG per ml in 20 per cent sodium cholate.
Galvanometer sensivity 1/20.

volume of colloid solution employed was 80 ml. In the determination of the solubility of progesterone in pure water the volume of the aqueous solution was 200 ml. As the colloid concentration increases the difficulties become greater. We did not use colloid concentrations above 25 per cent.

The maximum solubilities of the three hormones were determined in aqueous solutions of the association colloids already mentioned. The colloid solutions were in most cases saturated with hormone in the manner previously described [1,2] by shaking at 40° C. The solutions in polyoxyethylene sorbitan monooleate ("Tween 80") were prepared in a different manner. In these cases the hormone was dissolved in the anhydrous colloid by warming, this solution was then diluted to the required concentration, after which the precipitated hormone was removed by filtration. The values of the solubilities of the hormones in the "Tween 80" solutions are less accurate.

The solubility values are collected in Table 2 and the variation of the solubilities with colloid concentration is shown in Figs. 3—5. In solutions of potassium myristate, sodium oleate, sodium lauryl and sodium myristyl sulphate, the solubilities of the three hormones increase above the critical concentration for micelle formation linearly with the colloid concentration. In the sodium cholate solutions the solubilities increase slowly at first and gradually more rapidly until finally at higher cholate concentrations a linear

Table 1. Polarographic determination of ketosteroids separated from aqueous association colloid solutions.

Colloid concentration in per cent	Mg hormone per ml		Average error mg/ml	Average error per cent
	Added	Found		
Testosterone propionate solubilized in sodium lauryl and sodium myristyl sulphate solutions				
2	0.00	0.00		0
2	0.20	0.22 0.21 0.22	+ 0.02	+ 10 %
10	1.00	1.08 1.00	+ 0.04	+ 4.0 %
1	1.08	1.14 1.10	+ 0.04	+ 3.7 %
2	1.10	1.20 1.20	+ 0.10	+ 9.1 %
5	3.60	3.78 3.80	+ 0.18	+ 5.0 %
10	4.78	5.16 4.80	+ 0.20	+ 4.2 %
10	5.24	5.05 5.05	− 0.19	− 3.6 %
20	5.40	5.30	− 0.10	− 1.8 %
20	10.0	9.95 9.95 10.4	+ 0.10	+ 1.0 %
20	25.90	27.0 27.4	+ 1.3	+ 5.0 %
Progesterone solubilized in sodium cholate solutions				
5	0.22	0.21	− 0.01	− 4.5 %
14	1.03	0.99	− 0.04	− 3.9 %
10	1.20	1.18	− 0.02	− 1.7 %
20	1.52	1.48	− 0.04	− 2.6 %
15	1.66	1.60 1.64	− 0.04	− 2.4 %
10	1.67	1.59	− 0.08	− 4.8 %
20	3.30	3.26 3.26	− 0.04	− 1.2 %
20	3.32	3.31 3.26	− 0.04	− 1.2 %

Table 2. The solubilities of ketosteroid hormones in aqueous solutions of different association colloids in mg hormone per ml association colloid solution. Colloid concentrations in weight per cent. 40° C.

Association colloid solution		TP	DOCA	PRG
Potassium myristate	0.5 %		0.68	
	2.0 %		3.02	
	4.0 %		5.74	
	8.0 %		10.9	
Sodium oleate	0.5 %	1.16		
	1.0 %	2.67		
	2.0 %	5.10		
	5.0 %	12.75		
Sodium lauryl sulphate	0.5 %		0.87	1.04
	1.0 %		1.78	2.07
	2.0 %		2.06	3.97
	8.0 %		7.20	16.56
	20.0 %		21.3	
Sodium myristyl sulphate	0.5 %	1.20		
	1.0 %	2.64		
	2.0 %	4.92		
	5.0 %	11.0		
	10.0 %	21.8		
	20.0 %	44.3		
Sodium cholate	0.5 %			0.012
	1.0 %			0.032
	2.0 %			0.130
	5.0 %			0.683
	10.0 %			1.90
	15.0 %			3.09
	20.0 %			4.36
Polyoxyethylene sorbitan monooleate	5.0 %	0.60	0.41	0.24
	10.0 %	1.21	0.82	0.48
	20.0 %	2.44	1.64	0.98

relation is obtained as in the case of the other colloids. A similar behaviour has also previously been noted in the power of this colloid to solubilize hydrocarbons [5,6,7]. This is due, as shown earlier [5], to the fact that the micelle forma-

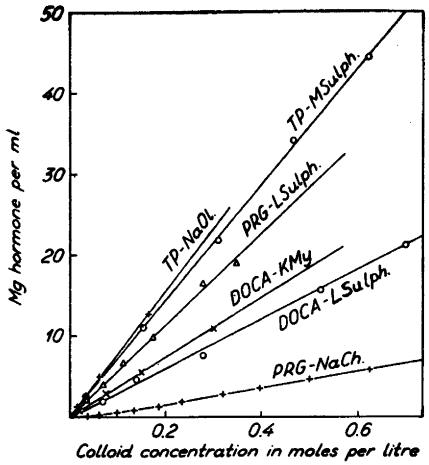

Fig. 3. The solubilities of ketosteroid hormones in various association colloid solutions. 40°C.

TP—NaOl. Testosterone propionate in sodium oleate solutions.

TP—MSulph. Testosterone propionate in sodium myristyl sulphate solutions.

PRG—LSulph. Progesterone in sodium lauryl sulphate solutions.

DOCA—KMy. Desoxycortieosterone acetate in potassium myristate solutions.

DOCA—LSulph. Desoxycorticosterone acetate in sodium lauryl sulphate solutions.

PRG—NaCh. Progesterone in sodium cholate solutions.

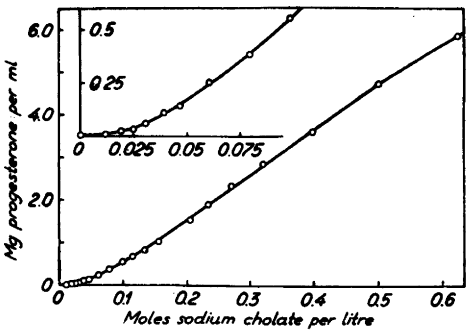

Fig. 4. The solubility of progesterone in aqueous solutions of sodium cholate. 40° C.

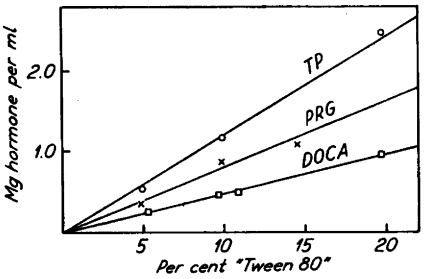

Fig. 5. The solubilities of TP, DOCA, and PRG in aqueous solutions of polyoxyethylene sorbitan monooleate ("Tween 80"). 40° C.

tion of sodium cholate occurs over a wider concentration range than in the case of the association colloids of the paraffin chain type and that the former takes place in several stages in which the micelles formed have different properties.

The saturation capacities of the micellar substances (the ratio of the amount of solubilized hormone in mg to the amount of micellar association colloid in moles) have been calculated from the linear parts of the solubility curves (Table 3). The data are still so limited that a comparison can not be made on this basis between the various hormones and between the different colloids. The differences noted are, however, fairly large. In the paraffin chain salt solutions testosterone propionate is the most soluble and desoxycorticosterone acetate the least soluble. The solubilizing power of the sodium cholate micelles

is much smaller than of the micelles of the other colloids although the hormone molecules partly have the same carbon skeleton as cholic acid.

Table 3. Maximum solubilizing powers of micellar substances for ketosteroid hormones.

Association colloid	Hormone	Mg hormone per mole micellar substance	Moles micellar substance per mole solubilized hormone
Potassium myristate	DOCA	37 200	10.0
Sodium oleate	TP	77 700	4.43
Sodium lauryl sulphate	DOCA	30 400	8.17
» » »	PRG	57 000	5.51
Sodium myristyl sulphate	TP	72 000	4.78
Sodium cholate	PRG	10 660	29.6
		Mg hormone per g Tween	Grams Tween per mole hormone
Polyoxyethylene sorbitan monooleate	TP	122	2 820
—»—	DOCA	82	4 090
—»—	PRG	49	6 410

If the micelles are assumed to contain 100 moles of the colloid substance, the number of moles of steroid hormone per micelle would be 10—20 moles in the case of paraffin chain colloids, but only about 3 moles in the cholate micelles. The degree of dispersion of the hormones solubilized in the association colloid solutions is thus relatively high.

From our results is seen that the steroid hormones in most cases are solubilized in larger amounts by the colloids than the polycyclic aromatic hydrocarbons.[7] For example, the solubility of progesterone is from about 40—50 times that of 1,2,5,6-dibenzanthracene in the solutions of most colloids. In the cholate solutions, however, the difference is smaller, the solubility of progesterone being only four times greater than that of dibenzanthracene. This is connected with the fact that whereas the solubilities of the polycyclic hydrocarbons per mole colloid are much higher in the bile acid salt solutions than in solutions of the paraffin chain salts, the opposite is the case, as we have seen, with progesterone.

These observations emphasize the special position of the bile acid salts among the association colloids. They further indicate, as do also other facts, that the solubilization differs in mechanism in the case of steroid hormones and

hydrocarbons. It seems probable that whereas the polycyclic hydrocarbons, like the lower hydrocarbons, are situated in the micelles between the hydrocarbon layers the hormones are, like alcohols and other hydropolar substances, situated between the molecules in the palisade layers of the micelles.

SUMMARY

Methods have been developed for the quantitative extraction of ketosteroids from aqueous solutions of association colloids. The isolated crystalline hormones can then be polarographically determined by the method of Eisenbrand and Picher. The methods have been used to determine the maximum solubilities of testosterone propionate, desoxycorticosterone acetate and progesterone in aqueous solutions of various association colloids.

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