Uptake of Steroid Hormones into Artificial Phospholipid/Cholesterol Membranes

THE effect of steroid hormones on the movement of ions[1,2] and glucose[3] across artificial lipid membranes has been studied, but their activity in such membranes has not been correlated with their concentration within the membranes. The problem of uptake of steroid hormones into lipid membranes has been the subject of speculation and experiment[4–7], and the work reported here has been carried out in order to examine this problem further.

The lipids used in this study were egg lecithin, which was chromatographically purified, after which it runs as a single peak in chromatographic systems with a characteristic R_F value; cholesterol, which was recrystallized from redistilled 'AnalaR' grade petroleum ether (40°–60° C); and chromatographically pure dicetylphosphate. Each of the tritiated steroids (in amounts of 5 μc.) was added to 1 l. of water.

Table 1. DISTRIBUTION COEFFICIENTS OF FIVE STEROID HORMONES BETWEEN A LIPID MYELIN SUSPENSION AND WATER

Membrane 1 (90 per cent lecithin, 10 per cent dicetylphosphate)		
Aldosterone	1·125 ± 0·017	(30)
Cortisol	1·322 ± 0·041	(76)
Deoxycorticosterone	1·840 ± 0·060	(200)
Oestrone	3·191 ± 0·062	(520)
Progesterone	4·080 ± 0·030	(730)
Membrane 2 (80 per cent lecithin, 10 per cent dicetylphosphate, 10 per cent cholesterol)		
Aldosterone	1·113 ± 0·024	(28)
Cortisol	1·302 ± 0·020	(75)
Deoxycorticosterone	1·502 ± 0·080	(119)
Oestrone	2·978 ± 0·014	(491)
Progesterone	3·861 ± 0·068	(710)
Membrane 3 (70 per cent lecithin, 10 per cent dicetylphosphate, 20 per cent cholesterol)		
Aldosterone	1·111 ± 0·033	(29)
Cortisol	1·277 ± 0·041	(72)
Deoxycorticosterone	1·390 ± 0·070	(93)
Oestrone	2·823 ± 0·049	(477)
Progesterone	3·650 ± 0·040	(694)
Membrane 4 (60 per cent lecithin, 10 per cent dicetylphosphate, 30 per cent cholesterol)		
Aldosterone	1·133 ± 0·021	(37)
Cortisol	1·270 ± 0·014	(75)
Deoxycorticosterone	1·406 ± 0·080	(96)
Oestrone	2·577 ± 0·055	(460)
Progesterone	3·320 ± 0·040	(647)

Values in parentheses are the corresponding lipid : water distribution coefficients.

The membranes were prepared by adding appropriate amounts of the lipids in chloroform solution to each of four 100 ml. flasks, giving a total of 250 μmoles lipid in the proportions indicated in Table 1. These were evaporated to dryness, 50 ml. of water was added to each and they were shaken for 1 h. The flasks were allowed to stand overnight and reshaken before use. Spherical myelins formed, which were examined by electron microscopy after staining with a 2 per cent solution of phosphotungstic acid and found to provide very good bimolecular structures as described in previous work[3].

After the final shaking, aliquots of 2 ml. were removed and dialysed against 40 ml. volumes of each steroid solution. These were left to stand overnight—which was found to be a suitable time for equilibrium to be established—after which 1 ml. of the inside and outside solutions were transferred to separate counting vials. The water was removed by standing on a hot plate, and scintillation fluid was then added to each vial and the tubes counted.

It was found that the presence of the small amount of lipid had no quenching effect on a tritium standard, and therefore the distribution coefficients for the steroids between the spherulite suspension and water could be directly obtained by taking the ratio of the counts for the corresponding inside and outside solutions (Table 1). The experiment was repeated and for each steroid with each membrane twelve values were obtained which have been used to determine a mean and a standard deviation.

The values of these distribution coefficients have been converted to a corresponding lipid : water distribution coefficient, which may be useful in the estimation of the distribution of the steroid hormones in the lipoidal environment of binding tissue[8].

The effect of steroid concentration was investigated and the distribution coefficient found to remain constant up to concentrations of steroid approaching their limiting water solubilities, which have been determined as: cortisol ($6{\cdot}2 \times 10^{-4}$ moles/l.); deoxycorticosterone ($1{\cdot}6 \times 10^{-4}$ moles/l.); aldosterone ($1{\cdot}2 \times 10^{-4}$ moles/l.); oestrone ($7{\cdot}6 \times 10^{-5}$ moles/l.); and progesterone ($2{\cdot}7 \times 10^{-5}$ moles/l.).

A comparison of these water solubilities with the distribution coefficients suggests that the distribution coefficient reflects a decreased solubility in water rather than an increased solubility in the lipid membranes. The presence of cholesterol in the membrane lowers the membrane solubility of the steroid hormone, the distribution coefficient varying linearly with the cholesterol content. Such observations may be relevant to problems of uptake and localization of the steroid hormones in cells.

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Production of Ethanol and Succinate by *Moniliformis dubius* (Acanthocephala)

LAURIE[1,2] found that formate, acetate and lactate were the chief excretory products from the carbohydrate metabolism of *Moniliformis dubius*, and we have found lactate and succinate to be the most important excretory products of *Polymorphus minutus*, an acanthocephalan parasite of domestic ducks[3]. Succinate has been reported as an intermediary metabolite of the carbohydrate metabolism of *M. dubius*[4,5], and so we decided to analyse for excretory products, in particular succinate, the medium in which specimens of *M. dubius* had been incubated.

M. dubius was maintained in the laboratory using cockroaches, *Periplaneta americana*, as the intermediate hosts, and Wistar rats as the final hosts. Adult worms 42–57 days old were recovered from the rats and washed thoroughly in Tyrode saline, before being incubated in Tyrode saline containing ^{14}C-glucose at *p*H 7·5 and 37° C under commercial nitrogen. Incubation was terminated after a certain time, and the worms were removed and weighed and the medium was analysed for volatile components, non-volatile acids and residual glucose. The results given below apply to an incubation, lasting 2·5 h, of 515 mg wet weight of worms with 5 mg of glucose in 3·4 ml. of saline.

Examination of a sample of the medium using a gas chromatograph with a flame detector revealed that the main excretory product of *M. dubius* was neither formate nor acetate but another very volatile non-acidic substance, and analysis showed that most of the excreted radioactivity was associated with this metabolite. The substance was found to have the same retention time as