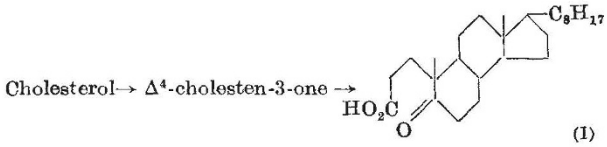
Table 1. Localization of Radioactivity in the Cholesterol Ring

Time after injection (days)	Dose given (μc.)	Specific activity (c.p.m./mgm. of cholesterol or its equivalent)			
		Crude extract	Cholesterol	Cholestenone	Keto-acid (I)
375 (rabbit)	5	45	47	35	1·8
502 (chicken)	3	22·9	23·4	14	0·96

A crude lipid extract in alcohol–ether (3 : 2) or chloroform–methanol (2 : 1) was obtained from the central nervous system of animals injected with cholesterol-4-^{14}C soon after birth. Pure cholesterol was obtained by recrystallization of cholesterol separated from the crude extract by column chromatography. Cholestenone and the keto-acid were prepared as described below.

and then oxidized with hydrogen peroxide by the method of Turner[4]. The keto-acid (I) obtained as a result of this elimination of a carbon atom at position 4 was purified and recrystallized. The melting point was in agreement with that reported in the literature[4,5]. Comparison of the radioactivity of the cholesterol with the radioactivity of the keto-acid (Table 1) shows that almost all the activity disappears on elimination of the 4-C atom. Furthermore, it was found that all the radioactivity in the original crude extract of the central nervous system was due to its content of radioactive cholesterol (Table 1). This latter result also suggests that the cholesterol molecule remains intact in the central nervous system for more than a year, for if the cholesterol-4-^{14}C had been degraded it would be anticipated that other radioactive lipids would be synthesized from the resultant fragments. These two groups of experiments, therefore, support the view that the cholesterol that persists in the central nervous system remains metabolically inert for more than a year.



(I)

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A Simplified Physico-chemical Method for Determination of Human Urinary Œstrogens

The methods for estimating urinary œstrogens are open to criticism and are not easily applied as a routine in the laboratory because of their complexity. Our chief aim in the present investigation is to develop a reliable, practical and simplified method for studying the excretion of urinary œstrogens by patients with cancer of the breast.

100 ml. urine of 24-hr. sample was adjusted to *p*H 5 with concentrated sulphuric acid. 10 per cent by volume of acetate buffer was added, followed by 300 Fishman units of β-glucuronidase/ml. urine. This was incubated at 37° C. for five days[1]. After completion of hydrolysis, the urine was extracted four times with 100 ml. peroxide-free ethyl ether. The ether extract was washed with (*a*) saturated sodium carbonate solution, *p*H 10·5, (*b*) 2 *N* sodium hydroxide solution which was neutralized to *p*H 10 with 8 per cent sodium bicarbonate solution and shaken again with the ether extracts, (*c*) 8 per cent solution of sodium bicarbonate, (*d*) water[2]. The ether extract was evaporated to dryness and the residue dissolved in about 80 ml. toluene; this was extracted four times with 0·25 volume *N* sodium hydroxide and washed twice with 0·05 volume water. Neutral steroids remain in toluene layer. Alkaline extracts and washings were acidified to *p*H 9 with sulphuric acid (6 *N*) and extracted four times with 0·25 volume ether. This was evaporated and the residue was transferred to small storage bottles with methyl alcohol and evaporated to dryness at 60° C. Counter-current distribution in 50 per cent methanol, 50 per cent water (upper layer)/carbon tetrachloride (lower layer)[3] was then carried out for 24 transfers. Œstrogen extract was dissolved in 0·2 ml. ethanol and heated in a water-bath with 1 ml. 90 per cent sulphuric acid at 80° C. for 10 min. This was diluted with 4 ml. 65 per cent sulphuric acid and cooled in crushed ice for 30 min. Fluorescence was measured in a Farrand photofluorometer model *A* using Corning glass No. 3389 and No. 5113 as the lamp filter (436 mμ) with Nos. 3387 and 4308 and a 483 mμ interference filter as the photocell filter[4–6].

The specificity, precision and accuracy of the newly developed technique are shown in Table 1. These results were obtained by running a series of recovery experiments for the three major naturally occurring œstrogens.

Table 1. Recovery of Œstrone, Œstradiol-17β and Œstriol added to 100 ml. of Normal Male Urine before Hydrolysis

Œstrogen used	Amount added (μgm.)	Percentage recovery	Partition coefficient*
Œstrone	100	85	3·4
	50	82	
Œstradiol-17β	100	91	2·1
	50	87	
Œstriol	100	93	15·3
	50	94	

* Between 50 per cent methanol and carbon tetrachloride.

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