THE BIOSYNTHESIS OF RADIOACTIVE ESTRADIOL

I. SYNTHESIS BY SURVIVING TISSUE SLICES AND CELL-FREE HOMOGENATES OF DOG OVARY

by

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The comparative biosynthetic pathways of closely related sterols are of particular interest in consequence of recent studies on precursors of cholesterol[1–4]. This study is concerned with the biosynthesis of estrogens. ^{14}C-labelled estradiol and estrone have been obtained from ovaries perfused with ^{14}C-acetate[5]. We are reporting an *in vitro* system in which ^{14}C-acetate is incorporated into estradiol and estrone. This system lends itself to investigation of intermediates, co-factors and conditions influencing synthesis.

EXPERIMENTAL

The ovaries used in these experiments were obtained from mongrel dogs. Adhering tissue was carefully removed and the ovaries placed immediately in an isotonic phosphate buffer solution at 1° C. Surviving tissue slices, 0.5 mm thickness, were prepared with a Stadie slicer. About one half gram of tissue was placed in each incubating flask together with two and a half volumes of buffer and 1 mg each of adenosine monophosphate, diphosphopyridine nucleotide and 2-^{14}C sodium acetate (1 mc/mM).

Homogenates were prepared after mincing ovaries in two and a half volumes of buffer containing K_2HPO_4 0.067 M, KH_2PO_4 0.042 M, $MgCl_2$ 0.006 M and nicotinamide 0.03 M at pH 7.0 at 0° C. The mince was then homogenized quickly in a loose-fitting Potter-Elvehjem glass homogenizer. The homogenate was centrifuged for seven minutes at 500 g to remove cells, nuclei and cell debris. The homogenate was divided among flasks containing the same co-factors and precursor as for the slices, so that the total volume of each flask was 5 ml.

The flasks were incubated at 37° C for 3 hours with gentle shaking; the gaseous phase was 100% oxygen. After incubation 10 mg of carrier estradiol, estrone or cholesterol was added separately to each flask. Metaphosphoric acid was added to pH 2. The material was extracted continuously with ether for 24 hours. The ether extract was evaporated to dryness and placed in a desiccator over KOH for several days. One ml of 10% acetic acid was added to each residue which was again dried in a desiccator over KOH. Each residue was dissolved in a minimum amount of absolute ether, decolorized with charcoal and alumina, and filtered. Petroleum ether was added to incipient turbidity and the material was kept at −20° C for 48 hours. The solid obtained was recrystallized until the specific radioactivity and melting points were constant and mixed melting point with authentic material showed no depression (m.p. 259° C for estrone; m.p. 176° C for estradiol). Aliquots of the obtained estrone and estradiol were chromatographed on celite by the method of BITMAN AND SYKES[6]. The samples followed the described elution rates and the specific radioactivity was not altered.

The *m*-bromobenzoate and 1-naphthoate derivatives of estradiol and estrone were prepared by the method of DOISY *et al.*[7] The materials obtained had correct melting points and mixed melting points with authentic material were not depressed (m.p. 195° C for di-1-naphthoate of estradiol; m.p. 155° C for *m*-bromobenzoate of estradiol; m.p. 208° C for mono-1-naphthoate of estrone, m.p. 221° C for *m*-bromobenzoate of estrone). Cholesterol was obtained as the digitonide. The radioactivity of all materials was determined, corrected to infinite thinness and for dilution, and expressed as cpm/mg C. Variations in the activity of derivatives of the same sample were within the counting error.

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RESULTS

Labelled estradiol, estrone and cholesterol were obtained in all experiments. The activity of the estradiol was greater in all cases than that of the estrone. The activity of both estradiol and estrone was far greater in ovaries obtained from animals during proestrus than in ovaries obtained during anestrus. The activity of the cholesterol obtained was about the same during the various phases of the oestrus cycle. The results are summarized in Tables I and II.

TABLE I

INCORPORATION OF 2-^{14}C ACETATE INTO ESTRADIOL AND ESTRONE BY TISSUE SLICES AND HOMOGENATES OF DOG OVARIES

	Recovered radioactivity							
	Estradiol (Cpm/mg C)				*Estrone (Cpm/mg C)*			
	Diluted, and recovered	*di-1-naphthoate*	*mono-m-bromobenzoate*	*Chromatographed*	*Diluted and recovered*	*mono-1-naphthoate*	*mono-m-bromobenzoate*	*Chromatographed*
Exp. 1	374	351	314	366	40	47	32	38
Exp. 2	285	295	256	—	19	14	—	—

TABLE II

INCORPORATION OF 2-^{14}C ACETATE INTO ESTRADIOL AND ESTRONE BY TISSUE SLICES AND HOMOGENATES OF DOG OVARIES IN PROESTRUS AND ANOESTRUS

		Recovered radioactive		
		Estradiol Cpm/mg C (range)	*Estrone Cpm/mg C (range)*	*Cholesterol Cpm/mg C*
Proestrus dogs	Tissue slices	760–896	31–46	—
	Homogenates	96–134	14–23	76
Anoestrus dogs	Tissue slices	71–94	3–17	—
	Homogenates	19–63	1–5	77

DISCUSSION

The conditions and techniques for liver slices and homogenates found to be optimum for cholesterol synthesis[1] were employed with success in this study.

The higher activity of estradiol than estrone in our experiments lends support to the idea that estradiol is the primary estrogen synthesized by the ovary.

The optimum conditions and co-factors for estradiol synthesis are under investigation and a particle-free, water soluble system is being developed.

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SUMMARY

1. 2-^{14}C-acetate is incorporated into estradiol, estrone and cholesterol by surviving tissue slices and homogenates of dog ovary.

2. Under comparable conditions more acetate was incorporated into estradiol than into estrone.

3. The activity of estradiol and estrone obtained from tissues of animals in proestrus was greater than that of animals in anestrus.

RÉSUMÉ

1. L'acétate-2-^{14}C est incorporé dans l'oestradiol, l'oestrone et le cholestérol par des tranches de tissus survivants et des homogénats d'ovaire de chienne.

2. Dans des conditions comparables l'oestradiol incorpore davantage d'acétate que l'oestrone.

3. L'activité de l'oestradiol et de l'oestrone préparés à partir de tissus d'animaux en pro-oestrus est plus élevée que celle d'animaux en anoestrus.

ZUSAMMENFASSUNG

1. 2-^{14}C-Azetat wird durch überlebende Gewebeschnitte und Homogenate von Hundenovaria in Östradiol, Östron und Cholesterol eingebaut.

2. Unter vergleichbaren Bedingungen wurde in Östradiol mehr Azetat eingebaut als in Östron.

3. Die Aktivität von Östradiol und Östron, die aus Geweben von Tieren in der Pro-Östrusperiode hergestellt worden waren, war grösser als diejenige der Tiere in der Anöstrusperiode.

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