

Paper Chromatographic Separation of some
Phloroglucinal Derivatives from *Dryopteris*
filix mas

From crude filicin, obtained via the oleoresin, filicic acid, flavaspidic acid and albaspidin were isolated, while filicinic acid was obtained by heating filicic acid in the waterbath with zinc dust and a 10% sodium hydroxyde solution for 6 h.

Paper. Use was made of Whatman No. 1 filter paper. The length of the employed strip was 270 mm and cut in the machine direction; the width was 70 mm.

Solvent. A 2 N sodium carbonate solution in which, immediately before chromatographing, 0.2 w/v% sodium sulfite had been dissolved.

Reagent. 1.5 ml of a solution of 1% sulfanilamide in 10% hydrochloric acid are mixed with 1.5 ml of a 5% sodium nitrite solution and gently shaken for 1 min. Next the whole partly neutralized with 1 ml 2 N sodium carbonate solution and water is added up to 50 ml. The reagent is always freshly prepared.

Ascending chromatography. 2 mg of the isolated substances are dissolved in 1 ml chloroform. By means of a pipette 3 µl of these solutions, are brought on the paper at 40 mm from the lower end of the strip at a mutual distance of 17.5 mm. In order to prevent difficulty in the spots being wetted by the solvent, first 1.5 µl is brought on the paper and after the chloroform has evaporated, 1 µl and finally 0.5 µl, instead of 3 times 1 µl. This is done to prevent the substance accumulating in the boundary of the spot.

When the spots are dry, the chromatogram is run at a temperature of 20°C. The paper is taken out of the cylinder as soon as the liquid front has reached a distance of 200 mm from the starting line, which takes about 3¼ h. Next the paper is dried for about 10 min in an oven at 80°C, sprayed with the reagent and dried again. Directly after the spraying the spots become visible and show some difference in colour for the different substances¹.

Result. See Table.

Substance	Colour	Average R _f value
Flavaspidic acid . . .	brownish-yellow	0.28
Filicic acid	brown	0.53
Albaspidin	violent-yellow	0.74
Filicinic acid.	orange-yellow	0.84

A mixture of these 4 substances shows the same average R_f values. The crude filicin itself, which is brought onto the paper in a concentration of 10 mg per ml chloroform, shows the R_f values of filicic acid and flavaspidic acid. In this concentration albaspidin is not visible. The crude filicin of some other fern species is being investigated.

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Résumé

L'acide filicique, l'acide flavaspidique, l'albaspidine et l'acide filicinique ont pu être isolés par chromatographie sur papier, suivant la méthode ascendante, avec comme

phase mobile une solution de carbonate de sodium 2 N, dans laquelle on a dissous 2 g de sulfite de sodium par litre. Les spots étaient rendus visibles en pulvérisant une solution de sulfanilamide diazotée. En même temps se manifestait aussi une différence de couleur pour les dites substances phlorogluciniques.

The Influence of Oestrogen and Thyroid on
the Pituitary and Blood Content of FSH and LH

Hyperthyroidism when experimentally induced, increases the duration of the oestrous cycle, and this is attributed to prolongation of the luteal phase (HAYASHI¹ and WEICHERT²). Thyroid administration produced an increase in the number and size of corpora lutea (SIDKI³; WEICHERT and BOYD⁴ and SIDKI and SOLIMAN⁵). Such observations indicate that thyroid-active materials possess an oestrogen like effect by favouring the production of LH (STEIN and LISLE⁶; CHU⁷ and OKANS and TAKANA⁸).

Under natural conditions, the increased oestrogen level during the follicular phase of the oestrous cycle was revealed to be concomitantly associated with increased thyroid activity and increased level of thyrotrophic hormone in the blood (SOLIMAN and REINEKE⁹ and SOLIMAN and BADAWI¹⁰).

The present investigation was thus devised to ascertain the influence exerted by oestrogen and thyroid on the FSH and LH content of the blood and hypophyseal glands of ovariectomized rats.

Ovariectomy was performed on 24 mature female rats and these were divided into four groups with six animals in each. Group I was kept as control. To the rats of group II, desiccated thyroid was administered at the level of 0.1% in the diet for 30 days. Parenteral administration of 1 µg of oestradiol benzoate (B.D.H.) daily for 30 days was conducted to the animals of group III. The animals of group IV were treated with oestradiol and, in addition, desiccated thyroid was fed for the same duration, after which all the animals were killed by decapitation under light ether anesthesia, and the blood of the animals of each group was pooled together and allowed to coagulate. The sera were procured by centrifugalization at a rate of 3,000 r.p.m. for 15 min and subsequently treated with acetone and ethyl alcohol in order to precipitate the proteins and the precipitate that arose was redissolved in saline so as to bring the sera back to their original volume. Such a procedure was adopted to annul any error that might arise from the presence of oestrogen in the serum of the rats. The hypophyseal glands of each group were cleanly dissected, weighed in a torsion balance, pooled together and thoroughly ground. The resulting suspension was so adjusted that every 0.1 ml of saline contained 1 mg of fresh hypophysis.

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7 J. P. CHU, Endocrinology 34, 90 (1944).
8 K. OKANS and H. TAKANA, Trans. Soc. Path. (Japan) 30, 239 (1946).
9 F. A. SOLIMAN and E. P. REINEKE, Amer. J. Physiol. 178, 89 (1954).
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¹ The spraying with reagent is done in the fume-chamber.

Table I
Influence of oestrogen and thyroid on FSH content rat blood and pituitaries

Treatment of Rats	Ovary Weights of Mice in mg/100 g Body Weight	
	Blood	Pituitary
Castrated	76.52 \pm 3.22	87.80 \pm 2.25
Castrated + Thyroid	62.78 ^a \pm 2.68	72.07 ^b \pm 2.48
Castrated + Oestradiol	65.47 ^a \pm 3.60	62.05 ^b \pm 1.69
Castrated + Oestradiol + Thyroid	49.32 ^b \pm 1.70	47.75 ^b \pm 1.13
Control Mice	17.25 ^b \pm 1.02	

\pm Standard error.

^a Significantly different from castrated rats at 1% level.

^b Significantly different from castrated rats at 5% level.

The FSH content of 0.5 ml of serum or 1 mg of hypophyseal substance was determined by the LH augmentation method adopted by BROWN¹¹. The above-mentioned amounts, together with 20 I.U. of chorionic gonadotrophin, were parenterally administered into 21-day-old female mice. 54 animals were utilized for this purpose and these were subdivided into 9 groups of 6 animals each. One group was retained as control and two of the remaining groups were allotted to each of the experimental groups, one set receiving serum while to the other hypophyseal suspension was administered.

The LH content, on the other hand, was determined by following the method adopted by SOLIMAN¹², in which groups of 5 immature female mice were primarily treated with 20 I. U. of pregnant mare serum, parenterally given. After 4 days when the graafian follicles attained full development, 0.5 ml serum or 1 mg hypophyseal substance was injected into the mice. The animals were then killed with ether 48 h later, and the number of the corpora haemorrhagica formed was considered as an index of the LH content of the test material.

As Table I shows, the FSH content of the blood and pituitaries of ovariectomized rats were the largest. Administration of either oestrogen or thyroid to ovariectomized rats induced a drop in the FSH content of both their blood and pituitaries, presumably due to decreased rate of formation and release of the hormone by the pituitaries. The combined administration of oestrogen and thyroid caused a further decrease in the level of FSH in the blood and pituitaries.

It appears from Table II that administration of oestrogen and thyroid, singly or in combination, significantly increased the concentration of LH in the blood of the experimental animals. The level of LH, however, was

greater on joint administration of the two hormones than when either of them was given separately. Oestrogen administration was accompanied with a pronounced increase in hypophyseal LH. Thyroid administration caused a mild increase in pituitary LH. It appears, then, that oestrogen favours the synthesis of LH by the pituitaries while thyroid administration increases the rate of release of this hormone. The LH content of the pituitaries of rats treated with the two hormones together was less than that of oestrogen-treated animals.

In the light of the data obtained from the present investigation, it is apparent that ovulation is associated with thyroid activity. Under natural conditions, there is an increased production of both oestrogen and thyroid hormones during oestrus which induces a drop in the FSH level in the blood with a prompt rise in the level of LH, a phenomenon that induces ovulation.

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Résumé

L'effet de l'administration du «Benzoate d'oestradiol» et des hormones thyroïdales seuls ou en combinaison sur le FSH et LH contenus dans le sang et les hypophyses des femelles rats castrés a été étudié. Il a été trouvé que l'oestradiol et l'extract de la thyroïde quand ils sont administrés seuls produisent une baisse, dans le sang, du FSH et une hausse du LH. L'administration combinée de ces deux hormones renforce cet effet.

Table II
Influence of oestrogen and thyroid on LH content of rat blood and pituitaries

Treatment of Rats	Average No. of corpora hemorrhagica	
	Blood	Pituitary
Castrated	0.50 ^b \pm 0.02	1.20 \pm 0.09
Castrated + Thyroid	1.80 ^b \pm 0.01	2.00 \pm 0.35
Castrated + Oestradiol	2.00 ^b \pm 0.01	2.50 ^b \pm 0.33
Castrated + Oestradiol + Thyroid	2.40 ^b \pm 0.11	1.40 \pm 0.11
Control Mice (20 I. U., PMS)	0.33 \pm 0.10	—

\pm Standard error.

^b Significantly different from castrated rats at 1% level.