A further in vitro experiment was performed, utilizing the rat pituitary perifusion system to ascertain the effect of Ind on TSH release. The findings are given in figure 3. The TSH release stimulated with 2.76×10^{-8} to 2.76×10^{-5} M TRH (left panel) was notably potentiated in the presence of 1.0×10^{-6} M Ind (right panel).

Discussion. The present study demonstrated the inhibitory effect of Ind on thyroid hormone release not only in vivo but also in vitro, in a perifusion system with rat thyroids. It is unquestionable that endogenous PG levels in the thyroid are lowered by Ind administration[5,6]. However, conflicting data have been presented concerning the effects of Ind on the release of thyroid hormones and of TSH. Some authors have reported that the thyroid hormone release was curbed by Ind[6,8,9], but others have said that it was not influenced[5,11,12]. Similarly, previous findings on the TSH release are divergent; that is, a deceleration[13,14] or an acceleration[9] of TSH release, or neither phenomenon was observed[5,6,12,15]. Haye et al.[16] claimed that the divergence may be explained by the hypothesis that there are two sorts of intrathyroidal PG pools. Thompson and Hedge[14] observed that therapy with a PG synthesis inhibitor (Ind or aspirin) suppressed the compensatory rise in TSH level following thyroidectomy or enfeebled the pituitary responsiveness to TRH. On the contrary, in our in vivo experiment, long-term Ind administration led to a marked increase of serum TSH and, simultaneously, the thyroid gland was swollen. However, serum T_3 and T_4 levels were both within normal limits. This perfect balance between TSH and thyroid hormones suggested a fully compensatory feedback mechanism.

In order to get data attesting to these effects of Ind observed in vivo, in vitro perifusion experiments were carried out. We obtained the following two characteristic findings: 1) Ind inhibited the TSH-stimulated thyroid hormone release and 2) it strengthened the TRH-stimulated TSH release. These in vitro results suggest independent action of Ind on pituitary and thyroid. Though it is questionable whether these results can be used for the interpretation of the in vivo results it is suggested that PGs play an important role in the pituitary-thyroid axis, especially in relation to thyroid hormone release.

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Estrogen and accessory sex gland lipids[1]

E. Umapathy

Department of Physiology, College of Medical Sciences, University of Benin, Benin City (Nigeria), 6 January 1983

Summary. Administration of estradiol-17β elicited differential responses on accessory sex glands of rats. In caput epididymis, the estrogen treatment led to an accumulation of glycerides and phospholipids while in cauda epididymis, there was no significant change. However, in seminal vesicles, depletion of phospholipids was observed. In prostate, the treatment, resulted in an accumulation of glycerides.

Key words. Rat, sex glands; estrogen treatment; lipid accumulation; epididymis, caput: epididymis, cauda; seminal vesicle; prostate.

Lipids form the major secretory products of the epididymis, besides sialoproteins, and the concentration of phospholipids is generally higher in caput epididymides of many species[2]. Epididymal lipids have been found to fluctuate during maturation of sperm[3]. It is also well established that the major amount of lipid in semen is contributed by the prostate[4] and that the formation and metabolic turnover of phospholipids in the prostate and seminal vesicles depend on androgenic stimulation[5].

Preliminary studies have shown a significant hormone-lipid interrelationship in male accessory sex glands[6]. In rates, estradiol has been shown to cause regression of the male accessory sex glands in general and prostate in particular[7]. The present study was undertaken to investigate the effect of estrogen on the male accessory sex gland lipids.

Materials and methods. Fourty male albino rats of the Wistar strain (100–110 days old; 190–200 g b.wt) were used in the present investigations. The animals ware divided into four groups of 10 each.

Group I: Control (one day) received peanut oil. Group II: Experimental (1 day) received estradiol 17β (6 µg/100 g b.wt) for a day. Group III: Control (7 days) received peanut oil. Group IV: Experimental (7 days) received estradiol 17β (6 µg/100 g b.wt) for 7 consecutive days.

Groups I and II animals were sacrificed by cervical dislocation 24 h after the treatment. Groups III and IV animals were sacri-

ficed by cervical dislocation on the 8th day. Caput, cauda epididymides, seminal vesicles and prostate (all lobes) were removed immediately, freed from adhering tissues, rinsed, blotted and weighed on a torsion balance for further processing.

Total lipids were extracted from the tissues by a mixture of chloroform: methanol (2:1), followed by purification of the extracts by the procedure of Folch et al.[8]. Aliquots of the extract were used for determination of total cholesterol[9], glycerides[10] and phospholipid phosphorus[11]. The amount of phosphatide was calculated by multiplying the phosphorus content (mg) by 25 so as to convert the inorganic phosphorus value to phospholipid[12].

Separation of neutral lipids was achieved by thin layer chromatography on silica gel G (British Drug House, London, England) using a 2-dimensional solvent system. Solvent system I, N-hexane-diethyl ether-glacial acetic acid (60:40:1, v/v). Solvent system II, N-hexane-diethyl ether-glacial acetic acid (90:10:1, v/v). The separated fractions of cholesterol and glyceride were identified by placing the dried plates in an iodine chamber. The separated fractions were eluted with 5 ml of chloroform. Individual neutral lipids were quantified by methods described in the literature[9,10].

Individual phosphatides were separated by thin layer chromatography on glass plates coated with silica-gel G using chloroform-methanol-ammonia (7 N) (115:45:7.5, v/v). Phosphatides were identified as blue spots appearing after spraying the sample with molybdenum blue[13].The identified spots were eluted with a mixture of chloroform: methanol:formic acid:water (97:97:4:2, v/v). The phosphorus was quantified as described earlier[11].

Analysis of lipid fractions was done at least in duplicate. The results were analyzed for statistical significance using Student's t-test.

Results. In caput epididymis, estradiol-17β treatment for a single day resulted in an increase in total lipids which was essentially due to an increase in free cholesterol, triglycerides and phosphatidyl ethanolamine ($p < 0.01$) as well as phosphatidic acid and phosphatidyl inositol ($p < 0.02$). After seven days of treatment, the increase in total lipids was very significant ($p < 0.001$) and was mainly due to an increase in triglycerides, sphingomyelin, phosphatidyl ethanolamine and phosphatidic acid ($p < 0.001$) (table 1a).

In cauda epididymis, the total lipids were found to have decreased in both short and long-term studies ($p < 0.02$) although this was not reflected in any of the fractions (table 1b).

In seminal vesicles, with both short and long-term treatment, phospholipids were decreased ($p < 0.01$), mainly owing to a fall in phosphatidyl ethanolamine ($p < 0.01$). However, in long-term treatment, free cholesterol ($p < 0.01$) and phosphatidyl choline ($p < 0.02$) contributed to the decrease in total lipids (table 2a).

In the prostate, triglycerides were markedly elevated in both short and long-term groups ($p < 0.001$). Though total phospholipids remained unaltered in the short-term group, they were found to increase in the long-term group ($p < 0.01$) (table 2b).

Discussion. Epididymis is a site of high metabolic activity due to its various activities, which range from sperm maturation to storage of sperms[14]. The effects of androgens on these activities have already been reported[15]. The two segments of the epididymis show differential responses following administration of androgens[16]. From the present study, it seems that estrogens also have a differential effect. The accumulation of triglycerides in caput epididymis found in both short and long-term studies indicates the non-utilization of glycerides. This could be due to estrogen having an inhibitory action on pituitary gonadotrophins and thereby decreasing testosterone biosynthesis. It is also reported that estrogenization produces atrophy of tubules and increases tubular total lipids and phospholipids[17]. A similar type of degenerative change was observed in caput epididymal lipids which clearly shows that estrogen might have caused these effects because of its anti-gonadotrophic action. In cauda epididymis, though a slight fall in total lipids is observed, it is not well reflected in the other lipid fractions. It appears that the effect of estrogen is more pronounced in caput than in cauda. This may be due to the differential responsiveness of the two segments of the epididymis or may be due to an elevated glycerylphosphoryl choline synthesis in caput as compared to cauda[2].

In rats, estradiol has been found to cause regression of the accessory sex glands through a direct action on these tissues[7]. It is quite possible that it could also have interfered with lipid synthesis in the seminal vesicles as evidenced by a fall in total phospholipids. This could be due to interference with the secretory processes. Accumulation of triglycerides in the prostate can be attributed to the fact that owing to interference with the secretory activities of the prostate, the main source of expendable energy in the form of triglycerides was not used up. However, an increase in phospholipids may be due to an increase in cellular transport, which involves membrane permeability associated with certain degenerative changes.

The overall picture suggests that estradiol brings about differential effects on accessory sex glands. In caput, glycerides and phospholipids were accumulated but in seminal vesicles, phospholipids were depleted, while in the prostate glyceride was not utilized.

Table 1. Estrogen and accessory sex gland lipids values are mg/g tissue, mean ± SD of 10 animals per group

	Short term		Long term	
	Control	Experimental	Control	Experimental
a) Caput epididymis				
Total lipids	102.8 ± 9.4	108.7 ± 9.9[c]	100.8 ± 9.4	135.5 ± 10.8[a]
Free cholesterol	2.1 ± 0.4	3.0 ± 0.5[c]	2.3 ± 0.3	2.4 ± 0.4
Triglycerides	18.5 ± 1.8	19.0 ± 2.3[c]	18.3 ± 2.2	26.2 ± 2.8[a]
Phosphatidyl inositol	1.4 ± 0.2	3.0 ± 0.3[b]	1.0 ± 0.3	1.8 ± 0.2
Sphingomyelin	2.2 ± 0.3	2.6 ± 0.2	1.0 ± 0.3	3.0 ± 0.5[a]
Phosphatidyl choline	8.3 ± 0.6	8.8 ± 0.8	6.2 ± 0.9	7.8 ± 1.2[b]
Phosphatidyl ethanolamine	3.8 ± 0.4	4.2 ± 0.5[c]	2.3 ± 0.4	4.6 ± 0.6[a]
Phosphatidic acid	3.5 ± 0.4	5.3 ± 0.6[b]	3.0 ± 0.4	4.5 ± 0.6[a]
b) Cauda epididymis				
Total lipids	72.5 ± 7.5	68.6 ± 6.8[c]	70.7 ± 6.7	62.9 ± 5.8[c]
Triglycerides	19.8 ± 1.0	19.5 ± 0.9	19.5 ± 1.0	19.8 ± 1.0

[a] $p < 0.001$; [b] $p < 0.01$; [c] $p < 0.02$

Table 2. Estrogen and accessory sex gland lipids values are mg/g tissue, mean ± SD of 10 animals per group

	Short term		Long term	
	Control	Experimental	Control	Experimental
a) Seminal vesicle				
Total lipids	*87.7 ± 3.2*	89.6 ± 4.3	83.8 ± 3.4	60.2 ± 3.2[b]
Total phospholipid	20.4 ± 1.5	16.8 ± 1.2[b]	20.4 ± 1.3	16.8 ± 1.2[b]
Free cholesterol	3.8 ± 0.4	3.8 ± 0.2	3.9 ± 0.4	1.8 ± 0.3[b]
Phosphatidyl choline	7.5 ± 0.8	7.2 ± 0.9	7.8 ± 1.2	6.0 ± 1.0[c]
Phosphatidyl ethanolamine	6.3 ± 1.0	4.2 ± 0.8[b]	6.3 ± 1.4	4.8 ± 1.0[b]
Phosphatidic acid	1.8 ± 0.2	1.0 ± 0.1[c]	1.8 ± 0.2	1.3 ± 0.3
b) Prostate				
Total lipids	48.2 ± 3.5	43.1 ± 4.2	46.9 ± 3.9	40.2 ± 2.8
Total phospholipid	14.3 ± 1.4	14.2 ± 1.4	14.0 ± 1.0	15.8 ± 1.2[b]
Triglycerides	2.0 ± 0.3	6.0 ± 0.5[a]	2.1 ± 0.3	7.2 ± 0.5[a]

[a] $p < 0.001$; [b] $p < 0.01$; [c] $p < 0.02$

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Selective herbivory on mosaic leaves of variegated *Acer pseudoplatanus*

P. Niemelä, J. Tuomi and S. Sirén[1]

Department of Biology, University of Turku, SF-20500 Turku (Finland), 9 March 1984

Summary. The color mosaicism was observed to correlate with selective herbivory on different leaf areas of individual leaves of the variegated sycamore *Acer pseudoplatanus*. The mosaicism affected the content of nutrients and of phenolic compounds and also the mechanical properties of the different leaf areas; this offers an explanation for the observed selective feeding by insect herbivores.
Key words. Somatic mutation; color mosaicism; leaf chemistry; herbivory.

The variation in both plant nutrients and secondary metabolites can affect the feeding behavior of insect herbivores, which are expected to favor resource patches with high levels of nutrients, and low levels of those plant allelochemicals that have adverse effects on their growth, reproduction and survival[2,3]. The heterogeneity of food resources for herbivorous insects includes variation among host plant species[4], among individual plants of the same host species[5], and even among leaves of the same individual plant[6]. Selective feeding by insect herbivores on leaves of variegated *Acer pseudoplatanus* was studied in order to test the hypotheses that 1) genetic mosaicism in plants can affect the quality of food resources for insect herbivores and 2) this variation can lead to selective feeding on the foliage of individual plants[7]. The color mosaicism of sycamore provides a special case in which individual leaves can represent mosaics as a resource for herbivores.

Material and methods. Three variegated trees of *Acer pseudoplatanus* (var. 'brilliantissima') were sampled on 27 July 1983 in a garden in Cambridge, England. The lower branches of the trees were studied and leaves partially damaged by three insect herbivore groups, lepidopterans, coleopterans and sawflies, were removed and dry material was later analyzed in the laboratory. Because the leaves were collected in late July, the material contained the consumption that had taken place during the first half of the growing season which is generally the most intensive feeding period of lepidopterans[2]. Leaves with evidence of extensive feeding were omitted from the analysis because it was impossible to estimate the proportion of different color areas in the damaged parts of these leaves. The amount of grazing damage, consumed leaf area and biomass were estimated in the total sample of 83 leaves in order to analyze the feeding preference between different leaf areas in natural conditions. The expected values for these parameters were calculated from the proportional distribution of leaf area and biomass among the different color areas of the leaves. The differences between the observed and expected values were tested by means of the χ^2-test. The phenolic and nutrient contents in the different color areas were analyzed by combining samples from the investigated leaves where these areas were available. The Folin-Denis method was used to analyze phenolics and standard methods for nutrients.

Results and discussion. The normal green leaves contain plastids both in spongy and palisade parenchyma, whereas white mutant leaves and leaf areas contain colorless plastids in all the layers of mesophyll. Mixed, mosaic-like, leaf areas contain colorless mutant plastids either in the palisade or spongy parenchyma[8,9]. Because the mosaicism of sycamore varied considerably in the variegated leaves of the investigated trees, we divided the area of each leaf into three major classes: 1) white areas with no or few green chloroplasts, 2) mixed areas consisting of an irregular mixture of white and green cells, and 3) green areas consisting of a more or less uniform population of cells with green plastids. On average, about a half of the leaf area consisted of a mixture of white and green cells and the other half was divided between white and green areas (fig. 1A). The proportional distribution of leaf biomass was slightly more skewed towards mixed and green areas (fig. 1B) because leaf biomass per unit leaf area was lower for white than for

Chemical composition of different leaf areas of variegated *Acer pseudoplatanus*. All values on a dry weight basis

	White	Mixed	Green
Biomass (mg/cm^2)	0.93	1.09	1.59
Nutrients (g/kg)			
N	34	31	33
P	5.2	2.9	2.7
K	30	19	18
Ca	23	26	28
Mg	3.2	3.0	3.2
Phenolics (%)	4.55	6.47	10.95