of potassium administered in these experiments proved to be marginally adequate, which may have masked any effect the pineal may have exerted. Wurtman *et al.*(5) reported a slight increase in potassium excretion in pinealectomized rats. They did not control potassium intake, however.

The concentration of plasma sodium in pinealectomized rats appeared to be slightly elevated (to 149 meq./l compared with 142 meq. l) but no differences in plasma potassium levels were noted. Yamada(6) found no change in plasma sodium or potassium levels after pinealectomy and ablation of the habenular nucleus.

In these experiments the pinealectomized rats retained close to 180 μeq. per day more sodium than the control groups while adrenalectomized rats lost about 180 μeq. per day more than the control groups. These results strongly suggest that the pineal gland has an influence on the regulation of body sodium.

Summary. 1. Pinealectomized rats retained more sodium than sham-operated or unoperated control rats. 2. The amount of sodium retained by adrenalectomized-pinealectomized rats was not different from that retained by adrenalectomized rats. 3. No effect of the pineal gland on potassium retention was observed in these experiments. 4. It was concluded that the pineal gland may elaborate a hormone which causes loss of sodium and evidence is presented to suggest that it acts via the adrenal gland.

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Influence of Sex and Hair Growth Cycle on Lipid Composition of Mouse Epidermis.* (27213)

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Recent evidence indicates that the lipid (phospholipid, neutral lipid, cholesterol, cholesterol esters, and triglyceride fractions) composition of epidermis of Swiss female mice at the 12th (anagen II) and 22nd (telogen) days of the hair growth cycle does not differ appreciably(1). The epidermis of the 2nd to the 5th day (anagen I to anagen III) of the cycle is several times thicker than that on the 12th to the 22rd days of the cycle(2). This pronounced increase in epidermal thickness is associated with some interesting chemical changes. For example Carruthers *et al.*(3) showed that the activity of cytochrome oxidase at the 4th day of the cycle is twice that of the 12th and 22nd days, and appears to be related to cell division. Also Griesemer(4) has shown that the activity of cytochrome oxidase, succinic dehydrogenase and ribonucleic acid content of the epidermis of the 4th day of the cycle of the rat are significantly higher than at 6 days (telogen). Sex also appears to play a role in the lipid composition of mouse epidermis since methylcholanthrene (MC) treated male epidermis has a much higher cholesterol content than similarly treated female epidermis(5). These differences in enzyme activity and lipid composition prompted an investigation of the lipids of the epidermis at 3 distinctly different stages of the hair growth cycle, the results to which are reported here.

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Methods. Hair growth was initiated in young Swiss mice (2-4 months of age) by plucking of quiescent (telogen) hair from the entire dorsum of each mouse(6). Skin samples of the entire plucked area were obtained from mice killed by cervical dislocation on the 4th, 12th and 22nd days following plucking. The epidermis was separated from the dermis at 50°C by the procedure of Baumberger *et al.*(7). Total lipid of the epidermis was extracted as previously described(1, 5) and the phospholipid fraction was separated from the neutral lipids with acetone (8). Silicic acid chromatography was employed to separate the neutral lipid fraction into cholesterol esters, triglycerides and cholesterol(9).

Results and discussion. The total lipid content of male epidermis of the 4th day is considerably lower (6.2%) than that of the 12th day (10.4%) and 22nd day (13.2%) of the cycle (Table I) whereas the % phospholipid is highest in the epidermis on the 4th day of the cycle (43.5) and lowest on the 22nd day epidermis (19.8). A similar situation exists in female epidermis with respect to total lipid and phospholipid at various stages of the hair growth cycle. However, there is twice as much total lipid and about half as much phospholipid in the female epidermis as in the male epidermis at any comparable stage of the hair growth cycle. MC treated male epidermis has a high phospholipid and a low total lipid content whereas MC treated female epidermis has levels of phospholipid and total lipid about the same as that of 4th day female epidermis. The water content of the epidermis of both sexes at different stages of the hair growth cycle is nearly the same except for the 12th and 22nd days of female epidermis which have a somewhat lower water content than the other epidermises.

Further work is required to explain the significant difference between total and phospholipid content of epidermis as related to the day of the hair growth cycle. It is possible that the high phospholipid and low total lipid content of 4th day epidermis may be associated with cell division at a time of maximum epidermis thickness. This assumption is in line with the high phospholipid content of hyperplastic (MC treated) epidermis of both sexes (Table I), and particularly with that of squamous cell carcinomas (Sq. Ca.) in which many cells are dividing.

TABLE I. Lipid and Water Content of Epidermis.*

Day of cycle and sex	Total lipid, %†	Phospholipid, %‡	Water, %§
4 ♂ (5)	6.2 ± .1	43.5 ± 3.3	61.2 ± .6
12 ♂ (3)	10.4 ±1.4	26.5 ± 4.1	61.4 ± 1.7 (4)
22 ♂ (3)	13.2 ± .3	19.8 ± 2.4	59.1 (2)
3 PMC ♂ (5)	6.8 ± .8	30.4 ± 5.1	63.2 ± 1.4
4 ♀ (3)	12.0 ± .4	18.8 ± 2	61.5 ± 1.6 (5)
12 ♀ (4)	19.2 ±1.6	15.5 ± 2.6	54.1 ± 2.7
22 ♀ (3)	23.6 ±1.2	11.8 ± 1.2	53.7 ± 3.4 (5)
3 PMC ♀ (3)	13.2 ±1.6	15.5 ± 2.6	60.9 ± 1.1
18-24 PMC ♀ (5)	12.6 ± .5	20.8 ± 1.3	62.6 ± 1.7 (6)
Sq. Ca. ♀ (3)	3.3 ± .1	50.3 ± 3.3	80.0 ± .8

* Epidermis from 50 to 100 mice pooled for each analysis.

† Expressed as % of wet wt of tissue.

‡ Expressed as % of total lipid.

§ Expressed as

$$\frac{\text{Wet wt of tissue} - (\text{loss of } H_2O + \text{extractable lipid})}{\text{Wet wt tissue}}$$

No. of samples analyzed indicated in parentheses. Portions of this table taken from data previously published(5).

The neutral lipid components of epidermis at various days of the hair growth cycle showed interesting differences in that 4th day male epidermis has a cholesterol content (27%) four times greater than that found in 12th and 22nd day male or 4th day female epidermis (Table II). Also the triglyceride content of 4th day male epidermis is very low (22.4%), but there is a large amount (49%) of lipid material eluted from the silicic acid with the cholesterol esters. Part of the difference found in lipid composition between the 4th day male epidermis and that of the others is due to the fact that only about 70% of the lipid material of the 4th day male epidermis could be eluted from the silicic acid column whereas with the lipid material of the other epidermises chromatographed,

TABLE II. Neutral Lipid Composition of Epidermis.*

Day of cycle and sex	Cholesterol esters†			Triglycerides†	Cholesterol†	
	Cholesterol ester	FAS‡	Remainder§		Cholesterol	FAS‡
	%					
4 ♂ (4)	1.9 ± .2	9 ±2	48.7 ± 2.3	22.4 ± 1.6	26.6 ± 1.7	5.3 ± .9
4 ♀ (4)	—	—	17.3 ± 2.6	76.9 ± 1.5	6.0 ± .8	5.2 ± .4
12 ♂ (4)	1.0 ± .1	23 ± .6	20.9 ± 2.3	70.9 ± 3.7	5.3 ± 1.9	5.3 ±1.3
22 ♂ (2)	1.6	11.1	29	62.5	6.7	6.7

* Epidermis from 50 to 100 mice pooled for each analysis.

† Results expressed as % of these lipids eluted from column following chromatography of neutral lipid fraction.

‡ % fast acting sterols (FAS) in cholesterol ester and cholesterol fractions.

§ Remainder of fraction eluted with cholesterol esters.

No. of samples analyzed indicated in parentheses.

the recovery ranged from 84 to 92%.

The amount of material eluted with the cholesterol esters is about the same in 4th day female and 12th and 22nd day male epidermis, but these tissues have a much higher level of triglyceride than the 4th day male epidermis. The nature of the substances eluted with cholesterol esters requires further study. A good part of it (70-80%) is saponifiable. The amount of lipid material eluted with the cholesterol esters is very low (2.6%) in epidermis of female mice treated 3, 18 or 24 times with MC and low in 12th and 22nd day female epidermis, but it may reach a level of 6-8% in MC treated male epidermis. It is of interest that cholesterol content of 4th day male epidermis is about the same as that found in squamous cell carcinomas and in rapidly dividing Ehrlich ascites cells(5). The significance of the high cholesterol content in 4-day male epidermis is not known.

Summary. The total lipid content of 4th day male epidermis is much lower than that of the epidermis of the 12th and 22nd days of the hair growth cycle whereas the phospholipid level is highest in the 4th day male epidermis and lowest in the 22nd day epidermis. Female epidermis at days 4, 12 or 22 of the hair growth cycle has twice as much total lipid and approximately ½ as much phospholipid as male epidermis at comparable stages of the hair growth cycle. A similar relationship with respect to the total lipid-phospholipid ratio and day of the cycle exists in female epidermis as in male epidermis. Four-day male epidermis is also characterized by a very high cholesterol content, a low percentage of triglycerides and a high level of lipid material which is eluted from silicic acid with cholesterol esters.

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