tones (SC-5233, Aldactone and SC-4988) were approximately equipotent in correcting aconitine-induced arrhythmias. Of these compounds, only SC-4988 did not possess significant DOC-blocking activity in the mammalian kidney. The other 2 compounds in the series, similarly weak as DOC-blockers, were inactive as antiarrhythmic agents. SC-8109 alone reversed the arrhythmia induced by electrical stimulation. DOC and quinidine sulfate were effective against both types of flutter. The relationships of structure-antiarrhythmic activity and of DOC blocking-antiarrhythmic activities of the steroidal spirolactones are discussed.

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Influence of Hair Growth Cycle on Lipid Composition of Mouse Epidermis and Dermis.* (26075)

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Recent evidence indicates that the hair growth cycle in the mouse not only influences the thickness and morphology of the entire skin, but also has some effect on its chemical composition. Changes in chemical composition of skin as related to hair follicle activity (1,2,3) are mainly obtained from histochemical procedures(4). Little is known concerning alterations in lipids in skin during the hair growth cycle. Although the adipose layer of skin doubles in thickness during periods of active hair growth, total fat content is not significantly increased over that in skin containing quiescent hair follicles(3). Lipid composition of epidermis and dermis including adipose and muscle layers for skin in growing (anagen VI) and quiescent (telogen) hair follicles(5) is given in this report.

Methods. Hair growth was initiated in adult Swiss mice by plucking of quiescent (telogen) hair from the entire dorsum of each mouse(5). Skin samples were obtained from mice killed by cervical dislocation on the 12th (anagen VI) and 22-23rd (telogen) days following plucking(5). Fat below the panniculus carnosus was carefully removed after which the epidermis was separated from the dermis at 50°C by the procedure of Baumberger *et al.*(6). Dermal specimens included the adipose layer and panniculus carnosus. The variation in dermal preparations produced by scraping of these layers from

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the dermis was thus avoided. For analysis the epidermis from 50 to 100 mice and dermis from 6 to 10 animals were used. These tissues were stored under alcohol at a −25°C until ready for extraction.

Total lipid was extracted from the epidermis and dermis at room temperature by the procedure of Hanahan *et al.*(7). To facilitate extraction of dermis, it was homogenized in 120-160 cc alcohol at full speed (Omnimixer) then stirred for 4 hours at room temperature. The residue from the ethanol extraction was subsequently homogenized in 160 cc alcohol-ether 3:1 V/V as above and stirred for 3 hours. Final extraction was accomplished by stirring the residue again for 3 hours in 160 cc of the alcohol-ether solution. The fat-free residue of the tissue was dried overnight at 110-115°C and weighed. Solvents were removed from the lipid extract under vacuo over N_2 at 40-50°C(7). Phospholipids were then separated from the total lipid mixture with acetone(7). Acetone soluble material was dissolved in petroleum ether (60-70°C) and 300 mg were fractionated on silicic acid by the procedure of Hirsch and Ahrens(8). This method gave the cholesterol esters, triglycerides and cholesterol. Fast acting sterols were estimated on some of the epidermal cholesterol and cholesterol ester fractions by the procedure of Moore and Baumann(9), cholesterol by the method of Abell *et al.*(10) and triglycerides by weight. The iodine number of triglyceride fatty acids was determined by the micro absorption Wijs method after Luddy *et al.*(11).

Results and discussion. Hair follicles in a quiescent or actively growing state were without effect upon per cent water, total lipid, phospholipid or neutral lipid content of either dermis or epidermis (Table I). The water and neutral lipid content of epidermis is lower and phospholipid content higher than that of dermis. The slight effect of the hair growth cycle on skin water content is in contrast to the findings of Butcher and Crokoest who found a correlation between growth of hair follicles and water content in rat skin (12). Cholesterol and cholesterol ester content of anagen and telogen epidermis is about the same and considerably greater than that of anagen and telogen dermis which have comparable amounts of these constituents (Table II). The fast acting sterols of the epidermis are limited essentially to the cholesterol ester fraction of which they are a considerable proportion, in agreement with the observations of Kandutsch *et al.*(13) and Frantz *et al.*(14). There is no significant difference in other lipid components between anagen and telogen dermis or between anagen and telogen epidermis. The lack of salient chemical differences in epidermis or dermis during anagen and telogen stages of the hair growth cycle seems most unusual in view of the findings of Chase *et al.*(15), who showed that in skin of C57BL/6CH strain mice the corium and adipose layers during the anagen stage of the hair growth cycle are respectively 800 and 300 μ thick whereas in the telogen phase these layers are 400 and 200 μ thick, respectively.

Summary. Water and lipid composition of epidermis and dermis (including adipose and muscle layers) were determined during the anagen VI (growing) and telogen (quiescent) stages of the hair growth cycle. These stages

TABLE I. Water and Lipid Content of Dermis and Epidermis as Influenced by Hair Growth Cycle.

Tissue		Water	Total lipid	Phospholipid	Neutral lipid
		%			
Dermis-anagen*	(5)	64.8 ± 3.1	16.4 ± 1.6	7.8 ± 2.1	92.2 ± 2.0
Epidermis-anagen*	(4)	54.1 ± 2.7	19.9 ± .7	13.9 ± 1.5	85.1 ± 1.5
Dermis-telogen†	(5)	62.6 ± 3.4	19.0 ± 1.5	6.3 ± 1.5	93.7 ± 1.5
Epidermis-telogen†	(4)	53.7 ± 1.0	23.6 ± 1.2	11.8 ± 1.2	88.2 ± 1.2

* Anagen-dermis and epidermis removed from skin of 12th day of hair growth cycle.
† Telogen-dermis and epidermis removed from skin of 22-23rd day of hair growth cycle.
No. of samples analyzed indicated in parentheses. Epidermis of 50 to 100 mice was used for each analysis.

TABLE II. Lipid Composition of Epidermis and Dermis as Influenced by Hair Growth Cycle.

Tissue		Cholesterol esters, %	Cholesterol, %	Triglycerides	Iodine value, Wijs
Dermis-anagen*	(9)	.27 ± .06	.75 ± .2	99 ±.16	71 ±13
Epidermis-anagen*	(5)	2.3 ± .6 †	3.1 ± .5	95 ±.6	59 ± 3
Dermis-telogen*	(6)	.44 ± .09	.5 ± .14	99.1±.2	63 ± 8
Epidermis-telogen*	(4)	1.8 ± .4 ‡	2.1 ± .4	96 ±.4	62 ± 4

* Anagen and telogen dermis and epidermis as indicated in Table I. No. of samples analyzed indicated in parentheses.

† Fast acting sterols were 18% of esters, 9.4% of total steroids.

‡ " " " " 27% " " , 13% " " " .

of the hair growth cycle represent times of maximum morphological differences in thickness of the corium and adipose layers. Yet total lipid, phospholipid, neutral lipid, cholesterol esters, cholesterol and triglyceride content of epidermis during anagen VI and telogen stages of the hair growth cycle are not significantly different. The same is true for the lipid composition of the dermis. However, independent of hair growth activity, epidermis has a lower water content and a much greater level of cholesterol, cholesterol esters and phospholipid than dermis. The fast acting sterols of epidermis are limited largely to the esterified fraction.

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A Heat-Stable Hemagglutinin in Mouse Tissue Extracts.* (26076)

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It has been observed repeatedly that hemagglutination by the polyoma virus takes place at "refrigerator," but not "room," temperature(1). In our laboratory we have been investigating the possible presence of this agent in normal and neoplastic mouse tissues, using hemagglutination *in vitro* as a method of identification(2). This paper describes a heat-stable hemagglutinin, derived from extracts of mouse tissues and active at both 4° and 23°C, which was demonstrated in the course of these experiments.

Materials and methods. Four kidneys, 26 livers, 2 lungs and 6 mammary glands were obtained from 34 mice of 20 inbred strains.

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