perimental infection with spotted fever, and he was able to demonstrate tick-to-mouse-to-tick transfer of the infection in this host by the simultaneous feeding of infected nymphs and non-infected larvae of *D. andersoni* on the same animal. The present paper provides the first direct evidence of the occurrence of spotted fever infections in meadow mice in nature. However, it should be emphasized that the findings of this study do not necessarily give an indication of the actual prevalence of spotted fever infections among wild meadow mice, or for that matter, among the other species of mammals dealt with in this study. Furthermore, the role played by these mammals in the maintenance of spotted fever in nature remains to be determined.

Summary. The recovery of a strain of rickettsia belonging to the spotted fever group, presumably *Rickettsia rickettsii*, from the tissues of a wild meadow mouse, *Microtus pennsylvanicus*, is described. The evidence supporting its identification as *R. rickettsii* is presented and the implications of these findings are discussed.

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Received March 15, 1954. P.S.E.B.M., 1954, v85.

Skin Sterols IV. Distribution of Δ^7-Cholestenol.* (20951)

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Δ^7-cholestenol, 7-dehydrocholesterol, and certain other sterols react much more rapidly in the Liebermann-Burchard test than cholesterol itself, and this difference in rate has been made the basis for a determination of total "fast-acting" sterols(1,2). 7-dehydrocholesterol, which occurs in the skins of most species examined(3), can be measured spectrophotometrically and the concentration of Δ^7-cholestenol and related substances calculated by difference. Commercial cholesterol contains 0.62% of the Δ^7 sterol, and the results of a selenium test suggest that sterols from certain other sources may contain as much as 4.34%(4). Much larger amounts of Δ^7-cholestenol, 23-25% of the total sterols, have been isolated from rat skins(5).

Experimental. Twenty-five to 500 g of fresh skin (single skin or pooled samples) were extracted with acetone and the sterols isolated as the digitonide by procedures previously outlined(5). Dry digitonide containing 50-500 μg of sterol was placed in a standard Evelyn tube and dissolved by warming with 2 ml of glacial acetic acid. After cooling, 4.2 ml of reagent (freshly prepared mixture of 20 ml of redistilled acetic anhydride and one ml of H_2SO_4 cooled to room temperature) was added and the color read in an Evelyn colorimeter at 1.5 and 38 minutes.

* Published with the approval of the Director of the Wis. Agric. Exper. Station. Presented in part before the Amer. Soc. of Biological Chemists, New York, April 15, 1952, (*Fed. Proc.*, 1952, v11, 261).

TABLE I. Fast-Acting Sterol in Sterols from Skins and Certain Other Materials.

Source of sterols	No. of animals	Total fast-acting sterol (%)†	7-Dehydro-cholesterol (%)†
♂ Albino rat	5	36.3	.84
♀ " "	5	30.9	
♀ Wild "	1	32.9	
♂ Cotton "	8	30.6	.29
♂ C_3H mouse	10	21.2	1.20
♀ Guinea pig	14	12.7	.37
♂ Chinchilla	2	5.7	.14
♂ Rabbit	1	3.3	.24
♂ Pig	1	5.6	4.70
♂ Dog	3	2.1§	.28
Calf	1	1.8	
♀ Human*	1	1.5	.43
Human atheromatous aorta‡	1	0.3	
Chicken (4 wk old)	10	1.4	
♂ Cat	1	1.2	
Sheep	1	8.8	.19
Wool	1	15.2	.14
U.S.P. Lanolin		8.6	.12
Egg yolk	2 eggs	1.4	
Halibut liver oil		n‖	

* Normal skin from a mammary flap, supplied by Dr. W. H. Jaeschke, Wisconsin General Hospital.

† Numbers refer to percentages of fast-acting sterol or 7-dehydrocholesterol in sterol preparations isolated from various sources indicated.

‡ Supplied by Dr. H. J. van Baaren, Department of Pathology, University of Wisconsin Medical School.

§ Skin sterols from a fourth dog (adult female) contained 12.3% of fast-acting sterol with 0.28% of 7-dehydrocholesterol; this was not included in the average for dogs, since the other 3 samples analyzed yielded totally different values, *viz.*, 1.9, 1.8 and 2.7% of fast-acting sterol.

‖ n = negligible.

The concentrations of fast-acting sterols (as Δ^7-cholestenol) and of cholesterol were then calculated by means of the appropriate equations† $F = 0.197\ R_1$ and $S = 0.751\ R_2 - 0.367\ R_1$(1,2). For the determination of 7-dehydrocholesterol in certain of the samples the sterols were regenerated from the digitonides(6), crystallized from 80% ethanol, and the absorption at 2815 Å measured in ethanol solution in a Beckman spectrophotometer. Rats were fed an adequate semisynthetic diet or one deficient in pantothenic acid(7). Certain weanlings and adults were made hyperthyroid by feeding 0.25% of iodinated casein (Protamone) for 4 weeks, while others were made hypothyroid by feeding 0.1% of 6-n-propyl-2-thiouracil‡ for 8 days and 0.02% of the drug thereafter(8).

† F and S refer to mgs. of the two types of sterols in 6.2 ml of reaction mixture and R_1 and R_2 are L units at 1.5 and 38 minutes respectively.

Results. Fast-acting sterols in skins of various species. In general sterols isolated from the skins of rodents contained the highest concentrations of the fast-acting sterols. Rat skin sterols contained about 35% calculated as Δ^7-cholestenol and only 1% or less of the preparation was 7-dehydrocholesterol. Sterols from most other rodents also contained substantial proportions of total fast-acting sterol (Table I). The fast-acting component of pig skin sterols was mainly 7-dehydrocholesterol. Sterols from sheep skin (not free of wool), wool, and lanolin gave a strong color at 1½ minutes but the final cholesterol color was a yellow-green rather than the characteristic green of cholesterol. It is therefore doubtful whether the color at 1½ minutes was due entirely to Δ^7-cholestenol. The triterpene alcohols of wool are reported to react with the Schoenheimer-Sperry reagent with maximum absorption at 458 mμ(9,10). Digitonides from sheep skin were low in 7-dehydrocholesterol. Skin sterols from most other species including man contained only small amounts of the fast-acting sterols, 1.2-1.8%. A sample of a human atheromatous aorta was practically devoid of the new sterol, as was halibut liver oil (Table I). Contrary to a previous report(4) eggs contained only low amounts.

Effect of age and sex. After 3 weeks of age the percentage of Δ^7-cholestenol in the sterols from the skin of male rats was significantly higher than that in females. In both sexes there was an increase in fast-acting sterol with age, most of the increase occurring prior to maturity. The concentration of 7-dehydrocholesterol on the other hand remained relatively constant. The percentage of fast-acting sterol also increased with age in the sterols of guinea pig skin (Table II), but was only about half that in rats at comparable ages.

‡ Supplied by Calco Chemical Division, American Cyanamid Company.

TABLE II. Percentages of Fast-Acting Sterol in Skin Sterols from Rats and Guinea Pigs of Various Ages.

Species	Age (days)	No. of animals	Total fast-acting sterol (%) ♂	Total fast-acting sterol (%) ♀	7-Dehydro-cholesterol (%) ♂	7-Dehydro-cholesterol (%) ♀
Rat	2	17	——9.5——		——1.13——	
	21	17, 16	21.0	19.0		
	37	7, 6	34.9	22.2	1.52	1.43
	50	5		24.1		
	63	5	34.2			
	365	11, 11	38.9	23.0	1.02	0.98
Guinea pig	5	2	——4.9——		—	
	Adult	14	—	12.7	—	0.37

Localization, metabolic rate and pantothenic acid deficiency. In agreement with a previous observation(1) skin from the back contained more Δ^7-cholestenol and 7-dehydrocholesterol than the skin from the abdomen (Table III).

Rats deprived of pantothenic acid contained a normal percentage of fast-acting sterols in the skin, in spite of a substantial deficiency shown by an average weight of 112 g at 6 weeks compared to 278 g for control rats.

TABLE III. Fast-Acting Sterols of Rat Skin from Various Parts of the Body: Effects of Basal Metabolic Rate and of Pantothenic Acid Deficiency.

	Sex	Skin	No. of animals	Age	Total fast-acting sterol (%)	7-Dehydrocholesterol (%)
C*	♂	Back	4	1 yr	43.3	1.33
C	♂	Abdomen	4		31.1	.52
C	♀	Back	7		26.9	1.48
C	♀	Abdomen	7		19.3	.42
C	♂	Entire skin	5	3-8 mo	36.3	
He†	♂	"	4		38.2	
Ho‡	♂	"	4		41.0	
C	♀	"	5		30.9	
He†	♀	"	6		38.9	
Ho‡	♀	"	5		29.0	
C	♀	"	5	7 wk	24.1	
He†	♀	"	5		34.1	
Ho‡	♀	"	5		25.1	
C	♂	"	5	9 wk	34.2	
PA.d.	♂	"	8		35.3	

* C = control; He = hyperthyroid; Ho = hypothyroid; PA.d. = pantothenic acid deficient.

† Avg of 3.83 L of O_2 consumed/24 hr/dm^2—as compared to 1.6 L for normal rats.

‡ Avg of 1.03 L of O_2 consumed/24 hr/dm^2—as compared to 1.6 L for normal rats.

Alterations in basal metabolic rate falied to alter the percentage of fast-acting sterol in the skin of male rats, but in females there appeared to be an increase in the fast-acting component of the hyperthyroid animals (Table III).

Discussion. The very large amounts of Δ^7-cholestenol in the skin of rodents and its presence elsewhere in only trace amounts suggests that the sterol may have a particular function in rodent skin. One possibility is that it may be in equilibrium with 7-dehydrocholesterol, serving as a storage reservoir from which precursors of vit. D may be derived as needed. It would thus be of value to the rat to have more Δ^7-sterol in the skin of the back than in the abdomen, and the high amounts in the skin of the ear(1), where there is relatively little hair, would be particularly useful. An accessory function of the new sterol rather than an indispensible one is suggested by the fact that the skins of many species do not contain it in any substantial amount. Something more than storage is suggested by the constancy in the ratio Δ^7-cholestenol to cholesterol throughout adult life, since true storage substances, *e.g.*, hepatic vit. A, show no such constancy. Nor does the alleged relationship to vit. D explain why there should be more Δ^7-cholestenol in the skins of males than of females. The increase in fast-acting sterol in the skin of rats of both sexes prior to maturity parallels a similar change in the sebaceous secretions of the human scalp, where there is a marked increase in the concentration of the fungistatic fatty acids at puberty(11,12).

Since these experiments were completed

Langdon and Bloch have postulated that Δ^7-cholestenol may be an intermediate in the biosynthesis of cholesterol(13). If this should be true, the large amounts of Δ^7-cholestenol in rodent skin would imply a less effective conversion to cholesterol in this tissue than in the rest of the body or in the skins of other species.

Summary. 1. The concentration of Δ^7-cholestenol in rat skin sterols increased rapidly from birth to 5-6 weeks of age but remained fairly constant thereafter. Skin sterols from adult male rats contained from 35-40% of Δ^7-cholestenol while skin from females contained 25-30%. These proportions did not change in severe pantothenic acid deficiency nor in hypothyroidism. Hyperthyroidism, however, appeared to increase the concentration of Δ^7-cholestenol in sterols from female rats. 2. The total fast-acting sterol in the skins of the wild rat and cotton rat were similar to those of the laboratory rat, while sterols from the skins of the mouse and guinea pig contained about 15% of a fast-acting component. The amounts of Δ^7-cholestenol were low in the skins of the pig, rabbit, dog, calf, chicken, and cat. A sample of human skin and a human atheromatous aorta contained negligible amounts of the new sterol.

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Received March 15, 1954. P.S.E.B.M., 1954, v85.

Effect of 5-Bromouracil on Utilization of Thymidine by *Streptococcus faecalis.** (20952)

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The introduction of a halogen in substitution for a hydrogen or an alkyl group in a metabolite not infrequently results in the formation of a compound with antimetabolite activity. 5-Bromouracil has been reported by Hitchings *et al.*(1) and by Weygand *et al.*(2) to inhibit the growth of *L. casei* and *S. faecalis*, respectively, in basal media supplemented with thymine. In addition, Hitchings *et al.* (1) have observed that 5-bromouracil, in the absence of thymine or folic acid, supported limited growth of *L. casei*. In view of evidence that thymine and its desoxyriboside are incorporated by different mechanisms in these microorganisms(3), studies have been made of the effect of 5-bromouracil on the growth of *S. faecalis* in a medium supplemented with either thymidine or thymine.

* This investigation was supported by a grant from the American Cancer Society, as recommended by the Committee on Growth, National Research Council, and in part by a grant from the National Institutes of Health, U. S. Public Health Service.

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