the failure of the response. This could be the result of the newborn rabbit's inability to sustain the transferred cells, to coordinate the cellular and vascular elements responsible for expression of delayed hypersensitivity, or to perform an essential step in development of the hypersensitive state. The capacity to accept passively transferred delayed sensitivity appears to mature by 4 weeks of age, with gradual transition between 2 and 3 weeks of age. This phenomenon may be common to all animals expressing delayed sensitivity. It should be studied further, not only because of its possible significance in understanding the basis for delayed allergy, but because of important implications which this deficiency may have in understanding the relative inability of the newborn to defend himself against infection and toxic stimuli.

Summary. The newborn rabbit is limited in its capacity to accept the passive transfer of delayed hypersensitivity, as is the case in the human newborn. The ability to accept passively transferred delayed sensitivity develops by 4 weeks of age in newborn rabbits.

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Biosynthesis of Squalene by Rat Preputial Gland.* (26141)

JAMES F. PATTERSON (Introduced by E. B. Astwood)
Depts. of Medicine, Pratt Clinic-New England Center Hospital and Tufts University School of Medicine, Boston, Mass.

During a study of effects of hormones on the rat preputial gland, it was found that this gland appears to synthesize and accumulate squalene. Preputial glands are present in both sexes of several species of rodents as a pair of small, clubshaped organs lying along the urethra, and emptying their oily secretion into the preputial sac. The function of these glands is unknown, but they contain large amounts of β-glucuronidase and acid phosphatase. A mouse preputial gland tumor incorporated C^{14} from acetate into sterols when incubated *in vitro* while normal mouse preputial glands were less active in this regard(1). Administration of androgens to rats of either sex causes a marked increase in size of preputial glands, whereas, hypophysectomy leads to atrophy. The preputial gland is similar in microscopic structure to the cutaneous sebaceous glands, and in the rat, its responses to administration of hormones are thought to be similar to those of the sebaceous glands(2).

Methods. Preputial glands were obtained by excision immediately after death (by blow on head) from normal Sprague-Dawley male rats which were 6 to 7 weeks old and weighed 160-200 g. The glands, which weighed from 20 to 75 mg, were dissected free of fat and other tissue, and were incubated whole or split longitudinally in 1 ml of Krebs-Ringer phosphate buffer (pH 7.4) to which had been added sodium acetate 1-C^{14} (12.5 μc of C^{14} in 25 μmoles of sodium acetate). Incubation was carried out in a Dubnoff shaking incubator at 37°C under a flow of oxygen and shaking at 100 strokes per minute for 3 hours. The tissue and incubating fluid were then heated in a steam bath overnight with alcoholic potassium hydroxide solution in tubes sealed under nitrogen. The nonsaponifiable lipids were extracted with petroleum ether and separated into squalene and sterol fractions by column chromatography on alu-

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TABLE I. Incorporation of C^{14} from Acetate into Squalene and Sterols by Various Tissues *In Vitro*.

Tissue	No. of samples	cpm/mg tissue	
		Squalene	Sterol digitonide
Rat preputial glands	25	78 (52 -108)*	2.8 (1.4- 6.2)
Human skin	34	32 (10 - 64)	1.4 (.4- 6.6)
Mouse preputial glands	10	1.2 (.5- 2.5)	5.3 (3.4- 6.6)
Rat liver	10	1.4 (.6- 2.5)	22 (12 -39)

* Range of values.

mina. The squalene was counted as an infinitely thin layer in cup planchets, and the sterols as an infinitely thin layer of the digitonide on filter paper in a thin end window gas flow counter. Results were expressed as counts per minute, per mg. of original tissue. It is recognized that this method of expressing the radioactivity does not give absolute comparisons of amount of incorporation into each lipid fraction, but it is felt that it is adequate to show differences in incorporation into these 2 lipids by different tissues. Further details of the method have been described(3) in which human skin was studied. Since incorporation of C^{14} into squalene was of special interest, the "squalene fraction" was further examined. In previous studies, it was found that the squalene fraction obtained by alumina chromatography of the nonsaponifiable lipids, contained squalene in good yield without detectable radioactive contaminants. To identify this fraction more accurately, the hexahydrochloride derivative was made. Commercial shark squalene was added to the radioactive squalene fraction and after rechromatography on alumina, the hexahydrochloride of the mixture was prepared(4). After careful washing and recrystallization, specific activity of the squalene hexahydrochloride was found to be 90% of that of the original squalene mixture, indicating that the radioactive squalene fraction was truly squalene. A larger amount of untagged squalene (about 4 mg) was prepared from 2.6 g of rat preputial glands (25 rats) in the same manner as the individual gland studies. The infra red spectrum of this substance (studied as a film without solvents)† was identical with that of shark squalene, and also with the spectrum for redistilled shark squalene(5).

Results. Preputial glands from young male rats incorporated C^{14} from acetate into squalene more actively than any other tissue that we have studied. Conversely, there was relatively little incorporation of C^{14} into sterols (Table I). (Preputial glands from female and male rats of all ages showed the same pattern, though actual amount of incorporation into squalene varied from 25 to 110 cpm/mg). Human skin exhibits the same pattern of incorporation of C^{14} into squalene and sterols. Representative samples of breast and scapular skin obtained at operation or biopsy are included in the table. Previous differential studies demonstrated that incorporation of C^{14} into squalene occurred mainly in the dermis, the site of sebaceous glands, while the epidermis carried on the more usual pattern of incorporation of C^{14} predominately into sterols(3). Mouse preputial glands (from young males, 26-30 g) on the other hand, followed the pattern of most other mammalian tissues in that they incorporated C^{14} more actively into sterols than squalene (Table I). Rat liver, which was incubated as 100 mg cubes, is included for comparison and shows the well known pattern of incorporation mainly into sterols. It is generally felt that squalene is a normal step in the synthesis of sterols by most tissues, and thus little of this substance accumulates. It is not clear why squalene accumulates in preputial glands or in human skin. It is possible that there is absence of the factors necessary for conversion of squalene to sterols or that inhibitors of the process are present in human skin and preputial glands. It is also possible that the squalene produced by these tissues

† Infra red spectrography was kindly performed by Dr. S. Wang, Dept. of Physiology, Tufts Univ. School of Med.

is biologically inactive because of some small difference in chemical structure. A final possibility is that the initial content of squalene in these tissues acts as a trap for radioactive squalene formed during the course of sterol synthesis. If this is so, the original source of their squalene is unexplained.

Summary. Preputial glands from rats, when incubated with C^{14} acetate show a marked incorporation of C^{14} into squalene, a sterol precursor, and little into sterols. In this regard, the preputial glands differ from many other mammalian tissues which show incorporation of C^{14} mainly into sterols and little into squalene. Human dermis, which contains sebaceous glands, is another mammalian tissue which shows preferential accumulation of the C^{14} into squalene. The mechanism of the difference is unknown, though it seems likely that factors necessary for conversion of squalene to sterols are missing in these tissues, or that an inhibitor of this reaction is present.

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Studies of Bovine Erythrocytes in Anaplasmosis. I. Flocculating Properties of Stromata in Water.* (26142)

GEORGE T. DIMOPOULLOS AND DAVID M. BEDELL

Dept. of Veterinary Science, Agric. Exp. Station, Louisiana State University, Baton Rouge

The erythrocytic membrane has been shown to be intimately connected with phospholipids as structural components(1). Chemical analyses of stromata in various anemias have shown that there are alterations in content of phospholipids(2,3). New evidence of surface structural changes obtained by visual methods and involving the phospholipids of the erythrocyte in bovine anaplasmosis was collected while studying the mechanism of the anemia produced. Observations showed that the flocculating characteristics of erythrocytic stromata prepared from blood of uninfected calves and calves infected with *Anaplasma marginale* were different from each other when suspended in water.

Materials and methods. Stromata were prepared from erythrocytes of 14 normal intact, 6 splenectomized uninfected, and 18 splenectomized infected calves. The latter group of animals was inoculated by the subcutaneous route with 10 ml of infective blood having approximately 30% of the erythrocytes showing anaplasma bodies. Jugular vein blood samples were collected periodically in 50-ml amounts in 0.3 ml heparin sodium solution (1,000 U.S.P. units/ml). The erythrocytes were thoroughly washed and packed in 0.85% sodium chloride solution by centrifugation at 700 × g for 30-minute intervals at 4°C. They were then mixed and lysed in 20 volumes of distilled water at 4°C which was saturated with carbon dioxide. The carbon dioxide saturated distilled water possessed a pH of 3.8 at this temperature. The material was allowed to settle in tall cylinders overnight at 4°C and the clear red supernatant fluid was aspirated off. The fluffy appearing stromata were poured into 250-ml centrifuge bottles and washed in unbuffered distilled water by centrifugation at 700 × g for 10-minute intervals at 4°C. This unbuffered distilled water had a pH of 5.4. Stromata have been prepared by lysing cells and

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