adult animals in which it will be possible to study the impact of the "graft versus host" reaction in absence of such complicating factors as neonatal immunological incompetence, neonatal failure of gamma globulin and protein synthesis, rapid growth and development, shifting blood and tissue volume, or of the devastating general effects of irradiation. Investigations of the effect of this "graft versus host" reaction on protein synthesis, immunological competence of the host, and the effect of prior immunological commitment of administered cells on capacity to develop the reaction, may now be effectively investigated.

Summary. 1. Runt disease in adult mice of the Z (C3H) strain made previously tolerant of A tissue by antigenic exposure at birth was obtained by injecting these animals intravenously with large numbers of immunological competent spleen cells taken from A strain donors. 2. The main pathological findings in these animals were: a) marked body weight loss; b) Splenomegally and c) Atrophy of lymphoid tissue followed by death.

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Anaerobic Conversion of Acetate to Squalene by Rat Skin.* (26407)

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When rodent skin is incubated with labeled acetate *in vitro,* sterol synthesis is substantial (1) and much of the label is found in sterols other than cholesterol(2). The relative activities of these sterols cannot however, be taken as a measure of precursor-product relationships since skin is so complex anatomically, and rate of penetration of acetate into different parts of the skin probably varies. Grinding of the skin has thus far yielded inactive preparations. Another approach to the problem might be possible if an active sterol precursor could be established within the tissue without the sterols themselves becoming labeled. The present studies describe conditions for accumulation of labeled squalene in rat skin.

Methods. In each experiment, one gram of rat skin was cut into pieces of 10 sq mm and incubated for 3 hours at 37°C in 6 ml of saline-phosphate buffer which contained 2.0 μc/0.4 μM of acetate-1-C^{14}. The buffer (pH 7.4) was prepared by mixing equal volumes of isotonic saline and 0.1 M sodium phosphate solutions. Potassium chloride and magnesium sulfate were added to final concentrations of 0.0125 M and 0.0026 M respectively. Nitrogen was bubbled into the solution for 10 minutes; all incubations were carried out under nitrogen. At the end of incubation period, the pieces of skin were filtered from the medium and each gram was saponified for 4 hours under nitrogen in 10 ml of 50% aqueous ethanol (v/v) which contained 20% of KOH (w/v) and 7.5% of pyrogallic acid (w/v). The unsaponified fraction was ex-

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TABLE I. Effect of Glucose and Insulin on Incorporation of Acetate into Squalene and Sterols by Rat Skin.*

	Additions				Activities	
	Glucose, M	Insulin, u/flask	Ferricyanide, M	Atmosphere	Squalene, cpm/g skin	Sterols,† cpm/g skin
1.	—	—	—	O_2	301	973
2.	—	—	—	N_2	186	3
3.	.005	—	—	"	443	—
4.	.017	—	—	"	618	—
5.	.05	—	—	"	768	21
6.	.17	—	—	"	622	—
7.	—	1.0	—	"	188	—
8.	.05	.25	—	"	803	—
9.	.05	1.0	—	"	858	12
10.	.05	2.0	—	"	862	—
11.	.05	—	.0075	"	1090	32
12.	.05	1.0	.0075	"	1620	31
13.	.025	—	—	O_2	492	1370

* Each flask contained 1 g of skin, 6 ml of saline-phosphate buffer, 2 μc/0.4 μM of acetate-1-C^{14} at pH 7.4. All incubations were for 3 hr at 37°C. Each value represents the avg of at least 8 samples from 3 separate incubations.

† Precipitated as the digitonides.

tracted into pentane which was washed, dried and concentrated under nitrogen. The fraction containing squalene was obtained by chromatography on aluminum oxide(3). Purification of the labeled sterols and assays of sterol and radioactivity have been described (4).

The squalene content of the first chromatographic fraction was estimated as follows: samples from whole skin (7750 cpm) or from "dermis" (3350 cpm)† were diluted to 50 mg with unlabeled squalene and reacted with dry HCl. The hexahydrochlorides of squalene were recrystallized from a mixture of absolute methanol and chloroform until the mother liquor and precipitate reached the same constant specific activity calculated as squalene. The specific activity of 4 samples of squalene from whole skin ranged from 117 to 135 cpm/mg and that from the "dermis" was between 57.3 and 62.8 cpm/mg. At least

$$75.5 \text{ to } 87.2\% \left(\frac{\text{Sp. Act. final} \times 100}{\text{Sp. Act. initial}} \right)$$

of the activity isolated in the first chromatographic fraction of whole skin extract was squalene; 85.5 to 93.7% of the activity isolated in the first fraction of "dermis" was squalene. No change in activity was observed when the crude fractions were reacted with thiourea.

Experimental. When rat skin was incubated with labeled acetate under nitrogen, the incorporation of label into the unsaponified fraction (sterol and squalene) was only 14% of that observed under aerobic conditions. Sterol synthesis virtually ceased under anaerobic conditions, and instead, labeled squalene accumulated (lines 1 and 2, Table I). This is in harmony with the finding that liver homogenates require oxygen for conversion of squalene to lanosterol(7). When glucose was added to the medium at concentrations of 0.005 to 0.17 M, a marked increase in squalene synthesis took place (Table 1, lines 3 to 6). Lower concentrations failed to affect squalene synthesis. Boiled liver filtrate (equivalent to 0.5 g liver/flask), glucose-6-phosphate (0.005 M), succinate (10^{-4}M), α-ketoglutarate (10^{-4}M) or ATP, DPN or TPN (1 mg each/flask) produced little or no stimulation.

Insulin (U-40, E. R. Squibb & Sons) enhanced the uptake of acetate into squalene in presence of glucose (lines 7 to 10, Table I). Amounts less than 0.25 units/flask were less active and 1 to 2 units/flask produced consistently higher results. In the presence of both ferricyanide and glucose, the effect of added insulin was even greater (lines 11 and 12, Table I). Upon addition of insulin, ac-

† The epidermal mucosa (0.3 to 0.5 mm) was removed by a single horizontal section made prior to incubation. The dermis in this case contained essentially all of the sebaceous glands (5,6).

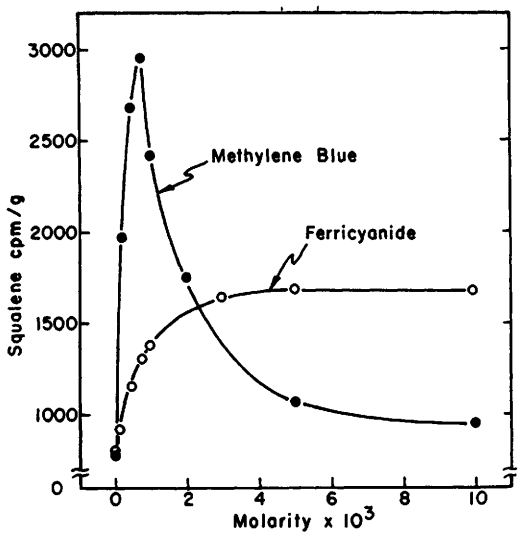

FIG. 1. Effect of methylene blue and ferricyanide on anaerobic incorporation of acetate into squalene. Each flask contained 6 ml of buffer, glucose (0.05 M), 2 units of insulin, 2 μc/0.4 μM of acetate-1-C^{14} and 1 g of skin and was adjusted to pH 7.4 after addition of the electron acceptor. Although there was no quantitative determination of extent of dye reduction, all of the methylene blue was in the leuco form when concentration was less than 10^{-3}M. Methylene blue was added as its chloride; ferricyanide as $K_3Fe(CN)_6$. Additions of potassium ion (KCl) in absence of ferricyanide produced no change.

tivity in the sterol fraction was increased by only 28 cpm/g skin.

When the buffer contained both glucose and insulin, addition of 10^{-3}M methylene blue produced a 2.5-fold increase in the activity of squalene (Fig. 1). However, with greater concentrations of methylene blue, the activity dropped off rapidly. Additions of ferricyanide to the same medium only doubled the incorporation over a rather wide range of concentrations.

In some experiments, after 3 hours of incubation under nitrogen, the pieces of skin were filtered from the anaerobic medium, washed with saline and resuspended in freshly-oxygenated, saline-phosphate buffer containing 27 mg of glucose and 4μM of unlabeled sodium acetate in each flask. The skin was then incubated for 5, 12, 60 and 90 minuates under oxygen. The squalene labeled *in situ* disappeared rapidly and activity accumulated in the sterol fraction (Table II). After 90 minutes of aerobic incubation 88% of the activity which was lost from the squalene fraction was recovered in the sterol fraction. As much activity was converted from squalene to sterol in 90 min as previously observed for 3 hour experiments with labeled acetate (lines 1 and 13, Table I; Table II). In subsequent experiments, significant amounts of labeled sterols were found after 2 minutes of aerobic incubation. Although the activity incorporated into the squalene fraction under nitrogen was greater when methylene blue was the electron acceptor, only a few counts were transferred to the sterols during aerobic incubation.

Conclusions. Others have observed the stimulation produced by glucose(8), insulin (9) and electron acceptors(6,10) on lipogenesis in various tissues under oxygen. The present data show that a combination of these materials enhanced the anaerobic conversion of acetate to squalene in pieces of rat skin. The amount of activity incorporated (1620 cpm/g skin) was sufficient to serve as labeled squalene for subsequent conversions of squalene into sterol precursors or the sterols themselves.

In contrast to human skin, rat skin contains relatively little squalene(11), and most of the activity incorporated into the unsaponified fraction of rat skin under oxygen is associated with the sterols rather than with squalene. Aerobic incubation of human skin with acetate, on the other hand, yields substantial amounts of labeled squalene(5,12). Presumably, the yield of labeled squalene by human skin could be increased by an anaerobic incubation in fortified buffers like those used in the present study.

TABLE II. Conversion of Squalene to Sterols by Rat Skin.

Length of incubation, min.	Activities		
	Squalene	Sterols	Δ Squalene
	cpm/g skin		
0	1730	43	—
5	1380	221	350
12	677	390	1050
60	474	853	1260
90	403	1160	1330

The skin was previously incubated for 3 hr under nitrogen. During the aerobic part, each flask contained 27 mg glucose and 4 μM unlabeled acetate.

Summary. 1. Rat skin incubated anaerobically accumulated labeled squalene from acetate and only negligible amounts of labeled sterols. Incorporation of acetate into squalene was enhanced markedly by the presence of glucose in the medium. 2. Insulin, ferricyanide and methylene blue enhanced squalene synthesis *in vitro* in presence of glucose. Increasing amounts of insulin or ferricyanide produced regular increases in squalene synthesis until a plateau was reached; methylene blue on the other hand showed a sharp maximum at 10^{-3}M, with a decline at higher concentrations. 3. Skin containing labeled squalene accumulated labeled sterol on subsequent incubation under oxygen.

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Activity of Pituitary-Adrenal Cortex Axis During Acute and Chronic Reserpine Treatment.* (26408)

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It has been demonstrated that reserpine treatment affects endocrine systems(1,2,3). It was reported that reserpine interferes with the estrous cycle in mice and rats(1,4), ovulation and menstruation in monkeys(5), prolactin production and secretion in rats, rabbits and women(6,7,8), and normal function of the pituitary-gonadal axis(9). Reserpine was found also to effect recession of exophthalmus(10), thyroid inhibition(11), and to have antithyroid activity(12).

It has been suggested that adrenal hypertrophy and ACTH hypersecretion occur during reserpine administration(1,13,14,15,16). On the other hand, inhibition of pituitary ACTH release after administration of reserpine(17,18), and suppression of adrenal hypertrophy in male mice(19) have been reported.

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In view of these conflicting reports we have studied the mode of action of reserpine treatment on the pituitary-adrenal cortex axis.

Materials and Methods. Reserpine stock solution was prepared according to the method described in Martindale's Extrapharmacopoea(1959), and diluted to the required concentration. Studies were carried out on adult male guinea pigs, adult male rats, and on human patients.

Exp. 1. Five adult guinea pigs (600± 30 g) were injected subcutaneously with 0.2 mg/kg reserpine daily for 7 days. The animals were then sacrificed, the adrenals weighed and histological sections prepared and studied.

Exp. 2. Ten adult male rats (200 ± 20 g) were injected subcutaneously with 0.01 mg/kg reserpine daily for 10 and 40 days respectively while 5 animals served as controls.