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12. During the insulin treatment, diabetic and control animals given insulin gained 103 ± 11 and 91 ± 20 g, respectively, whereas diabetic and control animals given saline gained 14 ± 12 and 54 ± 9 g, respectively (differences in weight gains between insulin-treated and saline-treated rats and between diabetic rats given saline and control rats given saline were significant at $P < .05$, Newman-Keuls test).
13. Preliminary studies indicate that daily administration of protamine zinc insulin (4 U per rat, subcutaneously), initiated 5 days after alloxan treatment, also prevents the increase in [^{3}H]spiperone binding observed 6 weeks after alloxan injection.
14. Short-term administration of DA agonists has been reported to decrease [^{3}H]spiperone binding in rats with supersensitive DA receptors but to have no effect on or to increase DA receptor sensitivity in normal rats [D. R. Howlet and S. R. Nahorski, *Brain Res.* **161**, 173 (1979); S. J. List and P. Seeman, *Life Sci.* **24**, 1447 (1979); P. Muller and P. Seeman, *Eur. J. Pharmacol.* **55**, 149 (1979)].
15. Neither glucose (1 n*M* to 6 m*M*) nor insulin (20 to 320 μg/liter), added to striatal membranes in vitro in a range of concentrations which exceeded those found normally in the brain, altered spiperone binding [S. R. Nelson, D. W. Schulz, J. V. Passoneau, O. H. Lowry, *J. Neurochem.* **15**, 1271 (1968); J. Havrankova, D. Schmechel, J. Roth, M. Brownstein, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5737 (1978)]. Furthermore, many of these concentrations of glucose and insulin probably greatly exceeded these encountered in the membrane preparations used in the investigations reported here, since all membranes were washed with buffer prior to analysis of spiperone binding.
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19. We thank G. Maengwyn-Davies for her helpful comments during preparation of the manuscript. We also thank Ayerst Laboratories, Inc., for supplying (+)-butaclamol. Supported by the Pharmacology Research Associate Program of the National Institute of General Medical Sciences (C.F.S.).

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28 August 1981

Correcting the Phenotype of the Epidermis from Chick Embryos Homozygous for the Gene Scaleless (*sc/sc*)

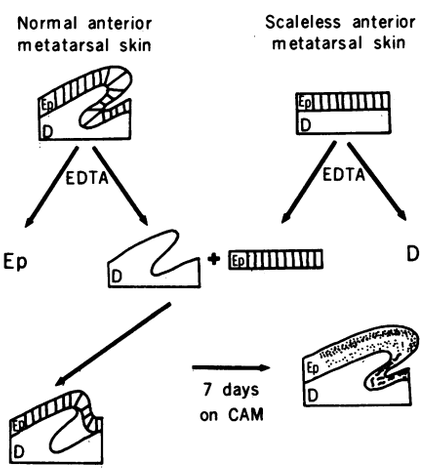
Abstract. *Scutate scales are completely missing in the scaleless* (sc/sc) *mutant chicken. Organ cultures consisting of epidermis from* sc/sc *embryos combined with normal (+/+) scale dermis of the same developmental age produce the scaleless phenotype, but the same scaleless epidermis in combination with normal dermis from more differentiated embryonic scales forms perfectly normal scales.*

The epidermal and dermal components of the avian skin undergo inductive tissue interactions that result in the morphogenesis of feathers and scales (*1, 2*). The scaleless mutant chicken (*3*) carries a recessive, autosomal mutation which, in the homozygous state (*sc/sc*), results in the lack of most of the feathers on the body and all of the scutate scales that are located along the anterior metatarsi. Biochemical, x-ray diffraction, and fine structural studies comparing normal scutate scale epidermis and the scaleless epidermis show that the beta stratum (made up of beta-type keratins), which characterizes the hard, platelike surface of the normal scutate scale, is totally missing from the scaleless epidermis (*4–8*).

Scutate scales first appear as discrete epidermal thickenings (scale placodes) along the anterior metatarsal surface of the legs and feet at 10 days of incubation (stage 36). Reciprocal epidermal-dermal tissue recombinations between normal and mutant anterior metatarsal skin at stages 36 and 37 have demonstrated that the scaleless defect is expressed initially by the embryonic epidermis (*9, 10*). Tissue recombination studies further show that the scaleless dermis itself becomes defective as development progresses (*11, 12*). It is not until stage 38, 2 days after initial scale placode formation, that the

Table 1. Development of overlapping scutate scales in recombinant grafts between scaleless anterior metatarsal epidermis (stage 36 to 42) and stage 40, 41, or 42 normal anterior metatarsal dermis cultured for 7 days on chick chorioallantoic membrane.

Stage of scaleless epidermis	Recombinant grafts		Macroscopic appearance of recovered recombinant grafts	
	Number done	Number recovered	With scales	Without scales
36	5	2	2	0
37	2	1	1	0
38	4	4	4	0
39	11	6	6	0
40	25	17	17	0
41	20	14	14	0
42	12	10	9	1



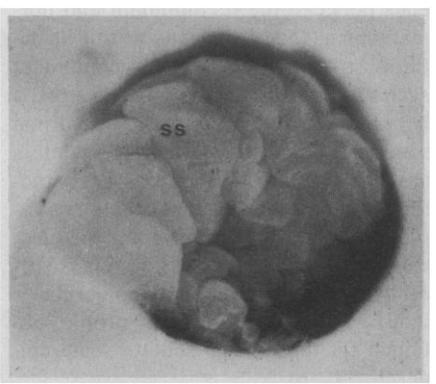


Fig. 1 (left). Diagram of the tissue recombination experiments between scaleless anterior metatarsal epidermis and normal scutate scale dermis. After 7 days of growth on chick chorioallantoic membrane these grafts developed typical scales with an outer epidermal surface (dots) and an inner epidermal surface (wavy lines). Abbreviations: *Ep*, epidermis; *D*, dermis; *CAM*, chorioallantoic membrane. Fig. 2 (right). Photograph showing the scales formed from scaleless anterior metatarsal epidermis recombined with normal scutate scale dermis and grown for 7 days on chick chorioallantoic membrane (×35); *SS*, scutate scales.

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normal anterior metatarsal dermis acquires its ability to induce normal scutate scales (with a beta stratum) from either presumptive feather epidermis (*13*) or the chorionic epithelium (*14*). It is at this stage of scale morphogenesis that an actual scale ridge is present for the first time (*11*, *15*). This ability to induce scales is never acquired by the anterior metatarsal dermis of scaleless mutants (*10–12*), nor does a scale ridge ever form.

We have reasoned that the lack of epidermal placode formation and morphogenesis (period of time from stages 36 to 38) in scaleless mutants results in the scaleless dermis not acquiring its later scale-inducing properties (*11*, *15–17*). Therefore, in the present study, scaleless anterior metatarsal epidermis, which never makes scale placodes, was allowed to interact with a normal dermis that had become a strong scutate scale inducer—that is, normal anterior metatarsal dermis beyond stage 38 of development. As illustrated in Fig. 1, we recombined scaleless anterior metatarsal epidermis (stages 36 to 42) with stage 40, 41, or 42 normal anterior metatarsal dermis. After 7 days of growth on the chick chorioallantoic membrane, the mutant epidermis of all stages developed typical overlapping scutate scales (Table 1), without itself undergoing placode formation. In conformity with earlier observations (*13*, *14*), we noticed decreased scale-inducing ability in normal anterior metatarsal dermis at stage 38 and total loss of this ability at stage 37 (*18*).

In addition to the macroscopic observation that scutate scales form in our recombinant grafts (Fig. 2), we have used other techniques to verify that our recombinant scales are true scutate scales. Histologically, there is no difference between the recombinant and normal scales (*15*, *18*). Extensive fine structural studies of several recombinant scales demonstrate the presence of a beta stratum, which typifies the normal scutate scale (*7*, *18*), whereas scaleless anterior metatarsal epidermis is characterized by the presence of an alpha stratum only (*8*, *18*). Furthermore, the electrophoretic profile of the keratins isolated from recombinant scale epidermis is indistinguishable from the profile obtained for the normal scutate scale epidermis (*4*, *18*).

Our results show that normal scutate scale dermis, which itself has undergone appropriate tissue interactions, can provide the necessary inductive influence for scale development to the scaleless epidermis and thereby correct this genetic defect. We find that, although the scaleless gene prevents the epidermis from ever making scale placodes, there is not an absolute requirement for placode formation in order for the scaleless genome to respond to later inductive cues—that is, cues that direct the differentiation of the epidermal surfaces of scutate scales and the production of their specific protein products.

Maderson (*19*) suggested that tissue interactions in embryogenesis serve as the basis for morphological changes during evolution. In fact, Kollar and Fisher (*20*) demonstrated the existence of quiescent genes for enamel synthesis in chick epithelium by recombining the chick epithelium with the inductive mesenchyme of first mandibular molars of mouse embryos. In the scaleless mutants, the genes for specific scale products (the scale beta keratins) also remain unexpressed, again, as the result of an abnormal tissue interaction.

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21. Supported by NSF grant PCM-8011745.

10 June 1981; revised 3 August 1981

Vertebrate Cell Cycle Modulates Infection by Protozoan Parasites

Abstract. *Synchronized HeLa cell populations were exposed to* Trypanosoma cruzi *or* Toxoplasma gondii, *obligate intracellular protozoan parasites that cause Chagas' disease and toxoplasmosis, respectively, in humans. The ability of the two parasites to infect HeLa cells increased as the HeLa cells proceeded from the G_1 phase to the S phase of their growth cycle and decreased as the cells entered G_2-M. Characterization of the S-phase cell surface components responsible for this phenomenon could be beneficial in the development of vaccines against these parasitic diseases.*

Some protozoan parasites require an intracellular environment for completion of part of their life cycle. This necessitates that the parasite find and enter a suitable host cell. The attraction of protozoan parasites to vertebrate cells has been demonstrated (*1*); the degree of attraction varies with vertebrate cell type. Once within the domain of a suitable host cell, the parasite attaches to and enters it. The attachment phase involves recognition by the parasite of host cell receptors (*2*). In this report, we describe a modulation of the attachment and subsequent entry phase of two obligate intracellular protozoan parasites, *Trypanosoma cruzi* and *Toxoplasma gondii*, is dependent on the position of the vertebrate host cell in its growth cycle. The influence of host cell cycle on susceptibility to infection was determined by exposing synchronized and nonsynchronized HeLa cells to parasites in the presence of [^{3}H]thymidine.

HeLa cells were synchronized by the method of mitotic shake-off (*3*). Nonsynchronized HeLa cells were harvested by mild trypsinization (*4*). Both cell populations were seeded at a density of 10^4 cells per milliliter (2 ml) in petri dishes (3.5 cm in diameter) containing two 12-mm square cover slips and incubated for 2 to 4 hours to allow attachment of the cells.

Trypanosoma cruzi (Ernestina strain) trypomastigotes were harvested at 3-hour intervals from bovine embryo skeletal muscle cell cultures (*5*) and adjusted to a concentration of 5×10^5 organisms per milliliter. *Toxoplasma gondii* (RH strain) tachyzoites (*6*) were harvested by peritoneal lavage of NIH general-purpose mice infected 3 days earlier (*7*), and adjusted to a concentration of 5×10^6 organisms per milliliter. Immediately before the organisms were used in the experiment, [*methyl*-^{3}H]thymidine (1 μCi/ml; specific activity, 5.0 Ci/mmole;

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