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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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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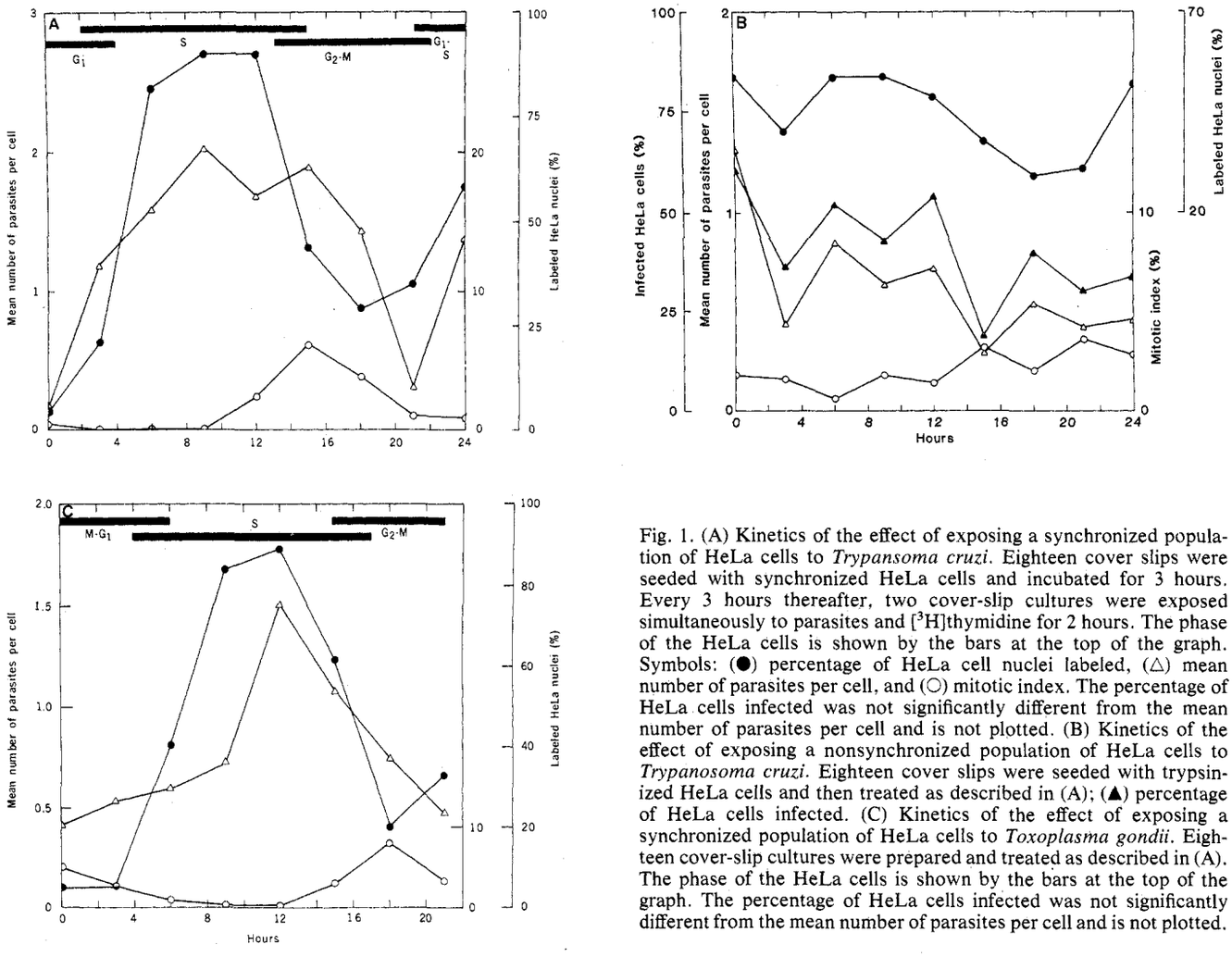

Fig. 1. (A) Kinetics of the effect of exposing a synchronized population of HeLa cells to *Trypansoma cruzi*. Eighteen cover slips were seeded with synchronized HeLa cells and incubated for 3 hours. Every 3 hours thereafter, two cover-slip cultures were exposed simultaneously to parasites and [^{3}H]thymidine for 2 hours. The phase of the HeLa cells is shown by the bars at the top of the graph. Symbols: (●) percentage of HeLa cell nuclei labeled, (△) mean number of parasites per cell, and (○) mitotic index. The percentage of HeLa cells infected was not significantly different from the mean number of parasites per cell and is not plotted. (B) Kinetics of the effect of exposing a nonsynchronized population of HeLa cells to *Trypanosoma cruzi*. Eighteen cover slips were seeded with trypsinized HeLa cells and then treated as described in (A); (▲) percentage of HeLa cells infected. (C) Kinetics of the effect of exposing a synchronized population of HeLa cells to *Toxoplasma gondii*. Eighteen cover-slip cultures were prepared and treated as described in (A). The phase of the HeLa cells is shown by the bars at the top of the graph. The percentage of HeLa cells infected was not significantly different from the mean number of parasites per cell and is not plotted.

Amersham) was added to the suspension.

At 3-hour intervals the medium was removed from a petri dish containing two cover-slip HeLa cultures and replaced with 2 ml of the parasite suspension. After 2 hours the HeLa cultures were removed, rinsed free of nonadherent parasites, fixed, prepared for autoradiography, exposed, developed, and stained (*8*). The presence or absence of nuclear labeling and the number of parasites per cell were determined for 100 HeLa cells per cover slip. In addition, the mitotic index of the HeLa cell population was estimated by classifying 200 cells.

The kinetics of vertebrate cell cycle modulation of the infection of HeLa cells by *T. cruzi* can be demonstrated when a synchronized population of HeLa cells is exposed to parasites (Fig. 1A). Initially, the majority of the HeLa cells are in the G_1 phase of the cell cycle and have a very low susceptibility to infection. As the HeLa cells progress into the S phase they become increasingly more susceptible to infection. By mid-S phase (9 hours) they are 14 times more susceptible (in the example shown) to infection than in the G_1 phase. The susceptibility to infection decreases with passage of the HeLa cells into G_2-M and increases again as the HeLa cell population begins a second, less synchronous entry into the S phase. The susceptibility of a nonsynchronous population of HeLa cells also varies according to the proportion of S-phase cells present (Fig. 1B). However, the marked changes in susceptibility observed with a synchronized HeLa cell population do not occur.

A similar pattern is observed when a synchronized population of HeLa cells is exposed to *T. gondii* (Fig. 1C). As the HeLa cells progress from G_1 to S they become markedly more susceptible to infection (four times more susceptible in the example shown). Also, as with *T. cruzi*, susceptibility to infection decreases as the cell population enters G_2-M. The susceptibility of nonsynchronous HeLa cell populations is similar to that observed in *T. cruzi*–HeLa cell interactions.

There are numerous reports describing cell cycle–related quantitative changes in the expression of vertebrate cell surface antigens (*9*). As vertebrate cells progress through the cell cycle, phase-dependent surface antigen levels increase or decrease. For example, cell surface antigens determined by the H-2 histocompatibility complex and Moloney leukemia virus appear predominately in the G_1 phase (*10*), whereas the expression of murine leukemia virus major envelope protein appears predominately in the S phase (*11*).

Thus, it appears that the infection of vertebrate cells by *T. cruzi* or *T. gondii* is modulated by the vertebrate cell cycle. Cell surface components acting as receptors are probably responsible for the phenomenon. It is possible that the components responsible for the enhancement of infection of HeLa cells by *T. cruzi* and *T. gondii* are different. In both instances, however, the components appear at highest concentration on S-phase cells. Our postulate is supported by the recent finding by Henriquez *et al.* (*12*) that in *T. cruzi*–vertebrate cell interactions "confluent cells are infected to a smaller extent than nonconfluent cells." A ma-

jority of the cells in a confluent culture are in G_1, whereas in a nonconfluent culture 30 to 60 percent of the cells are in the S phase (Fig. 1B).

Effective vaccines against *T. cruzi* and *T. gondii* have not been developed. The demonstration of vertebrate cell cycle modulation of infection and the probable existence of specific receptors for these parasites provides a new approach to the problem of disease prevention. Once isolated and characterized, the S-phase surface components responsible for the enhancement of infection could be used to block the complementary receptor on the parasites. This would interrupt the obligate intracellular vertebrate cell cycle and possibly produce sterile immunity in the host.

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4. HeLa cell cultures grown as described in (*3*) were rinsed with Dulbecco's minimum essential medium without serum and treated for about 30 seconds with Dulbecco's phosphate-buffered saline (without calcium or magnesium) containing 0.25 percent trypsin and 0.10 percent EDTA. A sharp tap on the flask detached the cells, which were immediately diluted tenfold in medium supplemented with 10 percent fetal bovine serum to inactivate the trypsin. After centrifugation (67*g*, 10 minutes), the cells were diluted for use as described in (*3*).
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Quantitative Autoradiography of [^{3}H]Muscimol Binding in Rat Brain

Abstract. *A simple quantitative autoradiographic technique for the study of neurotransmitter receptors that includes the use of a tritium-sensitive film permits saturation, kinetic, and competition studies of brain samples as small as 0.01 cubic millimeter. This technique was used to study [^{3}H]muscimol binding in rat brain. Unilateral γ-aminobutyric acid receptor supersensitivity was observed in the substantia nigra pars reticulata after production of localized lesions of the ipsilateral corpus striatum.*

The use of tissue homogenates in binding studies of the brain has provided a wealth of information about the location, pharmacology, and regulation of synaptic neurotransmitter and drug receptors (*1*). However, the use of homogenate techniques to study the details of regional variation in receptor numbers has been limited by the requirement that milligrams of tissue are needed for each assay. Studies of such structures as the rat's red nucleus (weight, 0.6 mg) or medial habenula (weight, 0.2 mg)(*2*) are thus impossible unless tissues from several animals are pooled. Therefore, light microscopy has been used with autoradiographic techniques to determine the location of receptors for diffusible ligands in the brain (*3*). Although these techniques provide information about variations in receptor density within an area of the brain, quantitation of the autoradiograms must be done by grain counting (*4*), which is laborious and difficult to standardize from one section or experiment to another. Quantitative techniques for autoradiography with x-ray film have been developed for other diffusible substances—for example, investigation of local cerebral glucose metabolic rates with 2-[^{14}C]deoxyglucose and ^{14}C-sensitive x-ray film—has gained widespread use (*5*).

We now report the use of tritium-sensitive film for quantitative autoradiography of [^{3}H]muscimol binding in rat brain. Muscimol, a potent γ-aminobutyric acid (GABA) receptor agonist, binds to synaptic GABA receptors (*6*). Our technique enables us to obtain directly from the film complete saturation, kinetic, and competition data comparable to those obtained from homogenate studies for regions of brain as small as 10 μg.

Frozen sections of rat brain (20 μm) were mounted on subbed slides and kept frozen at −20°C until use. Mounted sections were first subjected to three consecutive 5-minute washings in ice-cold 50m*M* tris citrate buffer (*p*H 7.0 at 4°C) to remove as much endogenous GABA as possible. The washed sections were then dipped in varying concentrations (5 to 80 n*M*) of [^{3}H]muscimol (15 Ci/mmole)(Amersham) in 50 m*M* tris citrate buffer (*p*H 7.0 at 4°C), with or without various concentrations of drugs. Nonspecific binding in the presence of 0.1 m*M* GABA was determined in adjacent sections. After incubation for 30 minutes at 4°C, sections were subjected to three consecutive 5-second washings with ice-cold buffer and then were blown dry. Slides were mounted along with appropriate standards in an x-ray cassette, and a sheet of Ultrofilm ^{3}H (LKB) was apposed to the sections. After a 12-day exposure at 4°C, the film was developed in D-19 (Kodak) for 5 minutes at 20°C, fixed, and dried. For quantification, the autoradiograms were projected in a photographic enlarger. Density readings were made with a photosensitive diode located at the center of the enlarger's image plane (*7*); readings of different regions were made by moving the autoradiographic film across the enlarger's film plane. Eight readings of the equivalent of 10-μg samples from each region were averaged, and the concentration of radioactivity in the region of the section that had underlain the film was determined by a computer-generated polynomial regression analysis, which compared the film densities produced by the sections with those produced by the standards.

In preliminary experiments, the correlation between densities produced by commercially available ^{14}C plastic standards and sections of brain paste containing known amounts of [^{3}H]formalde-

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