from 5 to 8. Saline controls were negative and served to assure us that the ion-exchange membranes were not releasing any active contaminant.

There is an important additional and unexpected result. Activity appeared in both the cathode and the anode compartments (in seven out of seven trials), while activity in the center compartment decreased during the 30-minute period of electrodialysis in the four which were assayed at the end as well as at the beginning of dialysis. Figure 2 offers a tentative explanation of this result and constitutes an important formulation for further testing. As indicated, it is assumed that the smaller active moieties, that are absorbed onto the larger ones of the active molecules extracted from serum (especially schizophrenic serum), are composed of two species. Initially, these species are united into moieties with resulting negative charges, but they break up further into positively charged and negatively charged fragments. These migrate toward their appropriate electrodes. Assuming that the positively charged moieties, whether in isolation or combined with the negatively charged ones, are the synaptic inhibitors, then one would expect higher activity at the cathode, as was usual, than at the anode, where dilution results from the presence of the inert portion of the negative moieties. Our findings support this hypothesis. Some charged fragments which are potentially available are indicated at the bottom of Fig. 2.

Our data indicate that successful separation of the postulated smaller active moiety was achieved and that this moiety has migration characteristics that call for amphoteric properties along the lines suggested in Fig. 2. We appear to have found another instance of a carrier function for an "albumin fraction" of blood. Similar testing is being carried on with the "globulin fraction" with which taraxein activity has usually been associated, although it has not always been found in the same protein fraction (*5*). The electrodialyzates contain nitrogen and further chemical characterization of these substances is in progress.

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Sensitivity of Cultured Mammalian Cells to Streptomycin and Dihydrostreptomycin

Abstract. *Cultured mammalian cells killed by streptomycin were essentially unaffected by an identical concentration of dihydrostreptomycin.*

It was recently reported that cultured mammalian cells are sensitive to streptomycin in relatively high concentration (3 to 5 mg/ml) and that variants resistant to this amount of streptomycin had been obtained (*1*). We have observed that strains of cells sensitive to streptomycin are essentially unaffected by dihydrostreptomycin.

The strains of cells, media, and general conditions for conducting the tests have been reported previously (*1*). Inasmuch as the physical environment of the cells is a factor in determining their response to streptomycin (*1*) the tests were carried out with identical results in plastic petri dishes, glass petri dishes, and glass tubes. Medium containing 100,000 cells per milliliter of a sensitive strain was added to the culture vessel, the cultures were observed daily, and cell counts were made at the end of 7 days on a Coulter electronic particle counter.

The amount of streptomycin necessary to produce toxic effects on mammalian cells is so large in relation to antibacterial concentrations that our initial observations were thought possibly to be the result of sufficient impurity in the streptomycin to be toxic for the cells. Commercial streptomycin and dihydrostreptomycin preparations, as well as two highly purified samples of each (*2*), were tested for differences in the response of the cells (Fig. 1). Although the dihydrostreptomycin affected the growth response of the culture slightly, the cells attached themselves to the glass and appeared healthy during the course of the experiment. The differences in numbers recorded for the different dihydrostreptomycin preparations are not significant. The cultures containing streptomycin appeared ill within 48 hours; the cells became detached from the glass and appeared to be dead when observed at 72 hours. Thus, the counts recorded for the cultures containing streptomycin represent dead cells.

These experiments were repeated with six strains of cells with identical results. With two of these strains, determinations of dose response were made and the effect of streptomycin persisted undiminished down to a concentration of 2 mg per milliliter and tapered off with lower concentrations.

The reason for the difference in the action of streptomycin and dihydrostreptomycin is not known. In most work, but not all (*3*), on the effect of these substances on bacteria differences have not usually been noted. Indeed, many workers use the term streptomycin in discussing their work although they actually used dihydrostreptomycin (*4*).

Studies on the mode of action of streptomycin on bacteria suggest that the primary lesion occurs in the membrane (*4*, *5*), or on the ribosomes (*6*), but the former interpretation has recently been questioned (*7*). The ability of streptomycin to combine with the nucleic acids (*8*) would lend support to a hypothesis that involves their action on cell components containing nucleic acids. That relatively large amounts of streptomycin are required to destroy mammalian cells, in contrast to the small amounts required for bacteria suggests the pos-

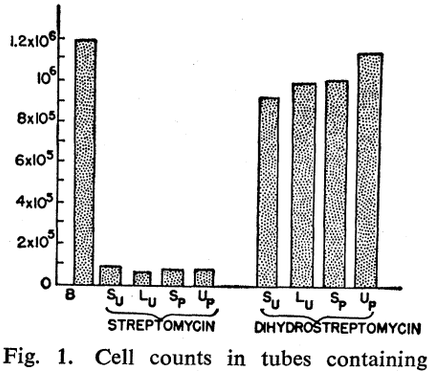

Fig. 1. Cell counts in tubes containing 1 ml of medium alone and with 5 mg of streptomycin or dihydrostreptomycin added. S_U, S_P, Squibb USP and purified; U_P, Upjohn purified; L_U, L_P, Lilly USP and purified.

sibility of a different mode of action on these two classes of cells. It is possible, however, that the mode of action of streptomycin on these two classes of cells is identical, but perhaps because of a difference in the permeability of the membranes of the respective cells to streptomycin, there is some interference in the passage of streptomycin to the active site in mammalian cells. Thus a much larger amount of streptomycin would be required to act on these cells. Such a hypothesis may also be evolved to explain the differences in the action of streptomycin and dihydrostreptomycin on mammalian cells. Perhaps these two substances may affect mammalian cells in an identical manner if they can get to the site on which the lethal action occurs, but, because of differences in the permeability of the membranes to these substances, only streptomycin can get to this site under the conditions of the experiment (*9*).

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Gene Action Mechanisms in the Determination of Color and Pattern in the Frog (Rana pipiens)

In urodeles, the cellular basis for pattern differences may be demonstrated by the usual techniques of embryonic transplantation, either of the neural crest, which gives rise to the pigment cells themselves, or the skin prior to its invasion by the migratory crest derivatives (*1*). When these techniques are applied to *Rana pipiens*, the transplants take, but are invariably rejected in the larva prior to the appearance of the definitive adult pattern. Rejection, which may or may not be obvious, is followed by repair from host sources, resulting in a host adult pattern. If pigment repair is incomplete by the time of metamorphosis, permanent pigment-free areas remain in the frog skin, indicating that melanoblasts are incapable of migrating in adult skin. Bovbjerg (*2*) has recently reported rejection of skin transplanted between sibling larvae, in agreement with the findings presented here. Autotransplants are not rejected.

Rejection has been avoided by two means. After exchange of right flank neural crest or dorsolateral skin in Shumway stage 15, the animals were joined in parabiosis (stage 16–17), either by the usual flank methods (*3*), or by the gill primordia. The latter method has proved superior for several reasons. The rich vascular exchange through the gills insures equal growth rates, while flank parabionts frequently become unbalanced, one animal essentially parasitizing the other. These rarely survive as frogs. Gill parabionts survive well and often spontaneously separate following resorption of the gills.

The second method involves exchange of the central belly area along with the underlying mesoderm at stage 17–18. The mesoderm in this region includes those cells destined to give rise to the elements of the blood (*4*). The successful exchange of these *Anlagen* constitutes a sort of permanent transfusion between the animals. The blood-island exchanges have resulted in tolerance in about 30 percent of the operated animals.

Methods of obtaining eggs and operative procedures were those of Hamburger (*3*), with the following exceptions. A 1-mm layer of 5-percent agar was used as the operating substrate, and the animals were kept in full strength Holtfreter's solution until corresponding hatching stages. Larvae were reared on parboiled spinach and the frogs on crickets.

Two well-known pattern mutants occur at low frequency in the Minnesota populations (*5*). The first variety, designated "burnsi," proved to be a single, incompletely dominant gene acting to inhibit the normal spotting pattern (*6*). The second mutant known as "kandiyohi" also is a single, dominant gene introducing mottled marks into the nonspotted areas of the skin (*7*). The two loci express their effects independently. Four classes may be obtained in equal frequency by crossing a burnsi heterozygote with a kandiyohi heterozygote (*5*). These are (i) the normal spotted frog, (ii) the burnsi frog with reduced spotting, (iii) the kandiyohi frog with normal spotting and mottled background skin, and (iv) the double mutant, kandiyohi-burnsi, a mottled frog with reduced spotting. Random exchanges of grafts between embryos from such a cross result in 75-percent combinations between animals of different genotypes, the remaining 25 percent being combinations between animals of the same genotype.

Exchanges of neural crest demonstrate the specificity of both the burnsi and kandiyohi loci as well as the double mutant. Dorsolateral skin exchanges come through strictly as host, as do simple blood-island exchanges and parabioses, the latter ruling out hormonal factors as primary genetic differences. In addition to the pattern

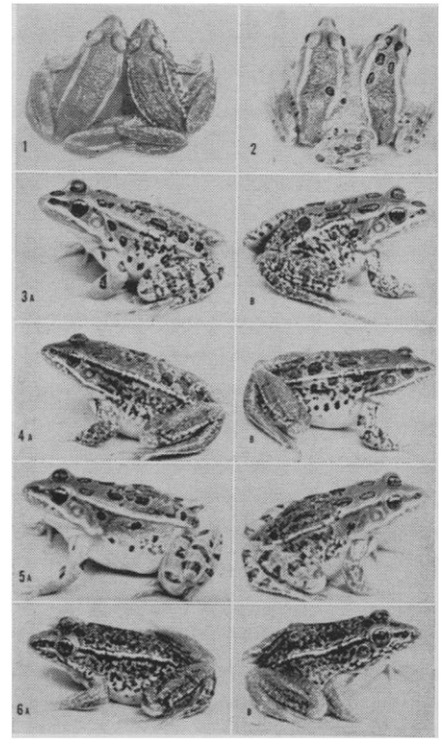

Fig. 1. The mutant types, burnsi (left) and kandiyohi (right), in gill parabiosis. Fig. 2. Right crest exchange followed by flank parabiosis between burnsi (left) and normal (right). Fig. 3. Left (*A*) and right (*B*) views, respectively, of a normal host with kandiyohi right crest. Fig. 4. Left (*A*) and right (*B*) views of kandiyohi host with normal right crest. Fig. 5. Left (*A*) and right (*B*) views of normal host with double mutant (kandiyohi-burnsi) right crest. Fig. 6. Left (*A*) and right (*B*) views of kandiyohi host with double mutant right crest. See the text for the explanation of tolerance induction.