drogenase (0.8 mg/ml), Holzer and Schneider (*12*) were able to demonstrate a small reduction of DPN upon the addition of unusually large amounts of this pyridine nucleotide (12 mg/ml). Although a similar reaction proceeded with somewhat greater facility in the case of glutamic dehydrogenase (*12*), it is unnecessary to add that these observations do not establish physiological roles for either enzyme in pyridine nucleotide transhydrogenase reactions.

We suggest that the term *pyridine nucleotide transhydrogenase* be reserved for enzymes exhibiting these activities as quantitatively primary functions, and which, by virtue of concentration in unmodified tissue fractions or high purification, possess a reasonably high specific activity (*13*).

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Direct Experimental Observation of Cells in Phenomic Lag

Abstract. By employing cells mutating at a very high spontaneous rate and a grid plate containing a medium totally selective against mutant cells, direct microscopic observation was made of mutant cells of recent origin passing through a limited number of phenomic lag divisions.

The concept that cells immediately following mutation are phenotypically normal and require several generations for mutant phenotype expression has been invoked to interpret discrepancies in mutation rates estimated by various methods (*1*) and to interpret the effect of intermediate cultivation after exposure to mutagens on the number of mutants expressed (*2*). Ryan (*3*) reviewed the evidence for phenomic lag and concluded that it was not compelling.

The likelihood of direct observation of a newborn mutant in phenomic lag is unreasonably small when the mutation rate is low. When the rate is high (for example, 10 to 40 percent; as in the cytoplasmic mutation to respiration deficiency in some yeast strains) the direct observation of phenomic lag becomes possible on a medium totally selective against the mutant. Under these conditions one predicts that normal cells should form colonies (complete development), old mutant cells or dead cells should fail to divide (no development), but mutant cells of recent origin should exhibit only one or a few divisions (limited development), if phenomic lag does in fact occur.

Intermittent observation of the development of many individual cells on an agar surface may be made if each cell can be relocated on a grid. A convenient grid plate may be constructed as follows. Ten razor blades are clamped together with the edges aligned on a plane surface. The blade edges are used to cross stamp the dry agar surface of a 2-day-old plate lightly, yielding a grid of squares approximately 0.1 mm on edge. One drop of a cell suspension (about 1×10^4 cells/ml) is placed directly on the grid. If the agar is dry the drop is rapidly absorbed, and microscopic observation may be begun almost immediately. The mechanical stage is modified to accommodate a petri dish, the corner of the grid is located under low and then high power and, as the grid is scanned, each cell or configuration (singlet, doublet, triplet, and so on) is drawn on a paper facsimile of the grid. At timed intervals the grid is scanned and the number of cells in each configuration is again recorded. Intermittent microscopic observation may be made of the open plate for 48 hours without visible interference from airborne contaminants. The tendency of growth to become confluent interferes with longer periods of observation.

Table 1. Number of microcolonies of various terminal sizes on lactate grid plates. Cells of strain 14940 were plated from shaken cultures growing in lactate nutrient broth at 35°C.

No. of cells in initial configuration*	No. of cells in final configuration*														Total microcolonies	Total macrocolonies
	1	2	3	4	5	6	7	8	9	10	11–12	13–14	15–16	17–20		
1	55	91	87	65	45	14	19	10	3	1	9	1	3	5	408	160
2		68	22	26	32	22	20	15	16	13	16	11	7	9	277	699
3			4	2	3	4	3	0	5	3	5	2	2	1	34	229
4				0	0	0	1	1	0	0	1	1	0	0	4	76
5					0	0	0	0	0	1	0	0	0	0	1	25
6						0	0	0	0	0	0	1	0	0	1	16
7							0	0	0	0	0	0	0	0	0	2
Total															725	1207

* Buds scored as cells.

Two plating media have been employed. One, a glucose nutrient agar, allows both respiration-deficient and respiration-sufficient cells to form colonies. The other, a lactate, nutrient agar allows only respiration-sufficient cells to form colonies (*4*). When a respiration-deficient clonal isolate of strain 14940 (*5*) growing in exponential phase in glucose nutrient broth was transferred to glucose grid plates, essentially all cells or configurations developed to form colonies. A low frequency (less than 1 percent) failed to produce a single bud and were considered dead cells. When aliquots of the same culture were studied on lactate grid plates, not a single new bud was formed in more than 1000 configurations. To test whether the respiration-deficient cells had died on the lactate agar, a doughnut-shaped ring of filter paper impregnated with glucose was placed on the agar surface surrounding the grid. After some lag, essentially all configurations budded on the plates to which glucose had been added. These cells were therefore still viable. Although previous observations (*4*) had indicated that respiration-deficient cells were incapable of forming macro-colonies on lactate agar, the observation of the complete failure to form new buds on the lactate grid plate is more critical evidence for the totally selective character of the medium against such cells. It does not, however, yield any information concerning the ability of respiration-deficient cells recently arisen from respiration-sufficient cells to produce buds in the selective medium.

Strain 14940 exhibits a high spontaneous mutation rate to respiration deficiency when it is growing exponentially in lactate nutrient broth at 35°C (about 40 percent). When samples of this culture were transferred to glucose grid plates, essentially all configurations (except 1 percent inviable) developed to microcolonies within 24 hours and to macrocolonies by 48 hours. On lactate grids however, many configurations (30.9

percent) developed only to a limited extent, as is shown in Table 1. The terminal microcolonies formed were shown to be mutant and viable. When glucose was added to lactate grids after 24 hours, growth was resumed. On control grids which were not supplemented by glucose, only a few microcolonies produced additional buds between 24 and 48 hours. Replating cells from microcolonies 24 hours after supplementation by glucose yielded only mutant colonies by tetrazolium overlay (*6*). Under different conditions or with other strains where the mutation rate was lower (about 1 to 2 percent), a parallel decrease in the frequency of colonies exhibiting limited development was observed.

The prediction of limited development for a considerable number of cells is thus fulfilled only with a strain showing a high mutation rate on a medium totally selective against the mutant. Further development is produced by delayed supplementation of the medium with a nutrilite which sustains mutant growth, indicating that development is not limited by death.

These observations are interpreted as the direct experimental observation of phenomic lag (*7*). When a respiration-sufficient cell produces a respiration-deficient bud, the latter still retains residual respiratory enzyme capacity and can proceed through a number of divisions until its respiratory capacity is diluted to the level of the respiration-deficient cell. Since the medium is totally selective against cells which lack respiratory ability, mutant cell division ceases when the phenomic lag is completed.

If one assumes essentially equal partition of the residual respiratory capacity during divisions of a newborn respiration-deficient mutant, no more than four or five phenomic lag generations should be possible. The grid plate experiments indicate that no configurations exhibiting limited development reached a terminal size compatible with more than five phenomic lag generations. The configurations exhibiting fewer phenomic lag generations probably arose from mutations which occurred one or more generations before plating.

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Interaction of Curare and Related Substances with Acetylcholine Receptor-Like Protein

Abstract. Existence of a receptor protein for acetylcholine has been postulated, and the hypothesis has been supported by experiments in intact cells. A protein from electric tissue has now been obtained in solution; there is striking parallelism between the ability of this protein to bind acetylcholine analogs and the effects of such analogs on electrical manifestations of intact electroplax.

The effect of acetylcholine in conduction and transmission of nerve impulses has always been attributed to its action upon a "receptor." Experimental evidence for the existence of a receptor has been obtained in studies with isolated electroplax of *Electrophorus electricus* (electric eel) (*1*). Recently Chagas and his coworkers have attempted to isolate the receptor (*2*). Their approach was based on the finding that radioactive triethiodide of gallamine (TRIEG)—a curare-like substance—is bound to a component (or components) present in an extract of the electric tissue of electric eel, a tissue undoubtedly rich in the receptor. Such complexes appeared to exhibit a fair degree of specificity with regard to competition between TRIEG and other substances known to have an affinity for the receptor (*2*). However, the fact that the formation of these complexes was reduced even in very dilute salt (0.02*M*) raises the question of whether the macromolecule responsible for the observed binding was indeed the receptor, inasmuch as curarization of intact electroplax is readily accomplished even in 0.18*M* salt (*3*). Moreover, the competition of various substances with curare that is observed on intact electroplax (*3*) differs in several respects from Chagas' results as to their competitive action in solution. Further exploration of this problem therefore appeared desirable.

The procedure followed (*4*) differs from that used previously in two important aspects. First, the tissue extract was subjected to ammonium sulfate fractionation and the resulting protein fractions were examined for their ability to bind curare and related substances. Since curare has two cationic nitrogen groups, it may react unspecifically with a number of macromolecules through Coulombic and van der Waals forces. Such binding was found to occur with nucleic acid and chondroitin sulfuric acid—components present to some extent in electric tissue extracts but eliminated by fractionation. Second, our method of studying the interaction is that of equilibrium dialysis (*5*) under controlled conditions of *p*H and ionic strength. Data thus obtained are less ambiguous than those from experiments in which binding has been determined after prolonged dialysis against large volumes of distilled water (*2*). All experiments were performed at 0°C and *p*H 7.5; it was assumed that equilibrium had been established when dialyzate analyses on successive days gave concentrations which checked to within 5 percent. Concentrations were determined by ultraviolet absorption. Calculations of the amount of material bound were based on the concentration of the outside solution of the particular substance when it was dialyzed against the protein as compared with the concentration when it was dialyzed against buffer. Analyses of inside solutions at equilibrium gave results which agreed well with those calculated by the indirect method.

The results are shown in Fig. 1. The following features appear to be pertinent. (i) There is a striking qualitative agreement between the degree of binding of curare and related substances in solution and the affinity of these compounds for the receptor, as measured by their effects on the electrical activity of intact electroplax (*3*). Thus, with respect to action on intact cell and the degree of binding in solution, these compounds have the following relationship, in descending order of effectiveness and binding ability: curare, dimethyl curare, prostigmine, eserine. In both types of studies, dodecylpyridinium proved to be

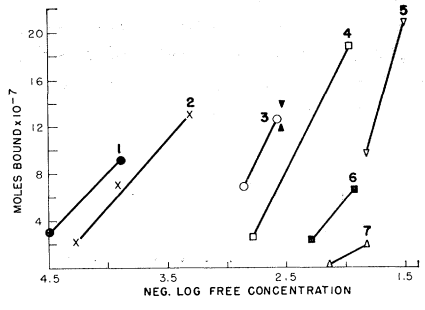

Fig. 1. Binding of curare (*d*-tubocurarine) and various related compounds to proteins obtained by ammonium sulfate fractionation of electric tissue. Protein concentration = 15 mg/ml, *p*H 7.5, 0°C. Curves 1 and 2, $\mu = 0.02$; curves 3–7, $\mu = 0.1$; curve 1, curare; curve 2, dimethyl curare; curve 3, curare; curve 4, dodecylpyridinium chloride; curve 5, prostigmine sulfate; curve 6, eserine salicylate; curve 7, methylpyridinium iodide. Curve 3 also shows data for binding of curare (0.004*M*) in the presence of (▼) prostigmine (0.05*M*) and (▲) decamethonium (0.02*M*).