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

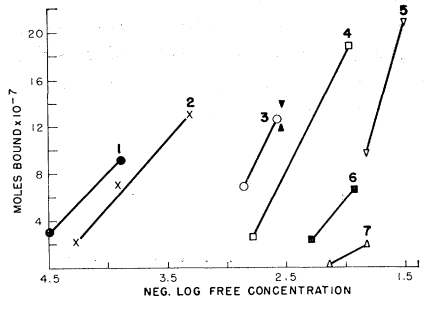

Fig. 1. Binding of curare (*d*-tubocurarine) and various related compounds to proteins obtained by ammonium sulfate fractionation of electric tissue. Protein concentration = 15 mg/ml, *p*H 7.5, 0°C. Curves 1 and 2, $\mu = 0.02$; curves 3–7, $\mu = 0.1$; curve 1, curare; curve 2, dimethyl curare; curve 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*).

much more effective than the methylpyridinium compound. It is to be noted that bovine serum albumin at a comparable concentration failed to bind any of these compounds, with the exception of dodecylpyridinium. (ii) The binding of curare, although salt-sensitive (compare curves 1 and 3), still occurred at a relatively high ionic strength ($\mu = 0.1$). As could be predicted from these results, a decrease in the ionic strength of the medium surrounding the electroplax resulted in a marked enhancement of the effect of curare (*6*). (iii) Addition of decamethonium and prostigmine did not result in the displacement of curare from the complex in solution. In agreement with these observations, it was found that these compounds, which depolarize the electroplax in low concentrations, are without effect on electroplax blocked by curare even when they are applied in concentrations several times as high as that of the curare (*3*).

The curare-binding component has been purified to a considerable extent by further fractionation of the extract with ammonium sulfate. A protein fraction has been obtained which, at a concentration of 0.5 mg/ml ($\mu = 0.1$; *p*H, 7.5), gives a precipitate with curare (2 mg/ml). Under similar conditions, chondroitin sulfate (3 mg/ml), deoxyribonucleic acid (2 mg/ml), and proteins of other fractions (15 mg/ml) fail to precipitate with curare. The precipitate, which contains about 50 percent of the proteins in this fraction, can be further fractionated by prolonged dialysis against 0.1 μ phosphate, *p*H 7.5. Part of the precipitate (about 20 percent) goes into solution, the remainder being solubilized by dialysis at *p*H 9. It is suggested that the latter material, which has a protein ultraviolet spectrum, may form highly specific complexes with curare and related compounds and thus has receptor-like properties. In line with the proposals of Nachmansohn and Wilson (*7*), it will be of considerable interest to determine whether those neurotropic agents considered to be "receptor activators" can induce configurational changes in this protein and whether the so-called "receptor inhibitors" are ineffective in this respect (*8*).

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8. I wish to express my gratitude to Dr. D. Nachmansohn for his continuous encouragement and interest in this work.

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Enzymatic Activity of a Genetically Altered Tryptophan Synthetase in Neurospora crassa

Abstract. Partially purified preparations of certain tryptophan-requiring mutants of *Neurospora crassa,* which contain a protein (CRM) antigenically related to wild-type tryptophan synthetase, possess indole glycerol phosphate-synthesizing activity. This activity can be inhibited by anti-CRM sera. It is suggested that CRM in such mutants represents a damaged tryptophan synthetase lacking the capacity to react with L-serine.

A number of microbial mutants unable to form L-tryptophan from indole and L-serine have been described (*1–3*). Crude extracts of such tryptophan-requiring (td) mutants of *Neurospora crassa* and *Escherichia coli* contain no tryptophan synthetase activity, but in most instances the presence of large quantities of a protein (CRM) antigenically related to tryptophan synthetase can be demonstrated (*2; 4–6*). Studies with *E. coli* (*2, 6*) have shown that CRM-containing preparations from certain tryptophan-requiring mutants will catalyze the conversion of indole glycerol phosphate to indole plus triose phosphate. These results indicate that CRM in *E. coli* appears to be functionally as well as structurally related to tryptophan synthetase. Experiments to date, in which both the *Neurospora* and the *E. coli* systems were employed (*5-9*), seem to support the view that the CRM proteins may represent genetically altered tryptophan synthetase molecules.

The present report (*10*), based on antienzyme studies with the *Neurospora* system, demonstrates that the reaction:

Indole + triose phosphate → indole glycerol phosphate

which is catalyzed by certain CRM-containing preparations, can be specifically inhibited by anti-CRM serum. This antiserum also inhibits the wild-type tryptophan synthetase activity (*5*).

Strain td_2 (*1*), a tryptophan-requiring mutant that accumulates indole and indole glycerol in culture filtrates (*8*), was used for most of these experiments, although preparations from several other strains appear, on preliminary screening, to give similar results. Partially purified td_2-CRM preparations, known to catalyze the reaction (*11*)

Indole glycerol phosphate → indole + triose phosphate

are able to convert indole and triose phosphate to indole glycerol phosphate. Maximum activity occurs only in the presence of fructose diphosphate, aldolase, and pyridoxal phosphate (*12*). The methods for determining indole disappearance and indole glycerol phosphate formation have been described (*2*). Although some indole glycerol phosphate is synthesized, it is apparent that considerably more indole disappears from the reaction mixture than can be accounted for by the indole glycerol phosphate formed. In addition, the rates of

Table 1. Effect of anti-CRM serum on the enzymatic activity of CRM. A constant number of td_2-CRM (5) units was added to each of several tubes containing increasing amounts of anti-td_2-CRM serum. The tubes were stored in an ice bath for 5 minutes, followed by the addition of the substrate mixture: (7.5 μmole of indole, 10 μmole of fructose diphosphate, 40 μg of pyridoxal phosphate, $2 \times 10^{-3} M$ glutathione, 0.2 ml of crude *Neurospora* aldolase, and 0.1*M* phosphate buffer, *p*H 7.8). The final volume per tube was 4.4 ml. Samples were incubated at 37°C for 1 hour. The reaction was stopped with 0.2 ml of 5 percent sodium hydroxide. Indole was extracted with 15 ml of toluene, and aliquots were assayed by the *p*-dimethyl aminobenzaldehyde method (*2*).

Antibody	Anti-CRM (unit)	CRM (unit)	Indole disappearing in reaction mixture (μmole)
Anti-td_2-CRM	45	77.7	0.40
Anti-td_2-CRM	60	77.7	0.02
Anti-td_2-CRM	120	77.7	0.0
td_1-absorbed anti-td_2-CRM	19	77.7	0.55
td_1-absorbed anti-td_2-CRM	38	77.7	0.45
td_1-absorbed anti-td_2-CRM	76	77.7	0.0
Anti-td_1	Equivalent volume	77.7	0.75
Nonimmune serum	Equivalent volume	77.7	0.75