ministered type A botulinus antitoxin, there develops a dilated fixed pupil on that side in the majority of surviving animals. Since parasympathetic efferent fibers are the only ones to synapse in the ciliary ganglion, and electrical and pharmacological data presented in this paper indicate an interruption of these pathways at the synaptic level, one may conclude that the toxin has been fixed at the sites of action in the ciliary ganglion by the time antitoxin is given systemically. The antitoxin then serves to prevent the further acton of the toxin at other sites such as neuromuscular junctions or other ganglionic synapses. Such an animal has a highly localized nerve defect, *i.e.* synaptic block of the parasympathetic efferent nerves to the eye. Other than in those animals which died, there were no gross signs or symptoms of impairment of other functions such as ocular motility, blinking reflex, corneal sensation or general neuromuscular dysfunction.

This nerve block persists for at least 6 months and then appears to be reversible in only a small number of animals. Thus ample time is available for experimentation during this period.

2. *Intravitreal injection of botulinus toxin.* By utilizing the same technic but using the intravitreal route for introduction of the toxin, a dilated fixed pupil is again produced, presumably due to a block of the nerve impulse at the post-ganglionic (cholinergic) nerve endings in the iris and ciliary body. The toxin diffuses forward from the vitreous and is selectively affective at this site. Since the pupil does dilate further to SCG stimulation (and topical cocaine-adrenalin) it is evident that the post-ganglionic adrenergic nerve endings to the iris are still intact. This data supports Ambache's contention that the toxin selectively blocks post-ganglionic cholinergic nerve transmission while leaving post-ganglionic adrenergic nerve transmission unaffected.

Summary. 1. It has been demonstrated that retrobulbar injection of type A botulinus toxin in rabbits followed in 15 minutes by systemic administration of type A botulinus antitoxin produces a dilated fixed pupil in the eye on the injected side. 2. The dilated fixed pupil results from a block of the parasympathetic efferent nerves synapsing in the ciliary ganglion. This block is produced by the botulinus toxin. 3. Such a block persists in the intact animal at least 6 months without interference to nerve transmission of sympathetic or somatic pathways elsewhere in the animal.

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Compatibility System Controlling Heterokaryon Formation in *Streptomyces coelicolor.** (24389)

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Most of the mutant characters which have been studied genetically in *Streptomyces coelicolor* have been morphological variations and growth-factor requirements. In the streptomycetes, heterokaryons and recombinants which were formed by interaction of 2 growth-factor dependent mutants have been found to grow readily without added supplements if the genes involved were nonallelic(1,2). This increased growth has been attributed to reciprocal sustenance of different nuclei in a common cytoplasm in the case of heterokaryosis, and in the case of recombination to reformation of the full complement of normal genes within a nucleus. Conversely, if the genes involved are allelic, no growth of the growth-factor de-

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TABLE I. Characteristics of Strains of *Streptomyces coelicolor* Used and Their Ancestors.

Strain No.	Source	Genotypic formulae*
S 16	wild type	prototrophic V^r
125	S16	meth$^-$ V^r
207	"	OHprol$^-$ V^r
199	wild type	prototrophic V^s
202	S199	cyst$^-$ prol$^-$ V^s
203	"	ura$^-$ arg$^-$ V^s

* Abbreviations employed are: V^r and V^s (resistance and susceptibility to bacteriophage P10); meth$^-$ (requires added methionine); OHprol$^-$ (requires added hydroxyproline); cyst$^-$ (requires added cysteine); prol$^-$ (requires added proline); ura$^-$ (requires added uracil); arg$^-$ (requires added arginine).

pendent mutants occurred in the absence of added supplements because no normal genes were present to compensate for the deficiencies(3,4). However certain other mixtures of growth-factor dependent mutants of *S. coelicolor* have failed to produce growth-factor independent colonies, *i.e.* prototrophs. With these mixtures the diversity of growth-factor requirements suggested that failure to form prototrophs was the result of factors other than allelism. A reasonable explanation was that mutations, which did not noticeably affect the morphology or growth of the organism but did affect the capacity of the organism to interact with one another, might have occurred among nutritional mutants obtained subsequent to ultraviolet irradiation. This report presents evidence for a compatibility system, which may have arisen by mutation, controlling heterokaryon formation in *S. coelicolor*.

Methods. The strains of *S. coelicolor* used were mutants which required added growth-factors in order to grow, *i.e.* auxotrophic mutants, and were derived from either strain S16 or S199. Characteristics of the strains used and their ancestors are shown in Table I. The media employed and the experimental details of the tests for capacity of auxotrophic pairs to produce prototrophs have been previously described(5). The test procedure may be summarized as follows: pairwise mixtures of auxotrophic mutants were grown together at 30°C on complete medium for 5 days, and then replica plated to minimal and complete media; after 3 more days incubation, plates were scored for the presence of colonies able to grow on minimal medium; these nutritionally independent colonies were picked from complete medium and purified by serial subculture of isolated colonies; finally susceptibility to bacteriophage P10 was determined, using a standard suspension containing 10^6 bacteriophage/ml.

Results. Strain S207 formed prototrophs with S125 but not with S202 or S203. Strains S125, S202 and S203 formed prototrophs with each other in all possible pairwise combinations. The numbers of prototrophs formed by these combinations are shown in Table II. During continued subculture of these prototrophs on complete medium, the parental types were recovered as colonies and sectors, however the parental types were not recovered with equal frequency.

Susceptibility of prototrophs from mixtures of S125 + S202 and S203 + S125 to bacteriophage P10 was determined. Of prototrophs tested, all 34 from the former mixture and all 42 from the latter mixture were sensitive to bacteriophage P10.

Thus, certain combinations of auxotrophic mutants of *S. coelicolor* did not produce prototrophic colonies. The failure of the auxotrophic pairs to produce prototrophs was not the result of allelism because 1) the genetic markers were so diverse that allelism would not be expected and 2) nonproductive pairs were productive in other combinations with each other, which would not be possible if the genes preventing prototroph formation were allelic. Therefore a compatibility system controlling prototroph formation exists. It has been concluded that this compatibility system acts at the level of heterokaryosis because 1) the growth-factor independent colo-

TABLE II. Number of Prototrophs Formed by Auxotrophic Pairs of *Streptomyces coelicolor*.

Mixture	No. colonies on minimal medium		
	Max	Median	Min
S125 alone	0	0	0
202 "	0	0	0
203 "	0	0	0
207 "	0	0	0
207 + S202	0	0	0
207 + S203	0	0	0
207 + S125	12	5	0
202 + S203	209	53	26
125 + "	1,000	451	138
125 + S202	3,000	1,000	584

nies from mixtures where bacteriophage P10 susceptibility could segregate were always sensitive and 2) upon continued subculture, the parental types were recovered from the prototrophs. The first point conclusively ruled out reciprocal feeding by a mixed population of cells because the resistant component could not be demonstrated as a discrete unit, indicating that the interaction was intracellular. Both lines of evidence argued against haplophase recombination because 1) bacteriophage P10 susceptibility did not segregate and 2) the prototrophs subsequently yielded parental dissociates. The remaining possibilities were heterokaryosis and parasexuality(6). The latter hypothesis would predict that nonparental types as well as parental types should be recovered from the prototrophs. Inasmuch as nonparental types have not been found, the only hypothesis which was consistent with all of the data was heterokaryosis.

Our data indicated that 2 factors determined heterokaryotic compatibility in *S. coelicolor,* as was found to be the case for heterokaryotic compatibility in *Neurospora crassa* (7). At least one of the compatibility factors arose by mutation because S207 and S125 displayed different compatibility responses even though these two strains had a common ancestor. It is not possible at this time definitely to conclude that the factors controlling heterokaryotic compatibility in *S. coelicolor* represent nuclear genes.

Summary. Heretofore, complementary pairs of growth-factor dependent mutants of *Streptomyces coelicolor* have interacted to yield colonies which grow readily in the absence of added supplements. Recently however certain combinations have been found which did not interact successfully. This failure of nutritionally dependent mutants to form growth-factor independent growth was not the result of allelism, but of a compatibility system controlling heterokaryon formation. The compatibility system was apparently determined by two factors, one of which probably arose by mutation.

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Anti-Progestational Activity of Estrogens in Rabbit Endometrium*† (24390)

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Established ability of estrogens to inhibit progestin-induced endometrial proliferation has been thoroughly reviewed by Courrier(1). This anti-progestational effect has been determined by histological methods, and its demonstration has been either qualitative or roughly quantitative. With development of the Lutwak-Mann test(2) for progestin-induced endometrial carbonic anhydrase increase, as a quantitative assay in the rabbit (3), we felt that accurate quantitation of the anti-progestational effect might also be achieved. We had previously shown that quantitative estimates of progestational potency made by carbonic anhydrase measurement correlated extremely well with concomitant measurements of the degree of pseudopregnant proliferation(3,4). Accordingly, in studying the estrogen effect we have measured in samples of the same uteri both carbonic anhydrase concentration and degree of pseu-

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