portant: a larger number of animals–15–had to be used, both for the tests and their controls. The animals had to be inbred and of equal age; they had to be young, since the tumor grows faster in younger animals (we used mice 3 to 4 weeks old). At the end, the animals had to be sacrificed, the tumors excised and weighed.

The thymus is a relatively rich source of retine and promine, but is a less advantageous material than aorta, tendon, or muscle, because of its high content of extractible matter. Tendon is difficult to handle and collect. We worked chiefly with aortas of calves. This material was frozen soon after the death of the animal and transported to the laboratory in frozen condition; it was reduced here to a snow which was dropped directly into methanol —100 liters of alcohol for 36.4 kg (80 lb) of tissue. Then the mixture was brought to the boiling point of methanol and cooled; the methanol was separated from solid matter on a basket centrifuge and clarified on the Sharples centrifuge.

The methanol extract was evaporated at low temperature to 3 liters, adjusted to *p*H 4 with HCl and shaken out, repeatedly with chloroform. The methanol extract was then adjusted to *p*H 1 and again shaken out with the same solvent. The united chloroform extracts, which contained both retine and promine, were shaken out with water of *p*H 1 which eliminated promine. The chloroform was evaporated, and the residue was dissolved in butanol, saturated with 1*N* HCl. The solution was subjected to chromatography on a cellulose-column wetted with 1*N* HCl saturated with butanol. Butanol saturated with 1*N* HCl was used as the moving phase. The first colored eluates were rejected, and the column was extracted with acid methanol. The methanol was evaporated under reduced pressure, and the residue was dissolved in chloroform. The retine was then shaken out with alkaline water (*p*H 9), and the watery extract was acidified (*p*H 1) and then shaken out with benzene which extracted fatty acids. After this the water was shaken out with chloroform which extracted the retine. After evaporation of the chloroform the residue was dissolved in peanut oil. The oily solutions were stored at −20°C. Precipitates forming were eliminated by centrifugation.

Our yields approximated about 50 percent. The final material injected had a dry weight of 2.2 mg/kg (1 mg/lb) of aorta extracted. We called a "unit" the quantity of retine which slowed down the growth of cancer to one-half. This corresponded to about 200 μg of dry weight. The aorta contains about 33 units per kilogram.

Our limited experience suggests that the promine and retine are of small molecular weight and have a high potency. Retine seems to contain one or more unstable links since it decomposes at room temperature in a week. It is more sensitive to alkali than to acid. It also seems to contain a group which dissociates at an alkaline reaction, though its solubility in alkali may have been due also to the accompanying substances, mainly fatty acids. Promine seems to have an alkaline group which makes the molecule more soluble on dissociation in acid water.

We found no harmful side effects either with retine or with promine. In this respect, these substances seem to differ from all the antimetabolites used in cancer therapy which are not specific in their action and interfere with some fundamental process or substance, common to all cells, so that even if cancer cells may be more sensitive, side effects do result. Retine and promine, being natural substances produced by nature, might perhaps specifically influence cell division; one might have here substances which will stop cancer growth and even produce regression without toxicity. Possibly even the growth-promoting substance may acquire a medical application, in analogy to weed killers which kill by promoting growth. One could, perhaps, introduce in the body some antimetabolite and then make the cancer grow fast and kill itself. The growth promoter seems not to induce malignancy by itself. It might also find application in accelerating the healing of wounds (*6*).

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Recombination Events in the Bacterial Genus Nocardia

Abstract. *Genetic recombination was demonstrated in the bacterial genus* Nocardia. *The compatibility mechanism governing recombination differs from that of* Escherichia coli *and the streptomycetes. Mutants of* N. erythropolis *or* N. canicruria, *of homologous origin, were incompatible, but mutants of heterologous origin were compatible. Such a compatibility system is suggestive of classic heterothallism which up to now has not been known among the bacteria.*

Members of the genus *Nocardia* are morphologically intermediary between less complex organisms, the Mycobacteriaceae, and more complex forms, the Streptomycetaceae (*1*). If one is allowed to speculate on phylogeny and evolution of bacteria, the nocardiae could be considered to be either the point from which divergence of the primitive and complex bacteria occurs or the endpoint of convergence (*2*). The inviting question concerning hereditary mechanisms of such an intermediary group is: Would studies of the nocardiae reveal genetic mechanisms similar to those found in the Eubacteriales, exemplified by *Escherichia coli* (*3*), or to those found in the streptomycetes (*4*), or would the genetic mechanisms of nocardiae have characteristics of both groups? In order to find an answer to this question, genetic studies have been undertaken with *Nocardia erythropolis* and *Nocardia canicruria*, members of the genus which morphologically typify the phylogenetically intermediate category (*1*).

These two taxonomically designated species grow relatively rapidly and form colonies in 5 to 7 days on a medium containing only mineral salts and glucose as well as on more complex but convenient laboratory media. They exhibited little colonial morphologic variation on the several media upon which they were tested. It seemed perfectly feasible to use bacterial genetic techniques which had been developed previously and to test for genetic interactions between complementary pairs of nutritionally characterized mutants of homologous or heterologous origin.

The velveteen method of replication (*5*), whereby identical plates of colonies are made from a master plate, was used for the indirect selection of auxotrophic mutants which were induced by an ultraviolet irradiation dose which killed more than 99.9 percent of the starting population. As a result of ultraviolet induction many replicated colonies capable of growth on the medium of mineral salts and glucose supplemented with amino acids,

purines, and pyrimidines, but not on the unsupplemented medium, were detected, purified, and characterized; they comprised a variety of single-deficiency auxotrophic mutants. By repeating this procedure with auxotrophic mutants rather than a wild-type population, strains with multiple auxotrophic characters were recovered. Mutants with additional markers were obtained by growing spontaneously occurring drug-resistant mutants on media containing drugs.

Tests to determine recombination between mutants showing nutritionally complementary characters were carried out. Suspensions of the marked strains were prepared, and samples containing approximately 1×10^6 bacteria per milliliter were placed on trypticase soy agar alone and in nutritionally complementary pairs. After 24 hours' incubation at 30°C, the resultant growth was transferred by velveteen replication to unsupplemented medium. These plates were incubated from 5 to 7 days at 30°C, and colonies of prototrophic organisms—that is, those not requiring supplements to the basic medium necessary for the growth of the auxotrophic mutants—were counted. Prototrophic colonies arising as a result of mixed growth of a pair could be considered to be the result of genetic interaction of the two strains used if the frequency greatly exceeded that of the two auxotrophs when plated alone. Streaking on nonsupplemented medium of both parental types in close but not overlapping proximity of all experimental pairs indicated that cross-feeding was not a primary cause of the effects hereafter noted.

Results of crosses attempted with nutritionally complementary pairs of *N. erythropolis* and *N. erythropolis* mutants derived from a single strain, *N. canicruria* and *N. canicruria* mutants derived from a single strain, and *N. erythropolis* and *N. canicruria* are presented in Table 1. Thus far no unequivocal data have been obtained justifying the conclusion that crosses of *N. erythropolis* and *N. erythropolis* mutants result in prototrophic colonies. Such data might be construed to indicate that the absence of prototrophs in such tests is the result of close linkage of genetic determinants used in the experiments. However, of 18 crosses noted in the table, in which a variety of mutants of homologous origin were tested, no prototrophs were observed. Furthermore, one cross (Table 1), *N. erythropolis* ade-1 his-3 by *N. erythropolis* gly lys, was replicated to minimal medium containing such two-factor supplements that all two factor nonparental prototrophic combinations could be recovered. It should be expected that the distal two, of four genetic determinants, which are automatically selected for in such an experiment, are of sufficient separation to allow recombinational events to occur. In these experiments there was no evidence of recombinational events. It would seem unlikely that all of the markers utilized are so closely linked that recombinational events resulting in prototrophic colonies are not detectable under the conditions of the experiments.

When crosses were attempted between nutritionally complementary mutants of *N. erythropolis* and *N. canicruria*, prototrophic colonies in significant excess of prototrophic reversion of individual parental types were observed as shown in Table 1. In crosses consisting of two to four factors, significant recombinational events were detected. The prototrophs recovered have been colonially typical of the strains of *N. canicruria*, although colonial morphology is not now considered sufficiently reliable to designate specific genetic character. In the cross *N. erythropolis* ade-1 his-3 str-s by *N. canicruria* ade-2 str-r, the recovered recombinant prototrophs were phenotypically str-r. When the streptomycin sensitivity of the parents was reversed —that is, *N. erythropolis* ade-1 his-3 str-r by *N. canicruria* ade-2 str-s—the prototrophs were sensitive to streptomycin.

Large numbers of crosses with many diverse auxotrophic mutants of *N. canicruria* have not yet been attempted. In crosses of *N. canicruria* ade-2 str-r

Table 1. Compatibilities of auxotrophic mutants of crosses of *N. erythropolis* by *N. erythropolis*, *N. canicruria* by *N. canicruria*, and *N. erythropolis* by *N. canicruria*. The suffix E indicates that the designated strain is an auxotrophic derivative of *N. erythropolis*; suffix C, auxotrophic derivative of *N. canicruria*. The numbers represented in the last column are the average total numbers of prototrophic colonies on plates replicated from mixed growth minus the average number of prototrophic colonies found on replicated control plates of several separate experiments; if control plates indicated reversions greater than one or two prototrophic colonies per three control plates, the experimental data were not generally incorporated into the table. *ade*-1 indicates that the genetic determinant was an adenine deficiency; the suffixing numeral indicates a mutant isolate designation. Abbreviations used: *ade*, adenine; *ala*, alanine; *arg*, arginine, *gly*, glycine; *his*, histidine; *ile*, iso-leucine; *leu*, leucine; *lys*, lysine; *met*, methionine; *str*-s, sensitive to 200 μg/ml streptomycin; *str*-r, resistant to 200 μg/ml streptomycin; *xan*, xanthine; ×, indicates a cross.

Strain designation	Genetic determinants		Prototrophic recombinants
39- 1E × 45-58E	ade-1	×his-1	0
39- 1E × 39- 8E	ade-1	×his-2	0
39- 1E × 39- 3E	ade-1	×gly	0
39- 3E × 45-58E	gly	×his-1	0
70- 4E × 96-16E	ade-1 his-3	×glys lys	0*
96-16E × 74- 1E	gly lys	×his-1 met-1	0
96-16E × 92- 2E	gly lys	×his-1 ade-	0
96-16E × 97-16E	gly lys	×his-2 leu	0
96-16E × 98- 2E	gly lys	×his-1 xan	0
96-16E × 98-19E	gly lys	×his-1 met-2	0
96-16E × 98-26E	gly lys	×his-1 arg-1	0
96-16E × 98-34E	gly lys	×his-1 ile	0
100-18C ×100-22C	arg-2	×ade-2	0
130- 2C ×100-22C	arg-2 str-r	×ade-2 str-s	0
100-18C ×130- 1C	arg-2 str-s	×ade-2 str-r	0
133- 8E† × 39- 3E	ade-1 his-3	×gly	0
133-10E† × 39- 3E	ade-1 his-3	×gly	0
133- 8E† × 96-16E	ade-1 his-3	×gly lys	0
133- 9E† × 96-16E	ade-1 his-3	×gly lys	0
133-10E† × 96-16E	ade-1 his-3	×gly lys	0
133-11E† × 96-16E	ade-1 his-3	×gly lys	0
100-18C ×134- 1C†	arg-2	×ade-2	0
100-18C ×134- 2C†	arg-2	×ade-2	0
130- 2C ×134- 3C†	arg-2 str-r	×ade-2 str-s	0
70- 4E ×100-22C	ade-1 his-3	×ade-2	23
74- 1E ×100-22C	his-1 met-1	×ade-2	7
70- 4E ×130- 1C	ade-1 his-3 str-s	×ade-2 str-r	15 all str-r
30- 1E ×100-22C	ade-1 his-3 str-r	×ade-2 str-s	14 all str-s
70- 4E ×132- 7C	ade-1 his-3	×ade-2 ala	9
45-58E ×132- 7C	his-1	×ade-2 ala	16
45-58E ×100-22C	his-1	×ade-2	14
39- 1E ×100-22C	ade-1	×ade-2	17
39- 1E ×100-18C	ade-1	×arg-2	0
45-58E ×100-18C	his-1	×arg-2	0

* All two-factor prototrophic recombinants selected for by replicating the mixed growth from complete medium to minimal medium supplemented with two growth factors such that all nonparental two-factor combinations could be recovered. † Strains designated 133- are 70-4E strains which were grown with 100-22C for 3 days, re-isolated, repurified and tested to assure auxotrophic requirements; strains designated 134- are 100-22C strains which were grown with 70-4 and re-isolated.

by *N. canicruria* arg-2 str-s, prototrophs have not been detected. Is close linkage responsible for failure to find recombination? Significantly, crosses of *N. canicruria* arg-2 with *N. erythropolis* mutants do not produce prototrophic recombinations, whereas *N. erythropolis* mutants crossed with *N. canicruria* ade-2 are generally fruitful (Table 1). Such evidence would seem to indicate that, if linkage is a factor, the *N. canicruria* arg-2 locus should be more closely linked with those loci thus far discovered in mutants of *N. erythropolis*. Thus, the *N. canicruria* arg-2 locus must not be closely linked with the *N. canicruria* ade-2 locus; accordingly, linkage is probably not responsible for undetected recombinational events in *N. canicruria* by *N. canicruria* crosses.

Our experiments seem to warrant the following conclusions. Crosses of *N. canicruria* or *N. erythropolis* mutants of homologous origin are not fertile or at least are not detectable by means of the procedures used in our experiments. Crosses of mutants of heterologous origin, *N. erythropolis* by *N. canicruria*, are fertile. Thus nocardial recombination seems to be governed by a compatibility system. Compatibility systems have been reported for both the streptomycetes (*6*) and *Escherichia coli* (*7*); however, nocardial compatibility differs in one respect. At least one member of a pair capable of genetic interaction in the *E. coli* or streptomycete systems is compatible with mutants of homologous origin (*6, 7*), but this has not been so in the nocardial system studied.

In the case of *E. coli* an infectious F agent is responsible for the compatibility (*8*). The possibility that an infectious F-like agent exists in the nocardial system was tested in the following manner. *Nocardia canicruria* ade-2 was grown in mixed culture with *N. erythropolis* ade-1 his-3 for 3 days. Several individual strains of the *N. canicruria* and *N. erythropolis* mutants were re-isolated and tested to ascertain nutritional requirements and to affirm their identities. These strains were then tested with complementary auxotrophs of the homologous species. All such mixtures failed to result in detectable recombinational events. Hence, in contrast to the system in *E. coli* controlling compatibility, our data indicate that mating types governing nocardial recombinational events are independent of an infectious F-like factor.

These recombinational events in bacteria are the first evidence suggesting heterothallism similar to that observed in the true fungi (*9*). Because the crosses *N. erythropolis* ade-1 his-3 str-s by *N. canicruria* ade-2 str-r and *N. erythropolis* ade-1 his-3 str-r by *N. canicruria* ade-2 str-s gave recombinant prototrophs having the streptomycin phenotype of the *N. canicruria* strain used, *N. erythropolis* could be regarded as the male and *N. canicruria* as the female. Preliminary data indicate that the frequency of recombination may be as high as 1×10^{-5} parent cells and that the recombinants do not readily segregate (*10*).

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Calorific Values of Microcrustacea

Abstract. *The heat content of copepods and other microcrustaceans and two species of algae in calories per gram varied from 4427 for immature crayfish to 5643 for the female* Diaptomus siciloides. *The algae, cladoceran, anostracan, conchostracan, and immature crayfish were all below 5000, whereas all the copepods were above 5300 ($\bar{x}$ 5467); thus copepods may contribute more energy to the food chain on a weight basis than other lower forms of crustacea.*

Studies of energy transformation have pointed out the need for determining the amount of heat that various organisms from a wide taxonomic range and a large variety of environments can produce (*1–3*). Some data are available for the plankton, but very few are available for copepods, either marine or fresh-water. We have studied calanoid copepods, a cyclopoid, cladocerans, and several other organisms.

Our interest in calorimetry has centered around energy transformations in populations of *Diaptomus siciloides* (*4*), *Mesocyclops edax*, and *Diaptomus leptopus*. The pertinent data in the present paper have been used in the computation of the energy budget for *D. siciloides* (*4*).

All determinations were made with a Parr model 1411 oxygen bomb calorimeter (*5*) and the standard Parr method, with modifications for small samples, was used. Millipore membrane (pore size, 5 μ) was burned with each sample as filler material. The filter membrane was first compressed into pellet form with a punch and die from a Parr pellet press (*6*) (except for the 5-μ disk onto which the sample was filtered). Twenty determinations on this pellet alone yielded a mean calorific value of 3104.6 $\pm$37.0 cal/g (coefficient variation of 1.19). Ten determinations on 0.45-μ membrane yielded a mean value of 2935.2 cal/g. To find the variation due to sample size, samples of this membrane varying from 6 to 95 mg were burned. A plot of the absolute variation from the mean against the sample size showed that the variation increased rapidly as the sample got smaller. Samples larger than 30 mg approached the mean with an accuracy of $\pm$ 7 to 17 cal/g. The membrane pellet burned usually weighed more than 30 mg, and the pellet and organisms together usually weighed more than 50 mg—in the 70-mg range most frequently. Determinations were made on an electrobalance.

Some of the animals from a sample were segregated onto a membrane which was then dried for 24 hours at 60°C, and other specimens were measured to determine their mean length and width. The weight of the animals determined the number used; we could maintain