6-Chloro-2-naphthylmethylcarbinyl Methyl Ether.—A solution of 10 g. of the carbinyl chloride and 40 g. of potassium hydroxide in 300 ml. of methanol was refluxed for two hours, poured into water, and most of the alcohol removed by distillation. On cooling, the oil (6.5 g., 77.5%) solidified and was then purified by distillation, b. p. 104° (0.5 mm.), 89.5–90.5° (0.11 mm.) and 87.0° (0.08 mm.); yield, 6.1 g. (72.7%), m. p. 37.5–40°. No suitable solvent for recrystallization was found, so a center cut from the distillation was analyzed.

Anal. Calcd. for $C_{13}H_{13}ClO$: C, 70.75; H, 5.94; Cl, 16.07. Found: C, 70.72; H, 5.98; Cl, 15.74.

6-Chloro-2-vinylnaphthalene.—A mixture of 3.3 g. of carbinol and 8 g. of potassium acid sulfate was heated under a pressure of 0.2 mm. A white solid sublimed at about 125°; yield, 2.8 g. (90%), m. p. 102–107°. Recrystallization from Skellysolve "F" and then 95% ethanol raised the melting point to 111.5–112.6°.

Anal. Calcd. for $C_{12}H_9Cl$: C, 76.20; H, 5.11; Cl, 18.79. Found: C, 76.39; H, 4.81; Cl, 18.80.

The **dibromide**, prepared in cold chloroform solution and recrystallized from 95% ethanol, melted at 98–99°.

Anal. Calcd. for $C_{12}H_9Br_2Cl$; C, 41.36; H, 2.58; halogen, 58.35. Found: C, 41.77; H, 3.11; halogen, 58.52.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NOTRE DAME CHARLES C. PRICE
NOTRE DAME, INDIANA GEORGE H. SCHILLING[2]

RECEIVED SEPTEMBER 7, 1948

(2) General Tire and Rubber Company Fellow, 1946–1947.

COMMUNICATIONS TO THE EDITOR

FRIEDEL–CRAFTS COPOLYMERIZATION

Sir:

Copolymerization studies have been used extensively to elucidate the nature of the propagation step in free radical reactions. We have now obtained some data on copolymerizations induced by Friedel–Crafts catalysts. The systems investigated contained stannic chloride, carbon tetrachloride, and derivatives of styrene. In the systems styrene/*p*-chlorostyrene and α-methylstyrene/*p*-chlorostyrene, the over-all reaction rate was lower when more *p*-chlorostyrene was present. The proportion of *p*-chlorostyrene in the copolymer was lower than in the monomer mixture from which it was derived throughout the entire composition range.[1] The results obtained with styrene/*p*-chlorostyrene are summarized in Table I. These composition results were plotted, correction being made for the finite conversions, and were then fitted by the theoretical copolymer composition equation. The following monomer reactivity ratios were found: $r_1 = 2.7 \pm 0.3$, $r_2 = 0.35 \pm 0.05$. The corresponding figures for the free radical polymerization are $r_1 = 0.74$, $r_2 = 1.02$.[2] Thus, while *p*-chlorostyrene adds slightly faster than styrene to both styrene and *p*-chlorostyrene *free radicals*, the reverse is true with respect to *carbonium ion* addition. Experiments involving different catalyst concentrations yielded the same values of r_1 and r_2, within experimental error.

Mayo and Lewis[3] report that polymerization of styrene–methyl methacrylate mixtures with stannic chloride yields almost exclusively polystyrene. Experiments with α-methylstyrene/*p*-chlorostyrene by the authors and by Mr. L. Arond indicated that α-methylstyrene is even more reactive with carbonium ions than is styrene. The order of reactivity with carbonium ions thus seems to be α-methylstyrene > styrene > *p*-chlorostyrene > 2,5-dichlorostyrene and methyl methacrylate.

Our interpretation of these results involves as a dominant factor the polarization of the double bond by substituents. Introduction of (electron-withdrawing) chlorine atoms into the benzene ring of styrene reduces the reactivity of the double bond for carbonium ions. Introduction of the electron-donating methyl group in the α-position *increases* the reactivity with carbonium ions. The low reactivity of methyl methacrylate can be

TABLE I

COPLYMERIZATION OF STYRENE (M_1) AND *p*-CHLOROSTYRENE (M_2) IN CARBON TETRACHLORIDE AT 32°[a]

Monomer concn. moles/l.	Time, hr.	Conversion, %[b]	Approx. rate[c]	M_2[d]	Cl in polymer, %	m_2[e]
1.864	5.1	48.3	8.5	9.08	1.86	5.56
1.798	1.8	..	..	19.66	3.71	11.3
1.709	2.4	12.2	5.2	39.27	7.03	22.2
1.501	7.0	..	..	50.00	9.70	31.4
1.663	5.1	24.1	4.7	49.89	9.97	32.4
1.605	7.4	..	..	60.63	12.39	41.35
1.615	5.1	17.1	3.4	60.70	12.40	41.35
1.543	3.8	16.9	4.5	69.33	14.13	48.1
1.505	7.3	13.6	1.9	84.16	19.43	70.3
1.484	5.6	20.5	1.9	89.21	20.39	74.6
1.555	0.4	7.6	19.0[f]	100.00	25.41	99.0

[a] The experiments were carried out in reaction cells sealed at 1 mm. pressure. All solutions contained 2% by weight $SnCl_4$ on the monomers. [b] By bromine addition to residual monomers. Both styrene and *p*-chlorostyrene add Br_2 quantitatively. [c] % conversion divided by time of experiment. [d] M_2 = mole % *p*-chlorostyrene in monomer mixture. [e] m_2 = mole % *p*-chlorostyrene in copolymer. [f] A mixture of nitrobenzene–carbon tetrachloride was used as solvent in this experiment.

(1) Mr. J. Stewart of Standard Oil Co. of N. J. obtains similar results with styrene/2,5-dichlorostyrene; apparently 2,5-dichlorostyrene is even *less* reactive than *p*-chlorostyrene (private communication).

(2) C. Walling, E. R. Briggs, K. S. Wolfstirn and F. R. Mayo, THIS JOURNAL, **70**, 1537 (1948).

(3) F. R. Mayo and F. M. Lewis, THIS JOURNAL, **66**, 1594 (1944).

attributed to the electron-withdrawing character of the —COOR group.

INSTITUTE OF POLYMER RESEARCH TURNER ALFREY, JR.
POLYTECHNIC INSTITUTE OF BROOKLYN
BROOKLYN, NEW YORK HARRY WECHSLER

RECEIVED JUNE 10, 1948

ISOLATION OF BIOCHEMICALLY DEFICIENT MUTANTS OF BACTERIA BY PENICILLIN

Sirs:

It is possible to isolate bacterial mutants with ease when the mutants can proliferate or survive in an environment in which these activities are not possible for the parent strain. There is therefore no difficulty in obtaining mutants, even of low frequency, which differ from the parent strain by resisting bacteriophage or antibacterial chemicals, or by having decreased nutritional requirements. Mutants with increased nutritional requirements, however, though a class of especially great biochemical interest, have been much less convenient to isolate. Recently developed techniques[1,2] permit a considerable improvement over the earlier practice of random selection, but still permit selection from only a few hundred colonies per agar plate.

The possibility of isolating these biochemically deficient mutants from much larger populations suggested itself on the basis of the reports[3,4] that penicillin sterilizes only growing bacteria. We confirmed this conclusion, and found that a tryptophan-less mutant of *E. coli* was completely resistant to the bactericidal action of penicillin in minimal medium unless tryptophan was added.

The technique was successfully applied to the isolation of new mutants. Ultraviolet irradiated bacteria were cultivated overnight in medium enriched with casein hydrolysate, washed, and exposed to penicillin (300 O.U./ml.) in minimal medium[5] for 24 hours. Large numbers of colonies (*ca.* 100, from an inoculum of 10^5 bacteria exposed to penicillin) were isolated on enriched agar; over 80% were mutants. These include replicates arising from each original mutant during intermediate cultivation; a variety of types, however, can be recovered on a single plate.

In earlier experiments bacteria had been exposed to penicillin following irradiation, without intermediate cultivation; no mutants were obtained. This failure depends on a lag in the adjustment of the enzymic composition of the cell to the new genic composition. Until the cell has gone through enough generations to dilute out the enzyme molecules which were formed by the gene prior to its mutation, the cell does not lose its capacity to form a given metabolite, and hence is not resistant to penicillin in minimal medium. Another factor which limits the survival of mutants is the syntrophic effect of metabolites secreted by the non-mutated cells growing in minimal medium. The density of the population exposed to penicillin is therefore best limited to 10^6 cells/ml.

By this technique mutants of *E. coli* ("Waksman" strain, ATCC 9637) have been obtained with individual or alternative requirements for all the naturally occurring amino acids except alanine and hydroxyproline; for several multiple sets of amino acids; for purines or pyrimidines and their derivatives; for most vitamins; and for unknown factors in yeast extract.

This procedure should make it possible for biochemists to isolate desired types of mutants at will. These mutants, which have some advantages over *Neurospora*, can be used for not only quantitative but also very simple qualitative microbiologic assay, as well as for discovery of new metabolites, and production of rare chemicals by mutants which accumulate the substrate of the blocked enzymic reaction. A more detailed account is being published.[5]

UNITED STATES PUBLIC HEALTH SERVICE
TUBERCULOSIS RESEARCH LABORATORY
CORNELL UNIVERSITY MEDICAL COLLEGE
NEW YORK 21, N. Y. BERNARD D. DAVIS

RECEIVED DECEMBER 1, 1948

CONCENTRATION OF BIOCHEMICAL MUTANTS OF BACTERIA WITH PENICILLIN[1]

Sir:

Existing methods for isolating biochemical mutants are still tedious, although mitigatory procedures have been described.[2] We have found that penicillin can be used to augment the proportion of mutants in a culture, greatly facilitating their isolation.

The method depends on the finding that penicillin lyses only growing cells with little permanent effect on resting suspensions.[3] This suggested that, if allowed to act on a mixture of mutant and non-mutant cells in a synthetic medium, penicillin might concentrate the mutants which are unable to grow in this medium.

These expectations were first tested in reconstruction experiments. Y-53 is a mutant of *Escherichia coli* requiring threonine, leucine and thiamin, and is lactose-negative; K-12 is its lactose positive wild type ancestor. Suspensions were assayed for mutants by planting on EMB-lactose agar[4] and counting the dark and light colonies as K-12 and Y-53, respectively. After preliminary study of various conditions, the following were adopted: Washed suspensions of young cells harvested from a complete medium

(1) J. Lederberg and E. L. Tatum, *J. Biol. Chem.*, **165**, 381 (1946).
(2) B. D. Davis, *Arch. Biochem.*, in press.
(3) G. L. Hobby, K. Meyer and E. Chaffee, *Proc. oc. Expl. Biol. Med.*, **50**, 281 (1942).
(4) E. Chain and E. S. Duthie, *Lancet*, **1**, 652 (1945).
(5) B. D. Davis, *Proc. Nat. Acad. Sci.*, in press.

(1) Supported by grants from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service, and the Rockefeller Foundation and The Wisconsin Alumni Research Foundation. Paper 384, Department of Genetics, College of Agriculture, University of Wisconsin.
(2) J. Lederberg and E. L. Tatum, *J. Biol. Chem.*, **165**, 381 (1946).
(3) G. L. Hobby, *Proc. Soc. Exptl. Biol. Med.*, **56**, 181 (1944).
(4) J. Lederberg, *Genetics*, **32**, 505 (1947).