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).

were mixed in the ratio K-12:Y-53 of 100 – 1000:1. These mixtures were inoculated into a synthetic medium[4] containing 300 O.U./ml. penicillin G to give an initial cell concentration of 10^8–10^9/ml. The suspensions were incubated with shaking for four hours, when there were about 10^4–10^5 viable cells. In a number of experiments, assays of these survivors have given ratios of K-12:Y-53 ranging from 2:1 to 1:100, and representing amplifications of mutants of several hundred to several thousand-fold. No change from the initial ratio was observed when the growth factors required by Y-53 were added to the treatment medium.

New mutants have also been obtained from several *Salmonella* strains, by treatment of cells grown from irradiated inocula. Approximately half the colonies surviving the treatment failed to grow when inoculated in synthetic medium. However, only about two-thirds of these proved to be stable mutants when subcultures on nutrient agar slants were retested. No satisfactory explanation of this behavior has been found.

The mutants so far characterized require a variety of growth factors, including histidine, methionine, tryptophan, leucine, threonine, proline, phenylalanine and tyrosine.

The method would lend itself to the isolation of specific mutants by the addition of irrelevant growth factors to the treatment medium. The method should be applicable to other bacteria, but the optimal conditions will have to be worked out for each organism.

Parallel experiments with streptomycin and streptothricin[5] gave no alteration in the mutant ratio. Very few antibiotics are reputed to have the differential activity on growing cells needed for this method.

(5) Kindly provided by Merck and Co.

DEPARTMENT OF GENETICS
UNIVERSITY OF WISCONSIN JOSHUA LEDERBERG
MADISON, WISCONSIN NORTON ZINDER

RECEIVED DECEMBER 1, 1948

THE LACTONES OF 2-HYDROXYMETHYLPOLYHYDROPHENANTHRYL-1-ACETIC ACIDS

Sir:

In the course of proof of structure of steroidal 16,17-ketols prepared by the method of Stodola, *et al.*,[1] we have submitted such a ketol (now known to be a 16-keto-17(α)-hydroxysteroid) to reaction with lead tetraacetate in aqueous acetic acid to rupture the C_{16}–C_{17} bond with cleavage of steroid Ring D to produce an aldehyde group at C_{13} and an acetic acid group at C_{14}.[2] Reduction of such an aldehyde-acid to the primary alcohol stage using hydrogen and Raney nickel catalyst (or hydrogen and Adams catalyst plus ferrous ion) yields a δ-hydroxyacid which easily lactonizes to the δ-lactone.

(1) Stodola, Kendall and McKenzie, *J. Org. Chem.*, **6**, 841 (1941).
(2) Huffman and Lott, THIS JOURNAL, in press.

By this method of synthesis we have prepared a series of 2-hydroxymethylpolyhydrophenanthryl-1-acetic acid lactones utilizing various 16-keto-17-(α)-hydroxysteroids as starting materials. Thus, from 3-methoxy-17(α)-hydroxy-16-keto-$\Delta^{1,3,5}$-estratriene[3,4] has been obtained the lactone of 7-methoxy-2-methyl-2-hydroxymethyl-1,2,3,4,9,10,11,12-octahydrophenanthryl-1-acetic acid (m. p. 176–177°).[5] *Anal.*[6] Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 75.85, 75.96; H, 8.03, 7.96. This lactone is easily demethylated with hydriodic acid[4] to give the free phenol (m. p. 285–287° dec.). Similarly, from 3(β),17(α)-dihydroxy-16-keto-Δ^5-androstene[7,2] has been prepared the lactone of 7-hydroxy-2,13-dimethyl-2-hydroxymethyl-1,2,3,4,-5,6,7,8,10,11,12,13 - dodecahydrophenanthryl - 1 - acetic acid (m. p. 205.5–206.5°). *Anal.* Calcd. for $C_{19}H_{28}O_3 \cdot H_2O$: C, 70.77; H, 9.38. Found: C, 70.82, 70.73; H, 9.41, 9.34. This lactone upon oxidation by the Oppenauer method furnishes the lactone of 7-keto-2,13-dimethyl-2-hydroxymethyl-1,2,3,4,5,6,7,9,10,11,12,13 - dodecahydrophenanthryl-1-acetic acid (m. p. 191–192°). *Anal.* Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.27, 75.22; H, 8.65, 8.75. The two isomeric lactones from 3(α),17(α)-dihydroxy-16-ketoandrostane and 3(β), 17(α)-dihydroxy-16-ketoandrostane have also been prepared; these melt at 228.5–229.5° and 201° respectively. *Anal.* Calcd. for $C_{19}H_{30}O_3$ (m. p. 228.5–229.5°): C, 74.47; H, 9.87. Found: C, 74.40, 74.32; H, 9.91, 9.84.

This series of lactones is not identical with that prepared by Westerfeld[8] and by Jacobsen[9] and co-workers. A mixed melting point determination using our 7-methoxy-2-methyl-2-hydroxymethyl-1,2,3,4,9,10,11,12-octahydrophenanthryl-1-acetic acid and the corresponding δ-lactone[10] (estrololactone methyl ether) of Dr. Jacobsen showed a depression of some thirty degrees.

(3) Huffman, *J. Biol. Chem.*, **167**, 273 (1947).
(4) Huffman, *ibid.*, **169**, 167 (1947).
(5) All melting points are uncorrected.
(6) Microanalyses performed by Dr. E. W. D. Huffman, Denver.
(7) Butenandt, Schmidt-Thomé and Weiss, *Ber.*, **72**, 417 (1939).
(8) Westerfeld, *J. Biol. Chem.*, **143**, 177 (1942).
(9) Jacobsen, *ibid.*, **171**, 61 (1947); Levy and Jacobsen, *ibid.*, **171**, 71 (1947); Jacobsen, Picha and Levy, *ibid.*, **171**, 81 (1947).
(10) Kindly supplied by Dr. R. P. Jacobsen.

DEPARTMENT OF BIOCHEMISTRY MAX N. HUFFMAN
SOUTHWESTERN MEDICAL COLLEGE MARY HARRIET LOTT
DALLAS, TEXAS JAMES ASHMORE

RECEIVED NOVEMBER 6, 1948

THE ION-EXCHANGE SEPARATION OF ZIRCONIUM AND HAFNIUM

Sir:

In the course of a rather cursory examination of the elution of tetra-positive ions from the cation exchange resin Dowex 50 with hydrochloric acid solutions, we have discovered a very effective method for separating zirconium from hafnium. In view of the great labor involved in preparing even reasonably pure hafnium compounds by