active solvents such as *n*-butanol and acetic acid (h) or lutidine mixture (i) were used.

Thus it became clear that the resolution was not due to the optical character of the solvent. For confirmation, a chromatopile separation was carried out in which a solution of 250 mg. of *dl*-tyrosine-3-sulfonic acid was dried on 20 sheets of 9 cm. No. 2 (Toyo) filter paper. The 20 disks were incorporated at 100 disks from the top of a 700 sheet pile packed in 15 cm. After 27 hours, the solvent (*n*-butanol:water:acetic acid:*l*-methyl-(β-phenylisopropyl)-amine. 15:5:1:1) reached the bottom and a further 24 hours were required for a total of 733 g. of solvent.

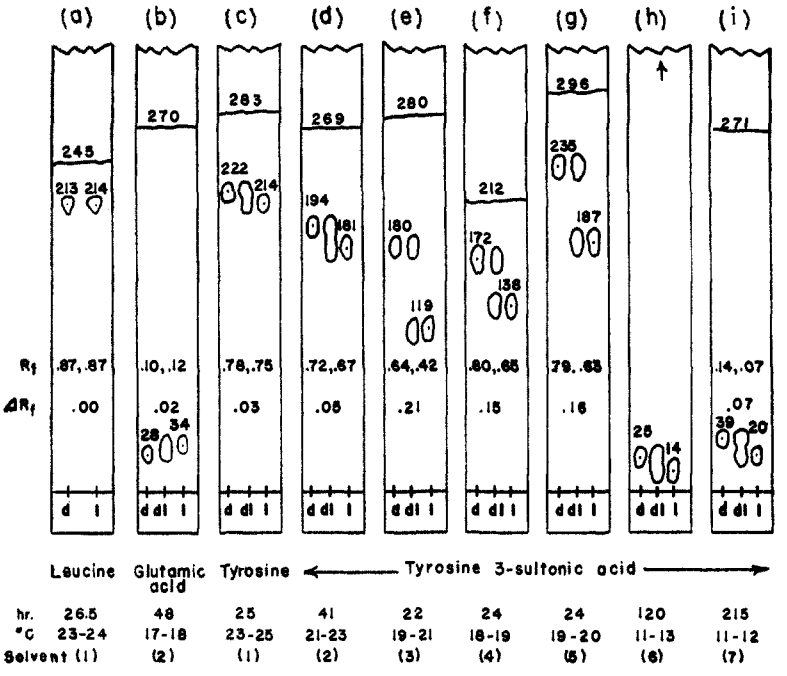

Fig. 1.—Uni-dimensional resolution of amino acids; solvent and compound excursion distances are recorded in mm.; solvent: (1) *l*-amine:AcOH:H_2O:*n*-BuOH, 1:1:1:1; (2) *l*-amine:water, 4:1; (3) *l*-amine:AcOH:H_2O: *n*-BuOH, 1:1:2:6; (4) *d*-amine: AcOH:H_2O:*n*-BuOH, 1:1:1:1; (5) *dl*-amine:AcOH:H_2O:-*n*-BuOH, 1:1:1:1; (6) *n*-BuOH:AcOH:H_2O, 4:1:1; (7) lutidine:AcOH:H_2O:-*n*-BuOH, 1:1:2:6.

After drying at room temperature, the approximate location of the two isomers was determined with ninhydrin. The amino acid was found in two parts, (I) at disks 180–255 and (II) at disks 275–330.

Each group of disks was eluted, and the amino acids were obtained as mercury salts which were decomposed with hydrogen sulfide to give crystalline products. The crystals from (I) tasted bitter and (II) very sweet. The rotation of (I) was $[\alpha]^{18}D$ −4.14° (2*N* NaOH, *c* = 1.69%); II. $[\alpha]^{18}D$ +4.47° (2*N* NaOH, *c* = 1.11%).

It is most reasonable to consider that these resolutions are due at least in part to the asymmetric character of the cellulose.

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RECEIVED APRIL 25, 1951

FORMATION OF FORMALDEHYDE FROM GLYCEROL-C^{14} BY PROPIONIBACTERIUM[1]

Sir:

It has been previously reported that following the fermentation of glycerol as well as other substrates by *P. arabinosum* in the presence of C^{14} formaldehyde, the isolated propionate was completely labeled, the highest activity occurring in the carboxyl position.[2]

Using glycerol 1-C^{14} as a substrate in a similar fermentation it has now been possible to isolate labeled formaldehyde from the medium.[3]

Eighty micromoles of unlabeled formaldehyde and 2.4 millimoles of glycerol 1-C^{14} per 100 ml. were fermented with resting cells using phosphate-bicarbonate buffer. At the end of 18 hours the formaldehyde was separated by neutral distillation. Twenty-eight micromoles were recovered as determined by the chromotropic acid method.[4] To this 124 micromoles of carrier formaldehyde were added and the dimedon derivative was made and found to have a constant specific activity when recrystallized from aqueous acetone and from aqueous alcohol. The specific activity was 1560 cts./min./mM. of carbon or 8,600 cts./min./mM. carbon in the original undiluted formaldehyde obtained from the fermentation. The average activity of the 1- and 3-carbons of the added glycerol-C^{14} was 13,000. Similar results have been obtained in a second experiment. Formaldehyde added to glycerol-1-C^{14} and isolated without fermentation was found to be inactive.

The propionate formed by resting cells of *P. arabinosum* from glycerol 1-C^{14} also has been degraded. It was separated by steam distillation, chromatographed on a silica gel column and degraded,[5,6] after conversion to lactate through bromination. The distribution of activity expressed as cts./min./mM. was: CH_3-(4760)-CH_2-(4840)-COOH-(8100). The activity of the CO_2 and bicarbonate of the buffer was 1630. This distribution is strikingly similar to that found when formaldehyde C^{14} was fixed in the propionate.[2]

From the results of the above experiments it seems that formaldehyde is formed largely from the 1,3-carbons of glycerol. Whether or not the 2

(1) This work was supported by grant AT-30-1-1050 from the Atomic Energy Commission under the auspices of the Office of Naval Research. Acknowledgment of many helpful suggestions is due to Dr. H. G. Wood.

(2) F. W. Leaver, THIS JOURNAL, **72**, 5326 (1950).

(3) The 1-C^{14} glycerol was kindly furnished by Dr. M. L. Karnovsky of Harvard University.

(4) B. Alexander, G. Landweler and A. M. Seligman, *J. Biol. Chem.*, **160**, 51 (1945).

(5) H. G. Wood and C. H. Werkman, *J. Bact.*, **30**, 332 (1935); *Biochem. J.*, **30**, 618 (1936).

(6) L. F. Goodwin, THIS JOURNAL, **42**, 39 (1920).

carbons of glycerol can be converted to formaldehyde to some extent cannot be definitely answered at present. The demonstration that formaldehyde is produced and utilized by *P. arabinosum* during the fermentation of glycerol indicates that formaldehyde or some compound in equilibrium with it is an active intermediate. The mechanism of propionate fermentation is under further investigation.

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RECEIVED APRIL 23, 1951

ENZYMATIC FORMATION OF BARBITURIC ACID FROM URACIL AND OF 5-METHYLBARBITURIC ACID FROM THYMINE

Sir:

The oxidation of uracil to barbituric acid and the formation of 5-methylbarbituric acid from thymine have been observed with a partially purified enzyme preparation obtained from strains of *Corynebacterium* isolated by enrichment culture from soil samples. A strain of *Mycobacterium*[1] was also found to metabolize uracil and thymine in the same way and because of its rapid growth was used in enzyme studies. The organism was grown for about 40 hours at 30° with shaking on a medium containing 0.1% uracil or thymine, 0.15% K_2HPO_4, 0.05% KH_2PO_4, and 0.02% $MgSO_4 \cdot 7H_2O$ in distilled water.

The enzyme was prepared by grinding the cell paste with alumina[2] and extracting with tris-hydroxymethylaminomethane buffer (0.02 *M*, *p*H 9.0). The clear supernatant obtained by high-speed centrifugation of the extract was fractionated with ammonium sulfate and the precipitate obtained between 35 and 50% saturation was collected.

With enzyme prepared from thymine-grown cells,[3] uracil was oxidized with the consumption of one atom of oxygen per one mole of substrate. The optimum *p*H of the reaction was about 9.0 and the presence of methylene blue was necessary. The ultraviolet absorption spectrum of the oxidation product was indistinguishable from that of barbituric acid (Fig. 1). The isolated crystalline material melted with decomposition at 243° as did an authentic sample of barbituric acid and a mixture of the two. The behavior of the uracil oxidation product on ion exchange (Fig. 1) and paper chromatography (propanol–water, 10:3) (R_f = 0.22) further indicated identity with barbituric acid.

With thymine as substrate, a product was obtained which on the basis of its ultraviolet absorption spectrum[4] appears to be 5-methylbarbituric acid. This product was differentiated from the uracil oxidation product by ion-exchange (Fig. 1) and paper chromatography (R_f = 0.36). Analogous to the relative behavior of uracil and thymine, the oxidation product of thymine follows the oxidation product of uracil (*i.e.*, barbituric acid) in the ion-exchange chromatogram and migrates farther on paper. Final identification awaits comparison with an authentic sample of 5-methylbarbituric acid.

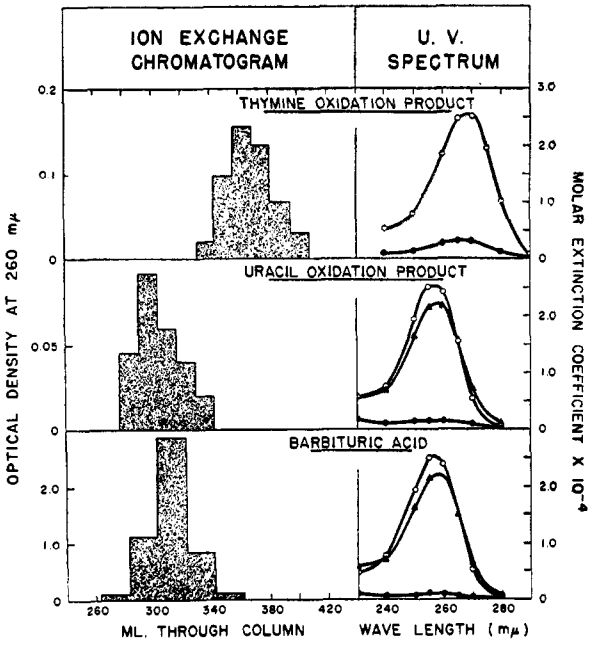

Fig. 1.—Ion-Exchange Chromatogram: 20 μmoles of thymine or uracil. 2.0 ml. of enzyme (2.4 mg. of protein). 2.0 ml. of 0.1% methylene blue. 15.0 ml. of tris-(hydroxymethyl)-aminomethane buffer (0.2 *M*, *p*H 8.7) and 2.0 ml. of H_2O. The incubation was at 30° for about 30 minutes. When the increase in absorption at 260 mμ reached a plateau, the incubation mixture was chilled and a 10 ml. aliquot was adsorbed on Dowex-1 (Cl^- form) column, 9.5 cm. × 1 sq. cm., and eluted with $NH_4OH.NH_4Cl$ buffer (0.2 *M*, *p*H 9.9). The thymine oxidation product was eluted between 255 and 370 ml. and the uracil oxidation product between 220 and 330 ml. Recovery based on u.v. adsorption was 107 and 88%, respectively. The presence of impurities in the reaction mixture interfered with the chromatographic pattern. Therefore, an aliquot (310–317.5 ml.) of the eluate of the thymine oxidation product containing 0.3 μmole and an aliquot (249–253.2 ml.) of the eluate of the uracil oxidation product containing 0.3 μ mole were each diluted with equal volumes of "tris" buffer and rechromatographed under the same conditions. The results are illustrated here: recovery, 100 and 50%, respectively. 5 μmoles of barbituric acid (Eastman Kodak recrystallized) were dissolved in 10.0 ml. of "tris" buffer (0.1 *M*, *p*H 8.7). Recovery: 90%. Ultraviolet spectrum: ▲—▲ *p*H 12.0. O—O *p*H 7.4. ●—● *p*H 2.3. The absorption coefficient of the oxidation product of thymine was calculated on the assumption that 100% of the substrate was converted to this compound when the increase in absorption reached a plateau.

This enzyme does not attack isobarbituric acid, postulated by Cerecedo[5] to be the initial product of uracil metabolism in mammals. It is also inert toward 4-methyluracil, dihydrothymine, dihydrouracil, 2-thiouracil, 2-thio-5-methyluracil and cytosine.

Bacterial oxidation of pyrimidines has been observed concurrently by others.[6,7] Wang and Lampen obtained an unidentified product from uracil and thymine oxidation by cell-free extracts.

(1) Isolated from rabbit feces by Drs. Schatz, Savard and Pintner and identified and kindly furnished by Dr. T. Stadtman.

(2) H. McIlwain, *J. Gen. Microbiology*, **2**, 288 (1948).

(3) Results with uracil-grown cells were essentially the same except that the growth of the organism was less favorable.

(4) R. B. Stuckey, *Quarterly J. Pharm. Pharmacol.*, **15**, 370 (1942).

(5) L. R. Cerecedo, *J. Biol. Chem.*, **88**, 695 (1930).

(6) T. P. Wang and J. O. Lampen, *Federation Proc.*, **10**, 267 (1951).

(7) F. J. S. Lara and C. B. van Niel, personal communication.