TABLE III

STRUCTURE OF THE PEPTIDES OBTAINED FROM THE CHYMOTRYPTIC AND TRYPTIC HYDROLYSIS OF β-MSH

Enzyme	Peptide no.[a]	Structure
Chymotrypsin	Ch-5	Asp.Glu.Gly.Pro.Tyr
Trypsin	T-2	Asp.Glu.Gly.Pro.Tyr.Lys
Chymotrypsin	Ch-3	Lys.Met.Glu.His.Phe
Trypsin	T-4	Met.Glu.His.Phe.Arg
Chymotrypsin	Ch-2	Arg.Try
Trypsin	T-3	Try.Gly.Ser.Pro.Pro.Lys.Asp
Chymotrypsin	Ch-4	Gly.Ser.Pro.Pro.Lys.Asp
Complete amino acid sequence		Asp.Glu.Gly.Pro.Tyr.Lys.Met.Glu.His.Phe.Arg.Try.Gly.Ser.Pro.Pro.Lys.Asp 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

[a] See Figs. 1 and 2.

TABLE IV

COMPARISON OF THE STRUCTURE OF β-MSH WITH THE RELATED AREA OF THE CORTICOTROPINS

β-MSH	Asp.Glu.Gly.Pro.	Tyr	.Lys.	Met.Glu.His.Phe.Arg.Try.Gly.	Ser.	Pro.	Pro.Lys.Asp.
Corticotropins	Ser.	Tyr	.Ser.	Met.Glu.His.Phe.Arg.Try.Gly.	Lys.	Pro.	Val.Gly.Lys.

corticotropins is well documented,[28,44] although the magnitude of the activation is open to some question. Inherently the corticotropins possess less than 1% of the melanocyte-stimulating activity of MSH, a fact which may be explained by the nature of the sequences adjacent to the heptapeptide core. Such sequences may be inhibitory, so that treatment of the corticotropins with alkali, by removing some of this inhibitory structure, may consequently potentiate the melanocyte-stimulating activity. In our experience, however, even when potentiation has occurred, the melanocyte-stimulating activity of the corticotropins is less than that found in MSH. This may be a result of the Lys.Ser. interchanges pointed out above. In other words, it may be that the undecapeptide discussed above is necessary for full melanocyte-stimulating activity.

Finally, the finding of a partial structure common to two different hormones is highly reminiscent of the findings in connection with oxytocin and vasopressin.[45] In the case of these latter hormones, two variations occur in a basic nonapeptide structure, and each hormone is endowed with some of the major activities characteristic of the other. Both of these hormones are produced by the hypothalamus and stored in the posterior pituitary. The corticotropins are produced by a group of the cells of the anterior lobe of the pituitary, whereas MSH is produced from an entirely different group of cells, those of the intermediate lobe of the pituitary. This lobe, however, is derived embryologically from the same anlage as the anterior lobe. A full discussion of the biological activity of β-MSH and the biological significance of the structure will be presented elsewhere.

Acknowledgments.—This work has been supported in part by grants from the National Institutes of Health of the United States Public Health Service (Grant No. G-2907) and the Albert and Mary Lasker Foundation.

(44) E. B. Astwood, in "The Hormones," Vol. III, Ed. by G. Pincus and K. V. Thimann, Academic Press, Inc., New York, N. Y., 1955.

(45) V. du Vigneaud, *The Harvey Lectures*, Series L, 1 (1956).

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND NUTRITION, COLLEGE OF AGRICULTURE, UNIVERSITY OF NEBRASKA]

The Enzymatic Synthesis and Disproportionation of 3-O-α-D-Glucopyranosyl-D-glucose[1,2]

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3-O-α-D-Glucopyranosyl-D-glucose (nigerose) is synthesized during the action of the transferring enzyme of *Aspergillus oryzae* on glucose and maltose. It was characterized by its specific rotation, its paper chromatogram mobility and its crystalline phenylosazone derivative. Evidence from isotope studies indicates that the compound is synthesized by a transfer of the glucosyl moiety of maltose to the three position of cosubstrate glucose molecules. On further treatment of nigerose with the transferring enzyme, it is disproportionated to new glucosyl oligosaccharides.

The α-D-(1→3) glucosidic linkage is a characteristic structural feature of a number of glucosyl polysaccharides and comprises approximately half of the glucosidic bonds in nigeran,[3] a variable frac-

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(2) A preliminary account of a portion of this work has been published in abstract form in *Federation Proc.*, **15**, 325 (1956).

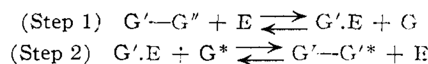
(3) S. A. Barker, E. J. Bourne and M. Stacey, *J. Chem. Soc.*, 3084 (1953).

tion of the bonds of bacterial dextrans[4] and a small but significant fraction of the linkages of waxy maize starch.[5,6] Little information is available on the mechanism of biosynthesis of the α-D-(1→3) glucosidic linkage. A number of years ago, the enzymatic synthesis of a glucosyl disaccharide for which an α-D-(1→3) structure was suggested was observed in digests of maltose and the transferring enzyme of *Aspergillus oryzae*.[7,8] More recently such a compound has been detected chromatographically in digests of maltose with a variety of other fungal enzymes[9] and with some yeast enzymes.[10] The oligosaccharide produced by the *A. oryzae* enzyme has now been isolated in chromatographically pure form and in sufficient amount for structural characterization. The compound is 3-O-α-D-glucopyranosyl-D-glucose (nigerose).[11] Nigerose on further treatment with the transferring enzyme is disproportionated to three reducing oligosaccharides, one of which is a new glucosyl trisaccharide with an α-D-(1→3) and an α-D-(1→6) linkage.

It has been reported by Peat, *et al.*,[12] and by Shibasaki[13] that nigerose is produced in small amounts during the action of enzyme preparations from *Aspergillus niger* on concentrated solutions of glucose. In our study, it was found that the *A. oryzae* enzyme synthesized considerably more nigerose from a mixture of glucose and maltose than from glucose alone. A transglucosylation reaction in which the glucosyl unit of maltose is transferred to the three position of glucose is probably responsible for the increased synthesis. Evidence from isotope experiments supports the transglucosylation mechanism for enzyme action. In view of the similarity in action patterns of the *A. oryzae* enzyme and of those enzymes responsible for the synthesis of glucosyl polysaccharides, transglucosylation may also be the type of mechanism involved in the synthesis of the α-D-(1→3) linkages in the glucosyl polysaccharides.

In the isolation of 3-O-α-D-glucopyranosyl-D-glucose (nigerose), use was made of its non-fermentability by bakers' yeast and of its fast migration rate on paper chromatograms. From an enzymatic digest of 10 g. of glucose and 10 g. of maltose, 0.2 g. of chromatographically pure oligosaccharide was obtained; specific rotation, +89°.[14] The oligosaccharide possesses a free reducing group reactive with several of the reducing sugar reagents. Paper chromatographic examination of partial and prolonged acid hydrolysates of the oligosaccharide revealed that glucose and unhydrolyzed compound were present in the former and glucose only in the latter. Determination of glucose in the hydrolysates showed that the oligosaccharide was quantitatively converted to glucose on hydrolysis. Melting point and X-ray diffraction pattern data of the crystalline phenylosazone of the enzymatically synthesized nigerose are in agreement with values previously reported for the osazone of the compound[3,14] and for the osazone of turanose,[14,15] which, of course, yields the same osazone as nigerose.

The nigerose which was synthesized in a digest of glucose-C^{14} and maltose was found to be radioactive. This result, together with the earlier findings on the mechanism of enzyme action on maltose[7] and on isomaltose,[16] point to a two-step transglucosylation mechanism for the synthesis of nigerose. The two-step mechanism is shown diagrammatically in the accompanying equations.



$$\text{(Step 1)}\quad G'{-}G'' + E \rightleftarrows G'.E + G$$
$$\text{(Step 2)}\quad G'.E + G^* \rightleftarrows G'{-}G'^* + E$$

The notations are as follows: G, glucose; G*, glucose-C^{14}; G′, glucosyl unit; G″ glucose unit substituted at position 4; G′*, C^{14}-glucose unit substituted at position 3, and E, enzyme. Evidence for the formation of a glucosyl–enzyme complex (G′.E) in this sequence of reactions was obtained from the isotope experiments. From glucose-C^{14} and non-radioactive maltose the transferring enzyme produced three radioactive oligosaccharides, namely, maltose, isomaltose and nigerose. Since under our experimental conditions little synthesis of oligosaccharides occurred from glucose-C^{14}, a reversibility of step 1 of the reaction sequence is indicated. Further, the incorporation of free glucose-C^{14} into maltose indicates a separate transitory existence of a glucosyl–enzyme complex. In another isotope experiment, isomaltose-C^{14}, maltose-C^{14} and nigerose-C^{14} were produced by the enzyme from glucose-C^{14} and nigerose. A disproportionation of the nigerose and the participation of glucose-C^{14} as a cosubstrate in the reactions is probably the mechanism of synthesis of labeled maltose and isomaltose, while a glucose exchange reaction between non-radioactive nigerose and glucose-C^{14} accounts for the synthesis of labeled nigerose These results indicate reversibility for steps 1 and 2 of the proposed reaction sequence.

The experiment in which glucose-C^{14} and nigerose were used as substrates for the enzyme showed that the nigerose was itself disproportionated to glucose and three glucosyl oligosaccharides (compounds I, II and III). Utilizing the relationship of papergram mobility and carbohydrate structure developed by French and Wild,[8] compound I appears to be isomaltose; compound III appears to

(4) A. Jeanes, W. C. Haynes, C. A. Wilham, J. C. Rankin, E. H. Melvin, M. J. Austin, J. E. Cluskey, B. E. Fischer, H. M. Tsuchiya and C. E. Rist, THIS JOURNAL, **76**, 5041 (1954).

(5) M. L. Wolfrom and A. Thompson, *ibid.*, **77**, 6403 (1955).

(6) The initial suggestion that linkages other than the α-D-(1 → 4) and α-D-(1 → 6) might be present in starch was made by M. Abdel-Akher, J. K. Hamilton, R. Montgomery and F. Smith, *ibid.*, **74**, 4970 (1952).

(7) J. H. Pazur and D. French, *J. Biol. Chem.*, **196**, 265 (1952).

(8) D. French and G. M. Wild, THIS JOURNAL, **75**, 2612 (1953).

(9) K. Shibasaki and K. Aso, *J. Fermentation Technol. (Japan)*, **31**, 311 (1953); *C. A.*, **48**, 7109 (1954).

(10) K. Shibasaki and K. Aso, *J. Fermentation Technol. (Japan)*, 395 (1954); *C. A.*, **49**, 2669 (1955).

(11) The trivial name "nigerose" has been suggested for this compound by Barker, *et al.*,[3] and will be used in this paper.

(12) S. Peat, W. J. Whelan and K. S. Hinson, *Chem. & Ind.*, 385 (1955).

(13) K. Shibasaki, *J. Fermentation Technol. (Japan)*, **31**, 354 (1953); *C. A.*, **48**, 7109 (1954).

(14) Specific rotations previously reported for this compound are: +81.8° (*J. Gen. Chem. (U.S.S.R.)*, **16**, 1923 (1946); *C. A.*, **41**, 6210 (1947)), +136° (ref. 3), +135° (*J. Fermentation Technol. (Japan)*, **32**, 399 (1954); *C. A.*, **49**, 2670 (1955)) and +87° (THIS JOURNAL, **78**, 1885 (1956)).

(15) C. S. Hudson, *J. Org. Chem.*, **9**, 470 (1944).

(16) J. H. Pazur, *J. Biol. Chem.*, **216**, 531 (1955).

be isomaltotriose and compound II appears to be a glucosyl trisaccharide with an α-D-(1→3) and an α-D-(1→6) linkage. Since compound II has not been previously prepared, it was isolated in chromatographically pure form and subjected to partial acid hydrolysis in order to get some indication as to its constitution. The hydrolysate contained three new reducing compounds as indicated by paper chromatography with R_f values corresponding to glucose, nigerose and isomaltose. This result, the characteristic R_f value, and the previous finding that the *A. oryzae* transfer reactions occur most readily to position 6 of the accepting glucose residues, point to O-α-D-glucopyranosyl-(1→6)-O-α-D-glucopyranosyl-(1→3)-D-glucose as the structure for compound II. Such a compound should be a very useful reference compound for structural analysis of the dextrans[4] and for immunochemical studies of the type described by Kabat.[17,18]

Nigerose must then be added to the list of compounds which can be disproportionated by the glucose-transferring enzyme of *Aspergillus oryzae*. This list[7,16] now includes maltose, isomaltose, isomaltotriose, panose and nigerose. Transfers of the glucosyl units by this enzyme may occur to position 6, position 4 or position 3 of the accepting glucosyl cosubstrates. Action patterns of this type for carbohydrate transferring enzymes involved in the synthesis of glucosyl polysaccharides may account for the presence of structural irregularities in such polysaccharides. In particular in the synthesis of amylopectin, Q-enzyme which is primarily responsible for the synthesis of the α-D-(1→6) linkages may occasionally effect a transfer to the 3-position of the accepting glucose residue, and hence it may be the enzyme responsible for the synthesis of the α-D-(1→3) link in amylopectin. This possibility is being explored in our laboratory.

Experimental

Enzyme.—Enzyme preparations of *Aspergillus oryzae*[19] have been previously shown to effect a hydrolysis and a disproportionation of maltose to glucose and to new oligosaccharides.[7] Since attempts to separate the hydrolyzing and transferring activities have been unsuccessful,[20] it is likely that one enzyme catalyzes both types of reactions. The best measure of enzyme activity is then the disappearance of maltose. A unit of activity is defined as that amount of enzyme which at 30° and at *p*H 6.5 will convert 1 mg. of maltose (at initial concentration, 0.2 *M*) to glucose and new oligosaccharides in a 3 hr. reaction period. The enzyme solutions employed in this study were prepared by dissolving the enzyme concentrate in water, removing insoluble particles by filtration and assaying by the method described below.

Assay tubes containing 1.0 ml. of 0.4 *M* maltose solution buffered to *p*H 6.5 with 0.1 *M* phosphate buffer and 1.0 ml. of the enzyme solution were incubated at 30° for 3 hr. The maltose in a 0.005-ml. aliquot of the digest was separated from other oligosaccharides by paper chromatography, located on the paper with copper sulfate reagent and extracted with 2 ml. of molybdic acid reagent.[21] The sample was diluted to 15 ml. with water and its optical density was measured at 750 mμ in a Beckman spectrophotometer. Values for maltose standards were also obtained by this procedure. Units of enzyme activity per ml. of solution were then calculated.

Isolation of Nigerose.—A solution of 10 g. of glucose and 10 g. of maltose in 40 ml. of water was shaken with 10 ml. of a solution of *A. oryzae* transferring enzyme (total activity, 440 units) and 10 ml. of toluene. After a reaction period of 96 hr. at room temperature, the aqueous layer was separated and heated at 100° for 10 minutes. Five g. of bakers' yeast was added to the cooled digest and fermentation was allowed to proceed for 48 hr. Qualitative paper chromatograms of the digest showed that the glucose and maltose had been essentially removed by this treatment and that a mixture of non-fermentable oligosaccharides remained in the solution. Samples of 0.2 ml. of the digest were placed on individual paper chromatograms and the fastest moving component was isolated by methods previously described.[14]

From the digest, 0.2 g. of chromatographically pure compound was obtained. The specific rotation of the sample was +89° (*c* 1, water). The apparent R_f value (2 ascents of the solvent) was 0.48; glucose and maltose values under the same conditions were 0.60 and 0.41, respectively. Acid hydrolysis of 1.20 mg. of the compound yielded 1.24 mg. of glucose as measured with Shaffer–Somogyi reagent 60.[22]

Nigerose Phenylosazone.—A mixture of 0.07 g. of the compound, 0.14 g. of phenylhydrazine hydrochloride, 0.21 g. of sodium acetate in 2 ml. of water was heated in a boiling water-bath for 30 minutes. The compound that precipitated from the solution on cooling was collected, air-dried and crystallized from 1 ml. of hot ethyl alcohol. The melting point of the crystalline product was 204–205° dec.; melting point of osazone of nigerose 203–205°[8,14] dec.; mixed m.p. 203–205° dec. The X-ray diffraction pattern[23] of the osazone was identical with the diagram for the phenylosazone of nigerose[14] and gave the following data: 5.42[24]–50[25]; 5.13–50; 4.68–30 (double); 4.23–70; 4.05–70; 3.58–20; 3.32–100; 3.16–10; 2.68–60; 2.20–30; 2.09–10; 1.93–20.

Enzymolysis of Glucose-C^{14} and Maltose.—A solution of 1 mg. of uniformly labeled glucose-C^{14} (total radioactivity 20,200 c.p.m.) and 5 mg. of maltose in 0.1 ml. of phosphate buffer of *p*H 6.5 was treated with 0.1 ml. of enzyme (total activity, 4.4 units). Samples of 0.05 ml. were placed on a paper strip and inactivated by heating at 100° for 10 minutes at 0 and 24 hr. reaction periods. The reducing sugars in the aliquots were separated by multiple ascent paper chromatography and located on the paper with copper sulfate reagent. The radioactive components were detected by radioautography and their radioactivities measured in a G. M. counting assembly. The values for glucose and the disaccharides were as follows: glucose, 16,600 c.p.m.; nigerose, 340 c.p.m.; maltose, 150 c.p.m. and isomaltose, 2460 c.p.m. Since under the above conditions new oligosaccharides were not produced in appreciable amounts from glucose alone, the major portion of the labeled compounds must have arisen *via* transglucosylation from maltose and glucose-C^{14}.

Enzymolysis of Nigerose.—A solution of 20 mg. of nigerose in 0.5 ml. of 0.1 *M* phosphate buffer *p*H 6.5 was treated with 0.5 ml. of *A. oryzae* enzyme (total activity, 22 units). Samples of 0.01 ml. were removed at 0 and 24 hr., placed on paper chromatograms and inactivated by heat. The remainder of the digest was also inactivated by heat at the 24-hr period. Paper chromatographic analysis of the 0 and 24 hr. samples revealed that the nigerose had been converted to glucose and to new oligosaccharides which moved on paper at a slower rate than nigerose. The apparent R_f values of the compounds (3 ascents of the solvent) were as follows: glucose, 0.75; nigerose, 0.60; compound I, 0.39; compound II, 0.27; compound III, 0.13. Under the same chromatographic conditions R_f value of isomaltose was 0.39 and of isomaltotriose, 0.14. Since the enzyme has been shown previously[7] to produce isomaltose and isomaltotriose by transfer of glucosyl units to glucose and to isomaltose and since the R_f values of compounds I and III are in agreement with the R_f values of isomaltose and isomaltotriose, it is likely that compound I is isomaltose and compound III is isomaltotriose.

The mobility of compound II did not correspond with any of the reference glucosyl oligosaccharides which were avail-

(17) E. A. Kabat, THIS JOURNAL, **76**, 3709 (1954).

(18) P. Z. Allen and E. A. Kabat, *ibid.*, **78**, 1890 (1956).

(19) Kindly supplied by Takamine Laboratories, Inc., Clifton, New Jersey.

(20) J. H. Pazur, *Federation Proc.*, **13**, 272 (1954).

(21) H. Tauber and I. S. Kleiner, *J. Biol. Chem.*, **99**, 249 (1932).

(22) P. A. Shaffer and M. Somogyi, *ibid.*, **100**, 695 (1933).

(23) The X-ray patterns were obtained at the Instrumentation Laboratory of the University of Nebraska.

(24) Interplanar spacings Å. CuK radiation.

(25) Relative intensities on basis of 100 for the strongest line.

able. It was isolated from the remainder of the 24-hr. digest by paper chromatographic methods previously described.[14] The isolated compound migrated on paper as one homogeneous spot and at a rate intermediate between those for maltotriose and panose. According to French and Wild[8] an oligosaccharide with α-D-(1 → 3) and α-D-(1 → 6) linkages would possess this type of mobility. Hydrolysis of the oligosaccharide should yield nigerose and isomaltose as the two disaccharide fragments and glucose as the monosaccharide fragment. Three reducing compounds were produced on hydrolysis as indicated by paper chromatography with R_f values (three ascents of the solvent) 0.74, 0.58, 0.38. Under similar conditions R_f values of glucose, nigerose and isomaltose were 0.75, 0.60 and 0.39. In consideration of these findings and the mechanism of action of the *A. oryzae* transferring enzyme, it is likely that the new oligosaccharide is O-α-D-glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 3)-D-glucose.

Enzymolysis of Glucose-C^{14} and Nigerose.—A solution of 5 mg. of nigerose and 2 mg. of glucose-C^{14} (total radioactivity, 31,900 c.p.m.) in 0.1 ml. of phosphate buffer (*p*H 6.5 and 0.1 *M*) was treated with 0.1 ml. of enzyme (total activity, 4.4 units). Samples of 0.05 ml. were taken at 0 and 24 hr. and analyzed for reducing and radioactive components by the methods of the previous sections. The radioactivities of the compounds in the 24-hour sample were as follows: glucose, 30,150 c.p.m.; nigerose, 420 c.p.m.; maltose, 180 c.p.m. and isomaltose, 1460 c.p.m.

LINCOLN, NEBRASKA

[CONTRIBUTION FROM THE DEPARTMENT OF MICROBIOLOGY, WAYNE STATE UNIVERSITY, COLLEGE OF MEDICINE]

Enzymatic Decarboxylation of the Aminobenzoates[1]

BY WILLARD G. McCULLOUGH, JOHN T. PILIGIAN[2] AND IDUS J. DANIEL[2]

RECEIVED JULY 19, 1956

From *E. coli* 0111:B4 cell-free enzyme preparations have been obtained which decarboxylate *p*-aminobenzoic acid and anthranilic acid to aniline. These preparations do not metabolize *m*-aminobenzoate. Dialysis of the enzyme preparations against 0.2 *M* acetate buffers at *p*H 5.0 results in a complete loss of the ability to mediate these chemical transformations. Restoration of the decarboxylating activity is obtained upon the addition of pyridoxal phosphate and iron(III), but the addition of either singly causes no reconstitution. These studies show that the above reactions are mediated by a pyridoxal phosphate-dependent enzyme(s) and that a metallic ion is an obligatory requirement of the system.

Introduction

During studies of *p*-aminobenzoic acid (PABA) metabolism in *Mycobacterium* species, Sloane, *et al.*,[3] demonstrated the conversion of PABA to aniline and to *p*-aminophenol. Small yields of the end-products were obtained and no detection of the liberation of carbon dioxide was possible. With *in vitro* preparations of *Mycobacterium* species Sloane[4] was able to demonstrate that the tetracycline antibiotics would block the conversion of aniline to *p*-aminophenol. The observed inhibition of the hydroxylating system was reversed by a PABA-metabolite which was obtained in crystalline form. The metabolite did not influence the antibiotic action of the tetracycline compounds in the standard antibiotic assay procedures. Studies of the acquisition of resistance by *E. coli* 0111:B4[5] toward the action of chlorotetracycline led us to the observation that the incorporation of pyridoxine and of the aminobenzoates into a chemically defined medium resulted in a partial reversal of the antibiotic action of chlorotetracycline.[6] *In vitro* studies of the relationship between vitamin B_6 and the aminobenzoates have demonstrated the existence in these cells of a pyridoxal phosphate dependent enzyme(s) which decarboxylates PABA and anthranilic acid. Experiments delineating this relationship are described in this paper.

Experimental

Production of Cells.—Cells of *E. coli* 0111:B4 were produced in a chemically defined medium[6] under aerobic conditions, harvested by centrifugation, washed three times by centrifugation with 0.067 *M* phosphate buffer of *p*H 6.0, and stored at 4° in the same buffer of such volume that each ml. of suspension contained 8 mg. of bacterial nitrogen.

Preparation of Cell-free Extracts.—For cell disruption a quantity of cells equivalent to 16 mg. of bacterial nitrogen was suspended in 5.0 ml. of 0.067 *M* phosphate buffer at *p*H 6.0 and was shaken with 10 g. of small glass beads for one minute at 4° in a bacterial disintegrator operating at a speed of approximately 2500 cycles per minute. This machine has been described in other studies.[7] Under these conditions essentially 100% of the aminobenzoate decarboxylase activity of the intact cells may be extracted in the *p*H range of 6.0 to 7.0. The homogenized mixture was decanted from the beads. The beads were washed with a small volume of buffer and the washing added to the mixture. The latter was then centrifuged at 3,000 *g* for five minutes and the supernatant was used as the extract. Microscopic examination revealed no whole cells and repeated attempts to culture these preparations failed.

Enzyme Resolution.—The cell-free extracts were dialyzed against 0.2 *M* acetate buffer at *p*H 5.0 and room temperature for 24 hours. A small amount of insoluble protein was removed by centrifugation and the extracts were adjusted to *p*H 6.0. These preparations are water-clear.

Incubation. The various cell preparations were incubated with the substrates at 35° on a rotary shaker[8] under aerobic conditions or in an atmosphere of nitrogen.

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(1) A preliminary report of this work was presented at the 128th National American Chemical Society Meeting at Minneapolis, September, 1955. Supported in part by a grant from The Upjohn Company, Kalamazoo, Michigan.

(2) Abstracted in part from theses submitted to Wayne University in partial fulfillment of the requirements for the degree of Master of Science, 1955 (J. T. P.) and 1956 (I. J. D.).

(3) N. H. Sloane, C. Crane and R. L. Mayer, *J. Biol. Chem.*, **193**, 453 (1951).

(4) N. H. Sloane, THIS JOURNAL, **75**, 6352 (1953).

(5) F. Kauffmann, *J. Immunology*, **57**, 71 (1947).

(6) W. G. McCullough, J. T. Piligian and I. J. Daniel, unpublished data; the cells used in this study were produced by incubating the cultures at 35° for 20 hr. on a rotary shaker (*cf.* ref. 8). The initial *p*H of the medium was 6.8 and 50 ml. of medium were contained in a 250-ml. erlenmeyer flask. The composition of the medium per liter is: glucose, 10 g.; $K_2C_6H_6O_7 \cdot H_2O$, 10 g.; NH_4Cl, 2 g.; K_2HPO_4, 6 g.; KH_2PO_4, 6 g.; $MgSO_4 \cdot 7H_2O$, 50 mg.; $MnSO_4$, 2.4 mg.; NaCl, 10 mg.; $FeSO_4$, 5 mg.; KNO_3, 300 mg.; and *l*-cystine, 40 mg. Incorporation of the aminobenzoates into this medium does not stimulate growth and does not affect the production of the aminobenzoate decarboxylase(s)

(7) L. M. Corwin, L. J. Schroeder and W. G. McCullough, THIS JOURNAL, **78**, 1372 (1956).

(8) New Brunswick Scientific Co., New Brunswick, N. J.