nitely establish the structure of its pyridylethylated derivative. This was done by condensing ethyl α-methylacetoacetate with 2-vinylpyridine and subjecting the product to ketonic cleavage. The semicarbazone of this material and that obtained by direct pyridylethylation of the ketone were identical. These reactions are outlined in the following scheme.

$$CH_3COCH(CH_3)CO_2C_2H_5 + 2\text{-}C_5H_4NCH{=}CH_2$$

$$\downarrow$$

$$CH_3COC(CH_3)(CH_2CH_2C_5H_4N\text{-}2)CO_2C_2H_5$$

$$\downarrow$$

$$CH_3COC(H)(CH_3)(CH_2CH_2C_5H_4N\text{-}2)$$

$$\uparrow$$

$$CH_3COCH_2CH_3 + 2\text{-}C_5H_4NCH{=}CH_2$$

Thus, with methyl ethyl ketone pyridylethylation appears to occur exclusively at the α-methylene carbon atom.

The mechanism of the pyridylethylation of ketones in the presence of sodium is probably analogous to that proposed by Doering and Weil[1] for the condensation of β-ketoesters and malonic esters with 2-vinylpyridine in the presence of alcoholic sodium ethoxide.

Work is now in progress in this Laboratory on the extension of these studies to the direct pyridylethylation of more ketones and a large variety of other types of active hydrogen compounds with 2- and 4-vinylpyridine.

Experimental

General Procedure for the 2-Pyridylethylation of Ketones.—A mixture of two equivalents of the appropriate ketone and one of 4- or 2-vinylpyridine was placed in a 500-ml. three-necked, round-bottom flask equipped with ground-glass joints and carrying a thermometer dipping into the mixture, a dibutyl phthalate-sealed stirrer and a reflux condenser protected from atmospheric moisture with a drying tube filled with Drierite. To the rapidly stirred mixture, 0.2 of an equivalent of small pieces of sodium metal was added rapidly. After a few minutes of stirring, a highly exothermic reaction started. If the reaction became too violent, it was checked by immersing the flask in an ice-water-bath. After the exothermic reaction subsided, the mixture was heated to and kept at its reflux temperature for the reaction times indicated in Table I. The mixture was then allowed to cool to room temperature and poured onto a mixture of ice and 1.3–1.5 equivalents of concentrated hydrochloric acid. Any acid-insoluble oil was removed and the aqueous solution extracted with several 50-ml. portions of benzene. The oil and the combined benzene extracts were dried over anhydrous sodium carbonate, the solvent distilled and the residue distilled first at atmospheric pressure and then in vacuum to give recovered ketone and neutral condensation products. The aqueous phase was then made strongly basic by the addition of 30% aqueous sodium hydroxide solution. After separating the oil which formed, the water layer was extracted with several 50-ml. portions of benzene. The oil and the benzene extracts were combined, dried over anhydrous sodium carbonate, the solvent removed and the residue fractionated in vacuum to give unreacted 2-vinylpyridine and the pyridylethylated ketones.

Preparation of Authentic Samples of Pyridylethylated Ketones.—Phenyl γ-(2-pyridyl)-propyl ketone, isobutyl γ-(2-pyridyl)-propyl ketone and methyl (α-methyl-γ-2-pyridylpropyl) ketone were prepared by the sodium-catalyzed pyridylethylation of ethyl benzoylacetate, ethyl isovalerylacetate and ethyl α-methylacetoacetate and subjecting the condensation products to ketonic cleavage by methods which have been published previously.[1,2] Mixed melting points on the semicarbazones of these compounds with samples prepared by the direct pyridylethylation of acetophenone, methyl isobutyl ketone and methyl ethyl ketone, respectively, showed no depression.

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[CONTRIBUTION FROM THE RESEARCH DIVISION OF ANHEUSER-BUSCH, INC., AND DEPARTMENT OF BOTANY, WASHINGTON UNIVERSITY]

Uridine as a Growth Factor for a Strain of *Streptococcus Faecalis*[1]

BY HARRISON A. HOFFMANN[2] AND PAUL L. PAVCEK

Hitherto unreported factors in yeast extract (Basamin) stimulate early growth of a strain of *Streptococcus faecalis*. The growth stimulating substances have been concentrated approximately a thousand-fold by extraction with aqueous acetone and subsequent treatment with charcoal and Superfiltrol. By means of ultraviolet spectrophotometry and paper-partition chromatography, one of the active components of the concentrate was identified as uridine. Commercial uridine and pure preparations obtained from Basamin evoke a half-maximum growth response as compared to the activity of the concentrate; however, surface-active agents such as Tween-80 and Triton A-20 act synergistically with uridine to give a growth response equivalent to that obtained from yeast extract.

A number of lactic acid bacteria grow well in a medium of known chemical composition. However, supplementation of the synthetic medium with extracts of natural substances such as yeast and liver significantly shortens the lag phase and the incubation time required for maximum growth of these organisms.

During an investigation of the nutritional requirements of lactic acid bacteria, it was observed that yeast extract (Basamin) provided a stimulation of the rate of growth of a strain of *Streptococcus faecalis*, which could not be duplicated by supplementation of the basal medium with any of a large variety of known compounds available at that time. Studies described here show that uridine is one of the factors in Basamin contributing to the early growth of the test organism, and present evidence for other factors in yeast extract which act synergistically with uridine.

(1) Presented in part, before the Division of Agricultural and Food Chemistry, at the April, 1951 Meeting of the American Chemical Society at Cleveland, Ohio.

(2) From the Ph.D. dissertation of H. A. Hoffmann, 1951.

Experimental

The organism used in this study is a strain of *S. faecalis*, presumably ATCC No. 8043. However, a more recent culture of the same number obtained from the American

Type Culture Collection shows a comparatively slight response to uncharacterized factors in Basamin. The sensitive strain has been designated *S. faecalis* AB. Cultures were maintained by weekly transfer on stock agar slants containing 1% glucose, 1% Basamin and 0.3% beef extract (Difco). The inoculum used for assay of the growth factor was prepared from an 18 to 24 hour culture grown in stock broth at 30°. The cells were washed twice by centrifuging and resuspending in sterile 0.9% saline. This washed suspension was then standardized turbidimetrically so that in the final dilution 0.1 ml. added to each assay tube contained approximately 30 thousand cells.

The basal medium shown in Table I is modified from Henderson and Snell[3]; it gives optimum reproducible growth curves during the first 15 to 18 hours incubation at 30° when supplemented with increasing levels of Basamin. Five ml. of the double-strength medium were used in each tube (18 × 150 mm.) and distilled water and sample added to make a total volume of 10 ml. Growth was measured turbidimetrically with a Fischer Electrophotometer (650 mμ filter), and values reported represent the average of duplicate tubes. Basamin was used as an assay standard. A growth response equivalent to that obtained with one mg. of Basamin in 10 ml. of culture medium was arbitrarily defined as one unit of activity.

TABLE I

COMPOSITION OF BASAL MEDIUM[a]

	Mg.		G.
L-Glutamic acid	100	Glucose	4
DL-Alpha alanine	100	Sodium citrate	1
DL-Aspartic acid	100	Sodium acetate	1
DL-Leucine	40	Potassium phosphate	
DL-Valine	40	(dibasic)	1
DL-Isoleucine	40	Ammonium chloride	0.5
			Mg.
DL-Methionine	40	Adenine	2
DL-Tryptophan	40	Guanine	2
DL-Phenylalanine	40	Uracil	2
DL-Threonine	40	Xanthine	2
L-Lysine HCl·H_2O	40	Thiamine	0.1
L-Arginine HCl	40	Riboflavin	.1
L-Histidine	20	Calcium pantothenate	.1
DL-Serine	100	Biotin	.002
Glycine	20	Folic acid	.004
L-Proline	40	Pyridoxamine HCl	.1
Glutathione	4	Pyridoxal	.1
L-Cystine	20	Niacin	.2
L-Tyrosine	20	*p*-Aminobenzoic acid	.04
L-Asparagine	50	Adjust *p*H to 6.9	
Salt solution[b]	2 ml.		

[a] Quantities for 100-ml. double strength medium; 5 ml. used per complete tube of 10 ml. [b] Dissolve in 250 ml. of dist. H_2O: $MgSO_4 \cdot 7H_2O$, 10 g.; $FeSO_4 \cdot 7H_2O$, 0.5 g.; NaCl, 0.5 g., $MnSO_4 \cdot 7H_2O$, 1 g.

Concentration Methods.—The factors which stimulate early growth of *S. faecalis* AB are found in a wide variety of natural materials (see Table II). Beer and Basamin were used as the source materials for the preparation of concentrates.

Attempts to effect separation by means of precipitation techniques with heavy metals, salts and organic solvents[4-8] resulted in large losses of the active material and poor separation. A successful procedure used in concentrating the factor from beer will be outlined in detail. This method of extraction and adsorption with few modifications has been employed for the preparation of the concentrates used in further experiments to be described.

(1) **Butanol Extraction.**—Four liters of ten-fold concentrated beer containing approximately 2 million units were placed in a Dakin-type extractor and extracted with 4-liter aliquots of *n*-butanol for a period of about 8 hours. The combined butanol extracts, totalling about 20 liters, were placed in the refrigerator at 5° overnight and the precipitate removed by filtration through Celite. This filtered extract contained 900 thousand units or a potency of 20 thousand units per gram.

TABLE II

RELATIVE FACTOR POTENCIES OF NATURAL MATERIALS

Material	Units/g.[b]
Malt sprouts (dried aqueous extract)	1500
Basamin	1000
Corn steep liquor	1300
Beer (Budweiser)	1400
Beer wort	790
Molasses (beet)	175
Whole liver[a]	2800
Liver-L (Wilson)[a]	2000
Liver, 1-20 (Wilson)	1400
Pepsin	530
Trypsin (Takamine)	6400
Trypsin, 1:300 (Wilson)	800
Zein[a]	400
Wheat gluten[a]	1900
Soy flour[a]	290
Celluflour[a]	None
Peanut flour[a]	1500
Beef muscle[a]	900
Whole egg[a]	1100
Egg albumin[a]	120
Yeast (bakers')[a]	2000
Casein (crude)[a]	300
Gelatin[a]	300
Tomato serum	750
Urine (human)	40

[a] Samples subjected to acid hydrolysis (121° for 90 min. at *p*H 2). [b] Weights on a dry solids basis.

(2) **First Charcoal Adsorption.**—The butanol extract was concentrated *in vacuo* to a volume of 2 liters, refrigerated overnight, and the resulting precipitate removed by filtration through Celite. The filtrate was then stirred at room temperature with 200 g. of activated charcoal (Darco G-60) for one hour and the mixture separated by filtration. The carbon pad was eluted at room temperature twice by stirring with one liter of distilled water. To avoid large losses of active material, the aqueous eluates containing 200 thousand units (80 thousand units per gram) were combined with the charcoal-treated butanol filtrate containing 700 thousand units (40 thousand units per gram), and this butanol-water mixture was steam distilled under reduced pressure to remove all butanol.

(3) **Second Charcoal Adsorption.**—The butanol-free extract from the preceding step, now at a natural *p*H of 3.5, was twice stirred for 30 minutes at room temperature with 50 g. of Darco G-60 and the final filtrate discarded. The carbon pads were combined and eluted twice at room temperature by stirring one hour with a liter of a mixture of water, ethanol and ammonium hydroxide (5:4:1 on a volume basis). The combined eluates were freed of ethanol and ammonia by boiling at reduced pressure and filtered to remove residual carbon. The filtrate contained in a volume of 500 ml. approximately 400 thousand units and the solids showed a potency of 100 thousand units per gram.

(4) **Adsorption with Superfiltrol F.O.**—The preparation from 3 was acidified with dilute sulfuric acid to *p*H 4 and stirred at room temperature with 50 g. of Superfiltrol F.O. for one hour. The filter was washed with water and the combined filtrate and washings brought to *p*H 7.5 with dilute sodium hydroxide, concentrated to about 20% of its original volume over a water-bath and again treated with 10 g. of Superfiltrol. At this point the preparation contained about 375 thousand units with a potency of 500 thousand units per gram representing 500-fold concentration and a recovery of approximately 40% of the active material.

(5) **Precipitation Methods.**—The preparation from 4 was concentrated *in vacuo* over a steam-bath to a volume of

(3) L. M. Henderson and E. E. Snell, *J. Biol. Chem.*, **172**, 5 (1948).
(4) F. R. Smith, *J. Bact.*, **46**, 369 (1943).
(5) D. W. Woolley, *J. Exp. Med.*, **73**, 487 (1941).
(6) H. Sprince and D. W. Woolley, *ibid.*, **80**, 213 (1944).
(7) R. Ballentine, G. M. Tuck, L. K. Schneider and F. J. Ryan, *Federation Proc.*, **4**, 85 (1945).
(8) B. L. Hutchings, N. Bohonos and W. H. Peterson, *J. Biol. Chem.*, **166**, 48 (1946).

about 20 ml., then further reduced to near dryness in a vacuum desiccator. A biologically inactive crystalline precipitate formed during the drying. This precipitate was only slightly soluble in water, and the active amorphous residue was separated by addition of minimal quantities of cold water and immediate decantation. An equal volume of acetone was added to the water soluble fraction and the mixture placed in the refrigerator overnight. A second crystalline precipitate which formed was also biologically inactive and discarded. After removal of acetone, the residual light-brown liquid contained about 350 mg. of solids with a 900-fold increase in potency over the starting material. One-half microgram of this concentrate in 10 ml. of culture medium promoted half-maximum response of the test organism.

Simple modifications of the described procedures were introduced with a view to reducing the number of operations: (a) ethyl acetate may be substituted for *n*-butanol in the continuous extraction in step 1, which eliminates the requirement of a charcoal adsorption in step 2; and (b) a Soxhlet extraction of Basamin with an acetone–water mixture, 19:1 (v./v.) is also satisfactory for the initial step and eliminates step 2.

The filter paper chromatopile technique of Mitchell and Haskins[9] was applied to further purification of the charcoal filtrates. A 20-ml. sample containing approximately 45 thousand units was taken up on 25 sheets of filter paper (9 cm. Whatman No. 1), dried in air, and placed 40 sheets below the solvent distributor. The pile contained a total of 1025 filter discs. Water-saturated *n*-butanol was used as the solvent. After 40 hours at 30°, the solvent front had descended 17.8 cm., and the pile was then separated into previously marked sections of 40 discs which were dried at room temperature. The top sheet of each section was extracted with 50 ml. of distilled water, and the extracts tested with *S. faecalis* AB. Growth promoting activity was located between discs 225 and 425.

Ninety-five per cent. of the active material was recovered by allowing 250 ml. of distilled water to percolate slowly through the 200 sheets supported in a buchner funnel. The percolate was then reduced *in vacuo* to a volume of about 10 ml. A precipitate formed during the concentration was removed by centrifugation and found to be biologically inactive. A second amorphous precipitate formed with the addition of acetone to 90% (v./v.) was also biologically inactive. The acetone was subsequently removed by evaporation and the remaining aqueous solution stirred with Superfiltrol F.O. to remove the brown color formed during the concentration. This preparation showed a twofold increase in potency over the starting material and a 1000-fold increase over Basamin.

Characterization.—The active material was completely dialyzable and passed rapidly through collodion and cellophane membranes. Biuret and ninhydrin reactions were negative, indicating the absence of peptide and α-amino groups. The Molisch (α-naphthol) reaction gave a strongly positive test, suggesting the presence of carbohydrate or other furfural-yielding substances. A Benedict test for reducing sugar was negative. The Bial orcinol–hydrochloric acid reaction for pentose was negative under normal conditions of testing, but with prolonged boiling gave a positive test, suggesting the presence of bound pentose.

Spectrophotometric examination of the material in the ultraviolet region gave an absorption curve characteristic of nucleic acid derivatives with a maximum absorption at 260 mμ at neutral *p*H.

Identification.—Commercial samples (Schwarz Labs., Inc.) of the nucleosides and nucleotides listed in Table III were added to the basal medium in molal equivalent quantities. Only those compounds containing the pyrimidine base stimulated growth. Results obtained from additional assays with varying concentrations of these pyrimidines in the basal medium are shown in Table IV. The relative activities indicated are: uridine > cytidine > uridylic acid > cytidylic acid.

Concentrations of pyrimidines greater than those listed in Table III (8×10^{-7}) did not stimulate greater growth, indicating that the response obtained with these compounds was not due to traces of uridine. To confirm this point chromatograms were run according to the method of Carter[10] using a solvent of *n*-butanol saturated with an aqueous solution of 10% urea to separate nucleosides and nucleotides. Uridylic acid and cytidylic acid spots on the chromatograms were located under ultraviolet light by contrast fluorescence. The spots were cut out, eluted with water, and the eluates assayed with the test organism. The cytidylic acid and uridylic acid chromatographically purified also stimulated growth of *S. faecalis* AB, and their relative activities were essentially in the same ratio (4:5) as that obtained with the initial commercial samples shown in Table III.

TABLE III

COMPARATIVE ACTIVITIES OF NUCLEOSIDES AND NUCLEOTIDES FOR GROWTH STIMULATION OF *S. faecalis* AB. 15.5 HOURS INCUBATION AT 30°

Supplement (8×10^{-7} molar)	Optical density × 100
None	0.1
Adenosine	.2
Adenylic acid	.2
Guanosine	.1
Guanylic acid	.2
Thymidine	.4
Cytidine	22.7
Cytidylic acid	15.2
Uridine	29.0
Uridylic acid	20.3

TABLE IV

COMPARATIVE ACTIVITIES OF PYRIMIDINE COMPOUNDS FOR GROWTH STIMULATION OF *S. faecalis* AB. 15 HOURS INCUBATION AT 30°

Molar concentration	Optical density × 100			
	Cytidylic acid	Uridylic acid	Cytidine	Uridine
4.1×10^{-8}	3.2	4.0	5.9	7.2
4.1×10^{-7}	4.8	5.2	8.3	10.9
4.1×10^{-6}	5.8	10.0	14.4	20.0

It was thus demonstrated by growth-rate studies that the compounds containing uracil or cytosine induced a biological response; however, the absorption spectrum of the refined concentrate gave no evidence for the presence of cytosine compounds. Growth factor preparations consistently gave maximum absorption at 260 mμ, while cytosine compounds have a characteristic peak at 275 mμ as demonstrated by Hotchkiss.[11]

Using the two-dimensional technique of Carter,[10] chromatograms were run for a qualitative determination of purines, pyrimidines and their corresponding nucleosides and nucleotides in the factor concentrate. The sample contained adenosine, uracil and uridine.

Isolation of Uridine.—A crude aqueous acetone extract of Basamin was used as the starting material for the isolation of uridine. The acetone extract was carried through step 4 of the previously described concentration procedures, and then a modification of methods described by Levene[12] was used. Fourteen ml. of 40% lead acetate solution was added to 200 ml. of the concentrate and the mixture allowed to stand overnight at room temperature. The precipitate consisting of nucleotides, if present, and inorganic phosphate was removed by decantation and filtration. The filtrate was brought to *p*H 10 with barium hydroxide, and after settling, the mixture was tested with the addition of lead subacetate solution to assure that precipitation was complete. The precipitate containing nucleosides in the form of lead salts was recovered by filtration, and carefully washed with water to remove occluded barium and lead salts. It was then decomposed by slow addition of dilute sulfuric acid with constant stirring until the *p*H remained steady at 0.2. Residual sulfuric acid was neutralized and precipitated by the addition of lead carbonate. The precipitate was filtered off and washed, and the filtrate and washings treated with hydrogen sulfide to remove residual lead. After removal of the lead sulfide, the filtrate was concentrated under reduced pressure to a volume of about 100 ml.

(9) H. K. Mitchell and F. A. Haskins, *Science*, **110** 278 (1949).

(10) C. E. Carter, THIS JOURNAL, **72**, 1466 (1950).

(11) R. D. Hotchkiss, *J. Biol. Chem.*, **175** 315 (1948).

(12) P. A. Levene and F. B. LaForge, *Ber.*, **45** 608 (1912).

This material by spectrophotometric determination contained approximately 150 mg. of pyrimidine calculated as uridine.

The solution was further concentrated to a thick sirup and then mixed with an equal volume of warm 95% ethanol. The precipitate formed showed no significant biological activity at high dilutions and was discarded. The alcoholic solution was again concentrated, finally in a centrifuge tube, to a volume of approximately 2 ml. The addition of absolute ethanol to a total volume of 10 ml. produced a second amorphous precipitate which showed good biological activity, but the nature of the precipitate indicated gross impurities. Five ml. of diethyl ether was added to the remaining alcoholic solution and the mixture placed in the refrigerator. After two weeks, a small quantity of long, needle-like crystals formed on the sides of the tube. The crystals were centrifuged down, washed with absolute alcohol, and dried. The yield was approximately 4 mg. The melting point was 167° (uncorrected) comparable to 166° for a commercial uridine (Delta).

The absorption curve for uridine isolated from Basamin is compared to those of the refined concentrate and a commercial sample in Fig. 1. The optical densities used to plot these curves are based on the ratio to a peak optical density arbitrarily taken as 1.0 at the absorption maximum.

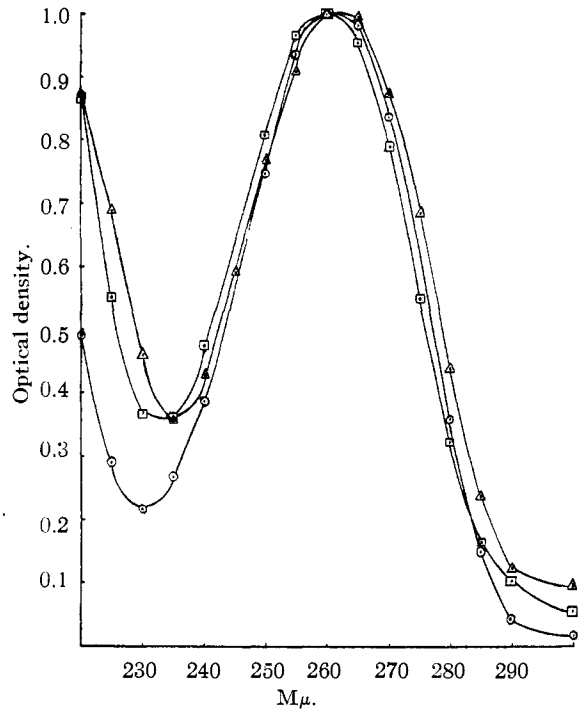
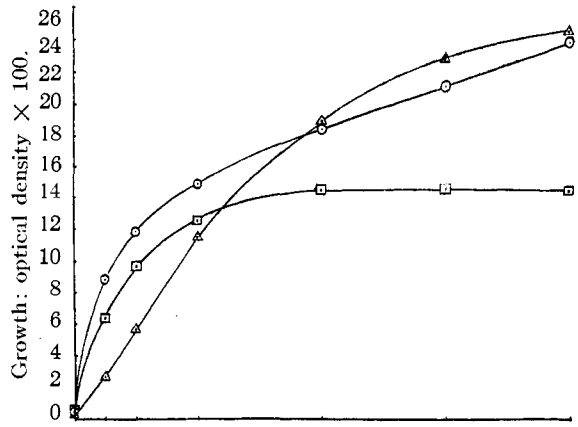


Fig. 1.—Comparative absorption curves of: ⊙, uridine (Delta); △, uridine from Basamin; and ⊡, factor concentrate.

The isolated uridine could not be distinguished from commercial samples in its growth stimulating activity for *S. faecalis* AB. A purity of at least 97% was indicated by two-dimensional paper chromatography.[10]

Synergism of Non-Ionic Surface Active Agents.—Only 50 to 60% of maximum growth response could be obtained with uridine as compared to that obtained with Basamin or the refined chromatopile concentrates. Comparative growth curves are presented in Fig. 2. Although compounds such as Tween-80 previously tested did not increase the growth response, they were tested again in the presence of uridine. It was found that the inclusion of either Tween-80 or Triton A-20 in the basal medium at the optimum level of 10 micrograms per tube provided a maximum growth response similar to that obtained with concentrates.

No attempt has been made to identify the substance in Basamin which simulates the action of Tween-80, but it seems likely that natural esters of fatty acids may act in a similar manner.



Concentrate	2	4	6	8 μg.
Uridine	1	2	3	4 μg.
Basamin	0.1	0.2	0.3	0.4 mg.

Fig. 2.—Comparative growth curves of *S. faecalis* AB with: ⊙, Concentrate; ⊡, Uridine; and △, Basamin; incubation 15 hr. at 30°.

Discussion

A requirement for the free pyrimidine bases uracil, cytosine, thymine and orotic acid has been demonstrated for a variety of microörganisms. However, references to a preference for nucleosides or nucleotides are few. Hutchings and co-workers[13] reported that nucleotides would support the growth of *Lactobacillus gayonii* and Loring and Pierce[14] have shown that nucleosides and nucleotides are more active than the free pyrimidines in supplying the essential growth requirement for a mutant strain of *Neurospora*. The order of decreasing activity of the pyrimidines for this organism, *i.e.*, uridine > cytidine > uridylic acid > cytidylic acid > uracil agrees with the findings reported here, except that *S. faecalis* AB cannot utilize uracil.

It must be emphasized however, that the pyrimidine compounds affect rate of growth in the case of *S. faecalis* AB. With the extension of the incubation time to 36 hours at 30°, there is no significant difference in terminal growth with or without supplementation of pyrimidine compounds or Basamin. Thus microbiological assays utilizing this organism do not require supplementation of the basal medium with uridine and Tween-80 when a long incubation time is employed.

There is no satisfactory explanation for the synergistic action of Tween-80 and Triton A-20 in the presence of pyrimidine compounds. A specific requirement for oleic acid is ruled out since Triton A-20 does not contain the oleic acid moiety in its molecule. It would appear therefore that these compounds function solely through their surface-active properties making pyrimidine compounds more readily available to the cell.

The limited data presented here suggest that uridine may be the pyrimidine precursor for nucleic acid synthesis in *S. faecalis* AB. Further experiments offering more evidence on this point will be reported elsewhere.

(13) B. L. Hutchings, N. H. Sloane and E. Boffiano, *J. Biol. Chem.*, **162**, 737 (1946).

(14) H. S. Loring and J. G. Pierce, *ibid.*, **153**, 61 (1944).

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[CONTRIBUTION OF THE NORTHERN REGIONAL RESEARCH LABORATORY[1]]

Starch Granule Swelling in Water Vapor Sorption

BY N. N. HELLMAN, T. F. BOESCH AND E. H. MELVIN

Microscopic measurements of the swelling of individual starch granules occurring with the sorption of water vapor at various relative pressures are reported for corn, potato, tapioca and waxy corn starch. The linear granule swelling in a water-saturated atmosphere over the vacuum-dry dimension is as follows: corn, 9.1%; potato, 12.7%; tapioca, 28.4%; and waxy corn, 22.7%. For all except tapioca starch, there is practically no hysteresis in the function of swelling *vs.* relative humidity of the atmosphere with which the starch is equilibrated. For all starches the function of swelling *vs.* moisture content shows an absorption–desorption loop with the desorption leg giving smaller granule dimensions for equal water content.

As part of an investigation of the water-sorptive properties of starch, it became of interest to determine the volume changes accompanying the sorption of water vapor. After preliminary studies, density methods were abandoned owing to ambiguity in interpreting the results when water was used as an immersion medium and difficulties of wetting, granule penetration, and degassing for non-aqueous immersion media. To avoid such uncertainties it was decided to follow the dimensional changes of individual granules directly. The starch granules were mounted in a conditioning chamber and microscopic measurements were made as they absorbed or desorbed water vapor to come into equilibrium with salt solutions.

Experimental

Materials.—Corn, potato, tapioca and waxy corn starches were chosen for investigation. The corn, potato and waxy corn starches were extracted at this Laboratory. The starch was separated from the ground-up source material by use of distilled water. Drying temperature of the extracted products in processing did not exceed 45°. The tapioca starch was a first-grade commercial starch.

Apparatus and Procedure.—The following techniques, though somewhat elaborate, were found to be necessary to obtain meaningful experiments. It was found early in this work that the increase in linear swelling which occurred when starch sorbed water vapor between two suitable low humidities may be only 1–2% of the vacuum-dry dimension. For the same humidity interval at high humidities, however, the linear swelling may be 5–10% of the vacuum-dry dimension. To demonstrate the approach and attainment of equilibrium for the small swellings at low humidities, it was therefore necessary to measure the granule dimensions with a very high precision, whereas for the large swellings at high humidities it was also necessary to control precisely the vapor pressure and temperature of the water vapor. A large number of simpler experimental arrangements were tried before success was achieved with the following apparatus and procedures.

A conditioning chamber, the thickness and length of a microscope slide, was constructed of brass. One-eighth-inch brass tubing rolled flat to $^1/_{16}$ inch (the thickness of the slide) was soldered flush into the slide and led to a $^1/_2$-inch diameter central hole. The central hole was covered top and bottom by $^3/_4$-inch circular cover slips which fit into recesses in the slide surface and were cemented to the slide by Plicene. A metal stop, covering two-thirds of the center hole in the slide and the same thickness as the metal slide in the region undercut to accept the cover slips, was inserted to prevent collapse of the cover slips under vacuum.

Before being cemented to the brass slide, the under side of the top cover slip was brushed with a very thin coat of a dilute mucilage solution. When the mucilage was nearly dry, the starch sample was dusted lightly onto the cover slip. The point of attachment of the starch granules to the mucilage layer could be seen with the microscope to be very small, yet this mounting procedure prevented the granules from moving in the subsequent evacuations and humidifications and presenting different profiles.

After assembly, the conditioning chamber was attached snugly to the microscope stage by short brass lugs screwed into the stage. A temperature-controlling coil was taped over the slide. The coil consisted of $^1/_8$-inch copper tubing bent into flat loops through which water could be circulated from a thermostat. The coil was shaped to allow free movement of the microscope objective in the region of observation.

The remainder of the equilibration apparatus was built around a T-bore, three-way stopcock. One arm was connected to a rotary oil vacuum pump, the second arm to a standard taper joint which connected to a water-jacketed small container for salt solution, and the third arm to the conditioning chamber on the microscope stage. A mercury manometer was connected to the arm going to the slide to permit measurement of the water vapor pressure at equilibrium. To permit flexibility of movement of the microscope, the entire apparatus was carried on a rod bolted to the microscope stage. The two portions of the equilibration apparatus were attached to each other by a very short section of rubber tubing wired at each end to assure a tight connection.

For the microscopic measurements, an oil immersion 97 × objective and a sensitive movement 15 × ocular micrometer with traveling spider silk bifilar cross hair were used. Because of the spherical shape of the starch granules, the apparent granule diameter changed with position of focus. It thus became necessary to make all measurements at a fixed depth of focus. To achieve this, the sample was illuminated with monochromatic light from a sodium vapor lamp that intensified the normally objectionable diffraction halos around each granule. It was then relatively simple to focus to a constant diffraction pattern about the granule, measure at the apparent granule boundary, and in this manner to achieve unusually high precision in the dimensional measurements. Ten readings of each dimension were customarily taken. The standard deviation of the measurements varied from 0.006 to 0.01 unit of the micrometer drum, and the standard deviation of the mean of the 10 readings was 0.002 to 0.003 unit. Since the average granule diameter measured was about 5 drum units, the precision of the dimensional determinations was about 0.1%. The calibration of the microscope equipment used was 1 drum turn = 0.002314 mm.

Equilibrations were accomplished by evacuating the salt solutions and slide simultaneously until the vapor pressure of the salt solution was reached. The stopcock of the equilibration system was then turned to connect the salt solution to the slide with the maintenance of the vacuum. Water from a thermostat at 25.10 ± 0.05° was circulated continuously through the salt solution jacket and the temperature controlling stage coil. Approximately five to eight hours were required for equilbration although generally 24 hours were allowed. Each granule measured was gener-

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