When compared with the sequence of reactions described in normal adrenal cortical and testicular interstitial cells the results are interpreted as indicating that the reaction catalyzed by 3 β-ol dehydrogenase may be the limiting step in androgen synthesis by the adrenal tumors but is not in the case of the testicular interstitial cell tumors.

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Received June 11, 1954. P.S.E.B.M., 1954, v86.

Stimulatory Effect of Carbohydrates on Aspartic Acid Deaminase Activity of *Bacterium cadaveris.** (21172)

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Two contrasting effects of glucose have been reported with respect to the aspartic acid deaminase system. The presence of this sugar during growth results in reduction of enzyme activity(1,2), whereas its addition to washed bacterial suspensions results in stimulation of deaminase activity(3). Presumptive evidence for the coenzymatic nature of material resulting from the acid degradation of glucose has been presented recently(4).

The present paper is concerned with the nature of the stimulatory effects of various carbohydrates on the aspartate deaminase system in resting bacterial suspensions, vacuum dried preparations, and cell-free enzyme fractions prepared by sonic vibrations.

Materials and methods. The organism studied was *Bacterium cadaveris* (Gale) cultured at 30°C for 16-18 hours in a medium composed of 1% yeast extract, 1% casitone, and 0.5% K_2HPO_4, adjusted to pH 7. The cells were harvested by centrifugation and washed once with distilled water. Resting bacterial suspensions were prepared by resuspending the washed cells in distilled water to give a cell concentration of 0.3 to 0.5 mg of bacterial nitrogen per ml. Phosphate aging was performed by the method of Lichstein(5) with the cell concentration adjusted to 0.5 to 1.0 mg bacterial nitrogen per ml. Dried cells were prepared by placing washed cell pastes over Drierite *in vacuo* for 24-96 hours; cell-free fractions by treating washed cells in a 9 KC Raytheon sonic oscillator for 10-15 minutes and collecting the supernatant fluid after centrifugation. The deamination experiments were performed in phosphate buffer at 37°C, using adequate controls without added aspartic acid. The reaction was terminated by the addition of 0.2 ml of 25% trichloroacetic acid, the tubes centrifuged and an aliquot of the supernatant fluid removed for ammonia determination by Nesslerization. Color was measured in a Klett-Summerson photoelectric colorimeter using a blue filter (400-465 mμ).

The carbohydrates tested for stimulating activity were glucose, acid degraded glucose

* This investigation was supported in part by RG 2916 from the National Institutes of Health, Public Health Service, and by Eli Lilly and Co.

TABLE I. Effect of Carbohydrates on Aspartic Acid Deaminase Activity of Phosphate Aged and Vacuum Dried Preparations of *Bacterium cadaveris*.

Additions	μg ammonia-nitrogen produced	
	Phosphate aged resting cells	Vacuum dried preparation
None*	23	28
Biotin, 0.1 μg + adenylic acid, 100 μg	38	46
Yeast extract, 1 mg	46	40
Glucose, 0.5 mg	58	30
" , 5.0 μg	35	28
Acid degraded glucose, 0.5 mg	65	26
Idem, 5.0 μg	33	30
Christman-Williams factor, 0.5 mg	63	27
Idem, 0.5 μg	50	28
None†	2.1	8.8
Biotin, 0.1 μg + adenylic acid, 100 μg	2.3	22.0
Christman-Williams factor, 50 μg	27.0	9.0
Christman-Williams factor, 50 μg + biotin, 0.1 μg + adenylic acid, 100 μg	40.6	21.5

* Reaction time 80 min., 37°C, M/20 phosphate buffer (pH 5). Resting cell suspensions aged in *M* phosphate 45 min. prior to use. Cell conc. = 0.1 mg bacterial nitrogen per ml for resting cell and 1 mg dry wt per ml for dried preparation.

† Conditions as above except resting cell suspensions aged 4 hr prior to use and cell conc. = 0.05 mg bacterial nitrogen per ml.

prepared according to the procedure of Christman and Williams(6), and the Christman-Williams factor prepared and kindly furnished by these workers.

Results. The data given in Table I demonstrate a marked contrast between the phosphate aged resting bacterial suspension and the vacuum dried preparation. All the carbohydrates tested were capable, even in low concentrations, of significant stimulation of the living cell. Since no consistent difference could be found in the activity of the various carbohydrate preparations, it would appear that acid treatment is not required for activity of glucose. This finding is in agreement with the exhaustive studies of Trudinger(3) but does not confirm the observations of Christman and Williams(6). It is pertinent to note that in contrast to the resting cell suspension none of the carbohydrate preparations was capable of stimulating the same system in the vacuum dried cell. That the aspartic acid deaminase was actually resolved may be concluded from the significant stimulations obtained with biotin, adenylic acid, and yeast extract. It is also of interest to note that the additive effect of this factor when combined with biotin and adenylic acid was demonstrable only in the intact cell.

Our findings with the dried preparation are contradictory to those reported by Williams and Christman(4) who demonstrated significant stimulation of this enzyme system by their factor in dried cells. A possible explanation for these differences lies in the method of drying. Williams and Christman employed either a 3-hour drying in a vacuum sublimation apparatus or 16 hours in a vacuum desiccator containing silica gel, whereas we employed more complete drying over a longer period of time. The data given in Table II reveal that until thorough removal

TABLE II. Effect of Drying Time on Carbohydrate Stimulation of Aspartic Acid Deaminase Activity in Vacuum Dried Preparations of *Bacterium cadaveris*.

Additions	μg ammonia-nitrogen produced	
	24 hr drying	72 hr drying
None	14.5	13.1
Glucose, 50 μg	15.4	15.1
Christman-Williams factor, 50 μg	24.6	13.3
Biotin, 0.1 μg + adenylic acid, 100 μg	26.5	24.9

Reaction time 60 min. Other conditions as for Table I.

TABLE III. Effect of Various Carbohydrates on Aspartic Acid Deaminase Activity of Sonic Extracts of *Bacterium cadaveris*.

Additions	μg ammonia-nitrogen produced		
	I	II	III
None	26.1	6.0	25.3
Biotin, 0.1 μg + adenylic acid, 100 μg	35.0		43.9
Yeast extract, 1.0 mg		19.6	36.4
Acid degraded glucose, 250 μg	24.4	6.0	26.0
Christman-Williams factor, 400 μg	29.6	6.1	28.6

Reaction time 60 min., 37°C, M/20 phosphate buffer (pH 7). Cell-free juice contains 0.1 mg nitrogen per ml.

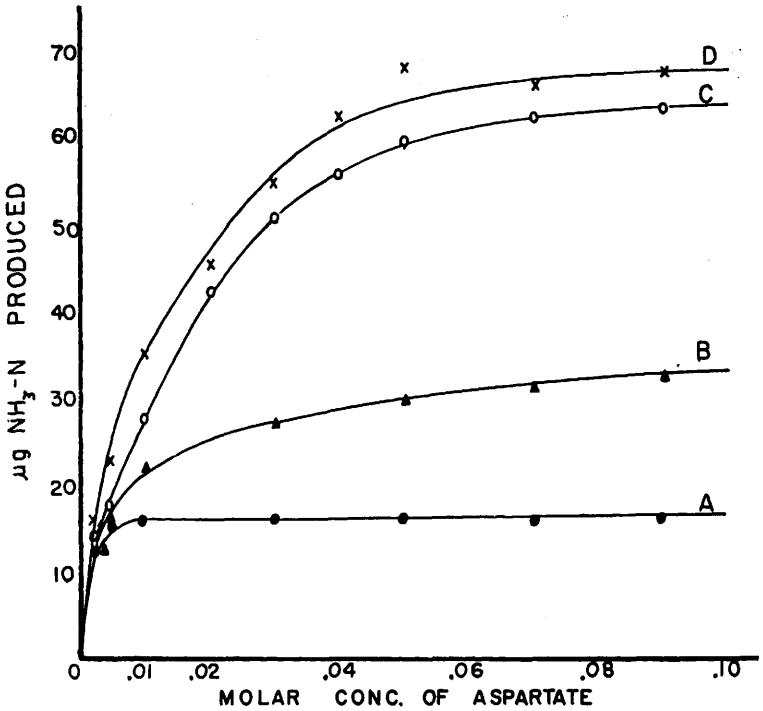

FIG. 1. Effect of substrate concentration on aspartate deaminase activity of *Bacterium cadaveris*. Reaction time 20 min., M/60 phosphate buffer (pH 7). A = living cell = 0.036 mg bacterial nitrogen per ml; B = living cell + 100 μg glucose per ml; C = vacuum dried cell = 1.0 mg dry wt per ml; D = sonic extract = 0.02 mg nitrogen per ml.

of moisture was accomplished the Christman-Williams factor did possess stimulating activity. The negative effects of these carbohydrates on sonic extracts of this organism may be seen by an examination of the results given in Table III, which are in keeping with those obtained with the vacuum dried preparations.

A plot of velocity versus substrate concentration (Fig. 1) reveals that the kinetics of the resting cell could be made to approach those of the sonic extracts and dried preparations if glucose was supplied to the cell. While a definite difference in magnitude was demonstrated between the velocity of the dried preparation, the sonic extract, and the glucose-treated resting cell, all fitted the Lineweaver-Burk equation giving Km values of 11.4 x 10^{-3}, 11.0 x 10^{-3}, and 5.5 x 10^{-3}, respectively, whereas the resting cell without glucose could not be made to fit the equation.

Discussion. It would appear from the data presented that the stimulatory effect of carbohydrates on the aspartic acid deaminase system may be due to an energy requirement for the passage of the amino acid substrate through the cell membrane. However, although the energy requirement for the passage of several amino acids including aspartic acid through the membrane of certain Gram positive bacteria has been demonstrated(7), no information exists with regard to aspartic acid in Gram negative species.

Summary. 1. Glucose, acid degraded glucose and the Christman-Williams factor exhibit significant stimulation of the aspartic acid deaminase system of the living cell. 2. Acid treatment is not required for the stimulatory activity of glucose. 3. None of these carbohydrates is capable of stimulating the thoroughly dried cell or the cell-free sonic extract. 4. The kinetics of the living cell approach those of the cell-free and dried preparation only if glucose is supplied. 5. It appears likely that the stimulatory effect of carbohydrates on this system is an indirect one perhaps concerned with the permeability

of the cell membrane to the amino acid substrate.

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Received June 17, 1954. P.S.E.B.M., 1954, v86.

Effect of Dihydrocholesterol and Soy Bean Sterols on Elevated Tissue Cholesterol. (21173)

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Dihydrocholesterol and soy bean sterols have attracted attention because they prevent accumulation of cholesterol in blood and tissues of animals receiving high cholesterol diets. While the exact mechanism of action remains undetermined, it appears that the principal effect of these substances is to diminish absorption of ingested cholesterol(1,2). It seemed important to determine whether mobilization of cholesterol already deposited in tissues can be influenced at all by adding soy bean sterols or dihydrocholesterol to the diet. Rabbits and mice in which tissue cholesterol had been elevated were therefore maintained on diets shown to be effective in preventing accumulation of cholesterol(3), and compared at intervals with animals maintained on control diets.

Experimental. Webster strain female mice weighing 20 g were used. They were fed *ad lib.* a fat-free diet, the composition of which has been covered in detail(3). Their liver cholesterol concentrations were elevated by supplementing this diet with 1% cholesterol and 0.5% cholic acid. After a 2-week period, the animals were divided into 2 groups. One group, the controls, received the unsupplemented diet *ad lib.*; while the other group received the same diet with the addition of 2% dihydrocholesterol* and 0.5% cholic acid, in place of equal percentages of alphacel. This diet has been shown to be efficient in preventing cholesterol accumulation(3) in the mouse. At definite intervals mice were sacrificed from both groups and their liver cholesterol levels determined by a method described previously (3).

In the second experiment, male albino rabbits weighing about 2,000 g were employed. Their plasma cholesterol levels were elevated by feeding *ad lib.*, for a 2-week period, a diet consisting of ground Rockland rabbit chow supplemented with 1% cholesterol and 8% corn oil. The animals were divided into 2 groups. The control group received, *ad lib.*, ground Rockland rabbit chow supplemented with 8% corn oil. The experimental animals received ground Rockland rabbit chow supplemented with 4% soy bean sterols and 8% corn oil. The latter diet when tested proved to be efficient in preventing absorption of dietary cholesterol. At definite intervals blood samples were obtained from both groups and the plasmas analyzed for total cholesterol according to the method of Sperry and Webb (4). The mice in both groups consumed 3.5 $\pm$ 0.5 g of food per day; the rabbits 150 $\pm$ 25 g per day.

Results. Table I gives the results of the experiments designed to show whether dihydrocholesterol is effective in mobilizing accumulated mouse liver cholesterol. It is apparent that dihydrocholesterol did not cause any acceleration of cholesterol mobilization.

* Generously supplied by the Schering Corporation, Bloomfield, N. J.