

following injection as compared with the immediate effect usually observed with serotonin. The period preceding an observable effect after histamine injection was even longer, and the duration of the effect was two hours or more. The toxic symptoms following administration of tryptamine were similar to those observed with serotonin; no toxic symptoms were seen following histamine injection.

Summary. Serotonin (5-hydroxytryptamine) has been found to depress the rate of oxygen consumption of the albino rat when injected intraperitoneally at dose levels as low as 1 mg/kg. A similar effect was observed with tryptamine and histamine (both of which are structurally related to serotonin) at much higher levels. This effect was not observed in the rabbit, guinea pig, or dog.

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1. Unpublished observations.
2. Freyburger W. A., Graham, B. E., Rapport, M. M., Seay, P. H., Govier, W. M., Swoap, O. F., and Vander Brook, M. J., *J. Pharm. and Exp. Therap.*, 1952, v105, 80.
3. Erspamer, V., and Asero, B., *Ricerca Scientifica*, 1951, v21, 2132.
4. D. I. Weisblat, private communication.
5. Brun, G. C., *Acta Pharm. et Toxic.*, 1950, v6, 74.

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Effect of Certain Carbohydrates on Nutritional Requirements of *Lactobacillus pentosus*. (19822)

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Lactobacillus pentosus, Strain 124-2, ferments both hexoses and pentoses(1-2). The organism has been grown on a synthetic medium containing glucose(3-5). However, when xylose replaced glucose in the synthetic medium, *L. pentosus* was reported not to grow(6). Growth was obtained when a supplement such as yeast extract was added and the existence of a "xylose factor" was postulated(6).

The present communication describes the study of an active principle in a liver extract supplement which promoted growth of *L. pentosus* on a synthetic medium containing either xylose or L-arabinose as a carbohydrate source.

Microbiological Methods. The organism used was *Lactobacillus pentosus*, Strain 124-2 (identical with *Lactobacillus plantarum* ATCC No. 8041). The synthetic medium employed was that described for the assay of nicotinic acid with *Lactobacillus arabinosus* (7) except that nicotinic acid was added and glucose was replaced by L-arabinose. Assays

were carried out using either conventional microbiological technics in test tubes or by the pad plate technic(8) in which test solutions were added to filter paper discs and placed on agar plates seeded with *L. pentosus*. In the latter instance the diameter of the growth zone was used as a measure of the biological activity.

Results and Discussion. The data in Table I illustrate that *L. pentosus* failed to grow in a synthetic medium containing L-arabinose as a substrate unless a supplement such as liver extract or yeast was added. Small amounts of glucose duplicated the growth stimulatory effect of the liver extract. However, when the organism was cultured in the absence of L-arabinose, liver extract was inactive, and in experiment 1, for example, 1000 times as much glucose was now needed for growth.

Other sugars were tested for growth of *L. pentosus* on an L-arabinose medium. The data in Table II show that glucose and mannose were the most active while galactose and maltose had slight activity. Fructose and

TABLE I. Response of *Lactobacillus pentosus* 124-2 to Liver Extract, Yeast Extract, or Glucose in the Presence or Absence of L-arabinose.

Additions to 10 ml basal medium	Optical density after 18 hr incubation— 200 mg L-arabinose added per tube			
	Exp. 1	Exp. 2	L-arabinose omitted Exp. 1	Exp. 2
0	.14	0	0	0
.01 ml liver extract	.66		0	
.03		.66		0
100 mg Difco yeast extract		.66		0
.001 mg D-glucose	.20	.06	0	0
.01	.41	.27	0	0
.10	.55	.40	0	0
1	.63	.49	.06	.06
10	.77	.65	.44	.46
100	.86	.78	1.05	1.10

TABLE II. Relative Activities of Various Carbohydrates in Promoting Growth of *Lactobacillus pentosus*, Strain 124-2, on an L-arabinose Medium, Using the Pad-Plate Method.

Substance	Relative activity (units/mg)
Water soluble liver fraction	1.0 $\pm$ 0
D-glucose	5.9 $\pm$.4
mannose	5.2 $\pm$.7
galactose	.3 $\pm$.1
fructose	<.02
Maltose	.8 $\pm$.2
Lactose	<.02
Sucrose	Inhibitory to glucose

One unit of activity is defined as the activity obtained from 165 μ g of glucose under standard assay conditions.

lactose had little or no activity and sucrose was mildly inhibitory to glucose.

In order to determine whether the growth-stimulating activity of liver was due only to its sugar content or to yet another substance, the liver extract was fractionated, and the total "xylose factor" activity, solids and glucose content were measured. Glucose was determined by reduction of copper sulfate(9) and by periodate oxidation(10). The results of these experiments are summarized in Table III.

The "xylose factor" activity was not removed by cation or anion exchange resins nor was it adsorbed to any extent on charcoal. The ratio of the "xylose factor" activity to the glucose determined by both methods was relatively constant in the various fractions whereas the ratio of the activity to total solids varied.

The fractions having substantial amounts of "xylose factor" activity were treated with

phenylhydrazine and in every case an osazone formed which had the characteristic crystal structure of glucosazone. The osazone from the charcoal effluent no. 2 was converted to the phenyltriazole. The melting point of the crystals was 193-194°, which is the melting point of phenyl-D-glucosotriazole(11).

Paper chromatograms were made with glucose and the liver extract using water-n-butanol-pyridine as a developer(12). The strips were cut lengthwise after development, and one half of each strip was treated with the sugar reagent 3-5-dinitrosalicylic acid(12), and the other half was bioautographed with *L. pentosus*. The single zone of microbiological activity coincided with the one area which gave the reaction for a sugar on each strip, so that the "xylose factor" activity in the liver extract was not separated from the sugar present.

In a personal communication, V. Shulimson and S. H. Hutner inform us that they have found that when *Streptococcus faecalis* is grown in media in which neutralized glucinolactone is substituted for glucose, a small amount of glucose or yeast autolysate becomes necessary. It remains to be determined whether the glucose content of this yeast preparation is responsible for the activity.

Summary. 1. *L. pentosus*, Strain 124-2, will grow on a synthetic medium containing L-arabinose as the carbohydrate source provided a small amount of glucose is added. 2. The supplement of glucose can be replaced by a liver extract. The active principle in the liver extract could not be separated by

TABLE III. Fractionation of the "Xylose Factor" Activity in Water-Soluble Liver Extract.

Fraction	Activity, units $\times 10^{-8}$	Dry wt (g)	Glucose (g)		Units/mg dry wt	Units/mg glucose (reduction)	Units/mg glucose (oxidation)
			Copper sulfate reduction	Periodate oxidization			
70% alcohol filtrate of water soluble liver fraction	100	99	27	5.3	1.0	3.7	1.9
Cation exchanger effluent*	125	50	28	97	2.5	4.5	1.3
Anion exchanger effluent†	67	27	16	21	3.7	6.3	4.8
Charcoal column effluent							
No. {	1	2.5	7.4	.8	3.4	.34	3.1
	2	15.4	5.8	4.7	4.2	2.7	3.3
	3	13.2	4.1	3.6	3.8	3.2	3.7
	4	10.0	1.9	2.2	2.3	5.3	4.5
	5	4.5	.7	1.1	1.2	4.1	4.0
	6	1.6	.5	.3	.6	2.7	5.3
	7	1.2	.2	.2	.3	6.0	6.0
	8	5.3	1.8	1.5	1.2	2.9	3.5
	9	ca .7	.6	<.1	.9	1.2	—

* Amberlite IR-120(H).

† Amberlite IRA-400.

ion exchange on synthetic resins, charcoal adsorption or paper chromatography from the sugar present in the liver extract. The total activity in the liver extract could be accounted for as the sugar content. Glucosazone was isolated from an active fraction of liver extract.

1. Fred, E. B., Peterson, W. H., and Davenport, A., *J. Biol. Chem.*, 1919, v39, 347.
2. Fred, E. B., Peterson, W. H., and Anderson, J. A., *J. Biol. Chem.*, 1921, v48, 385.
3. Dunn, M. S., Shankman, S., Camien, M. N., and Block, H., *J. Biol. Chem.*, 1947, v168, 1.
4. Shankman, S., Camien, M. N., Block, N., Marriñfeld, R. B., and Dunn, M. S., *J. Biol. Chem.*, 1947, v168, 23.
5. Krueger, K. K., and Peterson, W. H., *J. Bact.*, 1948, v55, 683.

6. Lampen, J. O., Peterjohn, H. R., *J. Bact.*, 1951, v62, 281.

7. Flynn, L. M., Williams, V. B., O'Dell, B. L., and Hogan, A. G., *Anal. Chem.*, 1951, v23, 180.

8. Williams, W. L., Esposito, R. G., and Pierce, J. V., *Fed. Proc.*, 1952, v11, 458.

9. Hawk, P. B., Oser, B. L., Summerson, W. H., *Practical Physiological Chemistry*, The Blackstone Co. Philadelphia, Pa., 1947, pp. 524, 526.

10. Adams, R., *Organic Reactions*, Volume II, John Wiley and Sons, Inc., New York, New York, 1944, pp. 341-375.

11. Cheronis, N. D., and Entriken, J. B., *Semi-micro Qualitative Organic Analysis*, Thomas Y. Crowell Co., New York, 1947, pp. 304-305.

12. Jeanes, A., Wise, C. S., and Dimler, R. J., *Anal. Chem.*, 1951, v23, 415.

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