

mary gland cells are more refractory to their direct growth stimulus than in other strains. This might be the case with the strains of mice which were deficient in their response to anterior pituitary materials. Since progesterone exerts its mammary growth effect through the anterior pituitary, it is possible that in some strains the pituitary is not optimally stimulated by progesterone, poorer mammary growth resulting. Further studies will have to be made to determine the possible roles of other endocrine glands such as the thyroid in the observed deficiencies in mammary growth.

Summary. Four strains of albino were studied as to mammary lobule-alveolar growth responses to anterior pituitary preparations and progesterone. Statistically sig-

nificant differences in strain responses were found. The Schwing and Swiss strains of mice were definitely superior to the Kansas and Sutter strains in their response to anterior pituitary preparations. The Swiss, Kansas, and Sutter strains of mice were all inferior to the Schwing strain in response to progesterone.

1. Turner, C. W., Chap. XI, *Sex and Internal Secretions*, Second Edition, Allex, Danforth and Dorsy, Williams and Wilkins, Baltimore, 1939.

2. Richardson, F. L., *Anat. Rec.*, 1951, v111, 669.

3. Richardson, F. L., *ibid.*, 1953, v117, 449.

4. Richardson, F. L., and Cloudman, A. M., *ibid.*, 1947, v97, 223.

5. Mixner, J. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.* No. 378, 1943.

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Effect of Some Pentoses on Growth of Lactobacilli.* (23130)

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Two recent reports(1,2) have emphasized the effect of certain pentoses in stimulating early growth of lactobacilli. In the report of Camien and Dunn(1) 3 pentoses were used individually. Often L-arabinose was somewhat more effective for *L. gayonii* and related species than was D-xylose or D-ribose, though the stimulatory effect of the individual pentoses differed somewhat with different cultures.

The present report deals with the growth response to a mixture of 3 pentoses shown by a collection of lactobacilli. The investigation was prompted by preliminary observations on a few cultures which indicated that homofermentative lactobacilli responded to pentoses quite differently than heterofermentative lactobacilli, particularly in a semisynthetic medium. It was found that this difference between the 2 fermentative types holds true, for the most part, for a larger number of lactobacilli and the degree of difference varies with the

test medium.

Materials and methods. Two culture media were used for the tests. One was a semisynthetic medium containing 0.5% acid digest of casein plus added tryptophane, cysteine, serine, threonine, purines, pyrimidines, vitamins, acetate, inorganic salts, and 1.0% glucose which was autoclaved in the medium. The detailed composition was given previously(3) but in the present study a few changes were made: the hydroxyproline of the previous work was omitted and xanthine was used in place of hypoxanthine. For testing the effect of pentoses, a mixture of L-arabinose, D-ribose, and D-xylose was sterilized by heat, but apart from the medium. This solution was added to tubes of previously autoclaved medium in an amount sufficient to give 0.5% of each pentose. In performing the tests, one series of tubes contained only the 1% glucose, tubes of the other series contained 1% glucose plus 0.5% of each of the 3 separately-sterilized pentoses. Certain departures from the foregoing pro-

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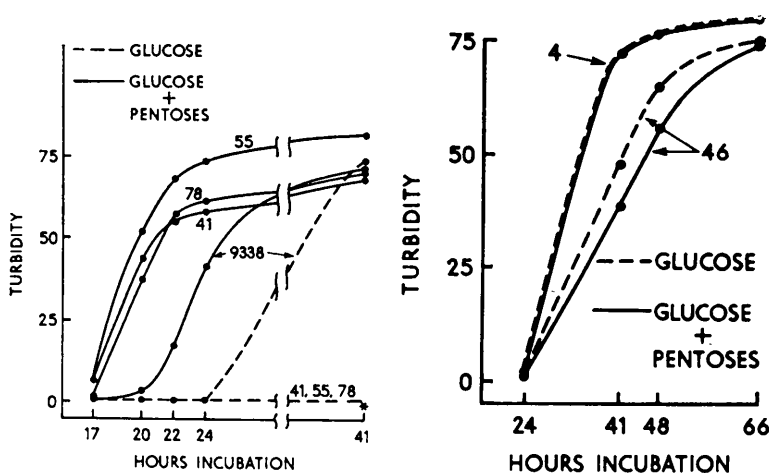


FIG. 1 (left). Growth response of 4 heterofermentative lactobacilli (*L. fermenti* strains 41, 55, 78, and ATCC 9338) in semisynthetic medium in presence of either glucose or glucose plus the 3 pentoses. Turbidity scale is based upon light transmission with a 580 $m\mu$ filter: 0 = clear tube, 65 to 80 = near maximal or maximal growth.

* Growth of cultures 41, 55, and 78 in glucose medium appeared in 41 to 48 hr and attained turbidity values of 65 to 73.

FIG. 2 (right). Growth response of 2 homofermentative lactobacilli (*L. casei* 46 and lactose-negative *L. casei* 4) in semisynthetic medium with either glucose or glucose plus pentoses.

cedure were made in some instances. In a few cases, 0.5% glucose was autoclaved in the medium while another 0.5% glucose was sterilized separately and added later. Also, in some later tests, the % of glucose in the one series was increased to equal total % of glucose plus pentoses. This and certain other departures from the usual procedure will be referred to later in connection with results. The second test medium was APT medium (Case) which is a modification of that of Evans and Niven(4). It is an enzyme digest of protein plus yeast extract, phosphate and other salts, polyoxyethylene sorbitan monooleate (Tween 80), and 1% glucose. For tests of the effect of pentoses, a separately sterilized mixture of L-arabinose, D-ribose, and D-xylose was added, as before, to previously autoclaved tubes of medium to give 0.5% of each pentose, in addition to the 1% glucose sterilized with the medium.

Thirty-seven lactobacilli were used. The homofermentative subgroup was represented by 12 strains of *L. casei*, 4 "lactose negative" *L. casei*, 2 *L. casei*-like, 5 *L. plantarum*, and 3 *L. acidophilus*; in the heterofermentative subgroup were 9 *L. fermenti* and 2 from

greenish discolored meat products.[†] Of the 37 cultures, 5 homofermentative and one heterofermentative were from the American Type Culture Collection. The others, aside from the 2 meat product cultures, had been obtained either from saliva or carious lesions and maintained for some years by monthly transfers in tomato juice yeast extract medium. Cells used for the tests were grown usually in the tomato yeast extract medium or, in some cases, in APT medium. For inoculation, either 0.001 or 0.0002 ml, secured by dilution of approximately 24-hour cultures, was used in most cases; in a few tests 0.01 ml was used. In each test, all tubes received the same amount of inoculum. Incubation was at 36°C, though 30°C was employed in a few instances.

Results. Growth of some representative lactobacilli in the semi-synthetic medium, with and without the 3 pentoses, is shown in the figures. The 4 heterofermentative lactobacilli in Fig. 1 ordinarily grew rather slowly in the semisynthetic medium, turbidity ap-

[†] For these 2 cultures the writers are indebted to Dr. J. B. Evans and Mr. R. H. Deibel of American Meat Institute Foundation.

TABLE I. Effect of Pentoses on Early Growth Response.

Species and No. of strains tested	Faster with pentoses		Essentially no diff.		Slower with pentoses	
	SS*	APT*	SS	APT	SS	APT
Homofermentative						
<i>L. casei</i> 18†	0	0	11	4	7	14
<i>L. plantarum</i> 5‡	0	0	0	0	5	5
<i>L. acidophilus</i> 3	0	0	3	3	0	0
Heterofermentative						
<i>L. fermenti</i> 9	9	9	0	0	0	0
From discolored meat products 2	0	0	2§	2§	0	0

* Letters refer to semisynthetic (SS) and APT medium.

† The 18 *L. casei* strains include 4 lactose negative *casei* and 2 *casei*-like cultures.

‡ *L. arabinosus* 17-5 (i.e., *L. plantarum* ATCC 8014) is one of the 5 strains.

§ Tests were made at both 30° and 36°C; one of these cultures failed to grow at 36°C. On repeated tests of these 2 cultures there were a few instances in which growth was a little slower in the APT medium in the presence of pentoses. Since the difference was slight and was not observed at other times, these 2 cultures are classed in the "no difference" category under the APT medium.

pearing only after 24 to 40 hours or more, although heavy growth was attained later. These organisms in repeated tests in the semisynthetic medium responded more readily when the 3 pentoses were supplied along with glucose. Similar results were secured with 5 other strains classed as *L. fermenti*.

Results of tests with several species or varieties of homofermentative lactobacilli were different. In some cases growth was somewhat slower when the 3 pentoses were included in the medium; in others, the rate of growth was approximately the same. Results shown in Fig. 2 are typical of other *L. casei* cultures as well as of *L. plantarum* and *L. acidophilus*. In no instance did the 3 pentoses stimulate growth of any of the homofermentative lactobacilli.

In APT medium, which contains yeast extract, all of the lactobacilli produced speedier growth than in the semisynthetic medium; nevertheless, the effect of the pentoses was still evident and the results paralleled those obtained with the semisynthetic medium. Included in these tests were different-sized inoculations from cultures of varying ages from 16 to 48 hours.

A summary of results with all cultures in both experimental media is given in Table I. Growth of the homofermentative lactobacilli either was not appreciably affected by the pentoses or it was slower in their presence. Results with individual strains of *L. casei* dif-

fered somewhat in this respect in the 2 media. In no case, however, was growth of *L. casei*, *L. plantarum*, or *L. acidophilus* accelerated in the presence of the pentoses.

Of the heterofermentative lactobacilli, all 9 *L. fermenti* (ATCC 9338 and 8 strains of oral origin) exhibited pentose stimulation. The 2 cultures from discolored meat products, however, were not so affected. It has been pointed out by others(4,5) that these lactobacilli differ in a number of respects from the familiar heterofermentative lactobacilli encountered commonly in lactic fermentation of milk or in the mouth. Their lack of response to the pentoses also seems to set them apart from *L. fermenti*.

Results of some additional tests involving departures from the usual procedure can be summarized briefly. At 30°C, instead of 36°C, growth was slower but pentose stimulation of *L. fermenti* was evident. The pentose effect showed no apparent correlation with pentose fermentation as determined by the usual fermentation tests. The majority of the *L. fermenti* cultures did not ferment xylose or arabinose but most of them fermented ribose. All of them showed growth stimulation by pentoses irrespective of the pentoses fermented. Of the homofermentative cultures, *L. casei* and varieties did not ferment arabinose or xylose but fermented ribose; *L. plantarum* fermented arabinose and ribose, or arabinose, xylose and ribose.

Yet these homofermentative lactobacilli which fermented readily at least 2 or, in some cases, all 3 pentoses showed no growth stimulation in the presence of the pentoses and some of them were inhibited.

An increase in the amount of glucose in the medium to equal the percent of glucose plus pentose did not alter the result: *L. fermenti* cultures in increased glucose were not stimulated as were the same cultures when supplied the pentoses and a smaller amount of glucose. The stimulation evidently is not simply a matter of an increased amount of fermentable sugar. When glucose was omitted from the medium, leaving the 3 pentoses, most of the lactobacilli failed to grow while a few grew very slowly. Autoclaving of the 3 pentoses along with glucose in the semisynthetic medium, instead of separate sterilization and later addition of the pentoses, did not decrease their stimulatory effect for *L. fermenti*; on the contrary, it seemed to augment it in some cases. No detailed study was made of the effect of individual pentoses on *L. fermenti*. A few tests showed that the growth acceleration produced by single pentoses differed somewhat from one strain to another, somewhat in the manner noted by Camien and Dunn(1).

Summary. Growth of the heterofermentative *Lactobacillus fermenti* (9 strains) was stimulated by a mixture of 3 pentoses: L-arabinose, D-xylose, and D-ribose when added to glucose-containing media. The pentose stimulation was observed in two different media but was more evident in a semisynthetic medium than in a medium containing yeast extract (APT medium). In contrast, no such stimulation was observed with any homofermentative lactobacilli classed as *L. casei*, *L. plantarum*, and *L. acidophilus*. Some of these cultures were slightly inhibited by the pentoses. Two heterofermentative lactobacilli from discolored meat products differed from *L. fermenti*; their growth was not appreciably affected by the pentoses.

1. Camien, M. N., and Dunn, M. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1955, v90, 183.
2. Moore, W. B., and Rainbow, C., *J. Gen. Microbiol.*, 1955, v13, 190.
3. Koser, S. A., and Thomas, J. L., *J. Infect. Dis.*, 1953, v93, 192.
4. Evans, J. B., and Niven, C. F., Jr., *J. Bact.*, 1951, v62, 599.
5. Niven, C. F., Jr., Castellani, A. G., and Allanson, V., *ibid.*, 1949, v58, 633.

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Distribution of Radioactivity in Human Maternal and Fetal Tissues Following Administration of C¹⁴-4-Progesterone.* (23131)

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During human pregnancy, the plasma concentration of progesterone is very low and does not parallel the rapid increase of its urinary metabolites(1). This observation raised some doubt as to whether pregnanediol and pregnanolone excreted into the urine during pregnancy are actually the metabolic products of progesterone. On the other

hand, it could be explained by the assumption that progesterone disappears quite rapidly from blood circulation in pregnancy. The latter explanation seemed to be more likely since Klein and Ober(2) were not able to increase plasma concentration of progesterone (as determined by the Hooker-Forbes test) in plasma of pregnant women by intramuscular administration of very large doses of progesterone (1000 to 1200 mg), although urinary pregnanediol is excreted in relatively large amounts following such an injection.

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