mately 50% diminution in urinary 17-ketosteroids. This diminution was not exaggerated by increasing the dose of Benemid to 4 g per day. (2) Spectrophotometric studies showed that Benemid did not change the androsterone-dehydroisoandrosterone ratio of urinary 17-ketosteroids. (3) Urinary estrogens were diminished in one subject. In another subject this diminution was questionable. (4) Urinary 11-oxycorticosteroids (neutral reducing lipids) did not appear to be affected by Benemid.

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Microbiological Assay Method for Thiamine Using *Lactobacillus fermenti* 36 as Test Organism.* (19104)

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Lactobacillus fermenti 36 has been proposed by Sarett and Cheldelin, and Cheldelin *et al.* for thiamine assay of foodstuffs(1,2). In studies with this method, it was observed that a striking stimulation in growth resulted when filbert nut meal extract was added to the assay medium containing either a suboptimum or an optimum amount of thiamine. Growth stimulants have been shown to exist in certain foodstuffs for the growth of *L. casei* in the microbiological assays for riboflavin and pantothenic acid when a suboptimum amount of vitamin is present in the assay medium by Bauernfeind *et al.*(3). Chu and Williams(4) and Pollack and Lindner(5) also reported the existence of stimulatory factors for *L. casei*. Wright and Skeggs(6) and Colio and Babb(7) have demonstrated the existence of a stimulatory growth factor in trypsinized vitamin free casein and malt sprouts extract, respectively, for *Streptococcus lactis R.* In this laboratory a series of experiments showed that there are a number of substances which will stimulate the growth of *L. fermenti* 36 in the presence of any level of thiamine.

The experiments reported here demonstrate the stimulatory effects of maltose and taka-diastase digested starch on the growth of *L. fermenti* 36 in thiamine assay medium. It also demonstrates that one may obtain an erroneous thiamine value by this method if the substances used are high in starch.

Experimental. In the microbiological assay of thiamine reported herein the procedure of Sarett and Cheldelin(1,2) was employed. The test organism used in this assay was *L. fermenti* 36. The extent of growth was determined quantitatively by measuring the turbidity after 16 hours of incubation at 37°C with a Klett-Summerson photo colorimeter, using a 54 filter. The readings are expressed in Klett units. In order to get a low blank value in this assay, a boiled alkali treated peptone was used in the medium. The

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TABLE I. Effect of Substances on Rate of Growth of *Lactobacillus fermenti* 36.

Substances added	Amt/10 ml, mg	Turbidity reading: 0 μg B_1, Klett units	.1 μg B_1, Klett units	.5 μg B_1, Klett units
Standard thiamine		19	165	171
Yeast extract (Bacto product)	1		174	
	2		195	
Liver extract (Wilson's 1:20)	1		195	
	2		226	
Papain digested casein	1		178	
	2		182	
Ascorbic acid	.4		193	
	.8		210	
Maltose	2		174	
	4		191	
Papain and takadiastase digested filbert nut meal extract	2.5	210	210	
	5	223	230	
	10	254	246	
	15	274	268	
	20	290	283	
Takadiastase and papain	100 μg each	30	168	
	200 " "	36	175	

takadiastase digested starch was prepared as follows: 50 ml of boiling water was added to one gram of soluble starch; 40 ml of sodium acetate buffer (pH 4.5) and 20 mg of takadiastase were then added to the solution after it had cooled; the mixture was allowed to digest 24 hours at 37°C under a layer of benzene. It was then steamed for 30 minutes to remove all the benzene and to inactivate the enzyme, neutralized to pH 6.6-6.8, and adjusted to 100 ml with water and filtered through a dried filter.

Results. Effect of B vitamins. Although *L. fermenti* 36 grows fairly rapidly on the assay medium, it was found that the growth rate was increased appreciably when filbert nut meal extract was added to the assay tubes. Vitamins were added individually to the assay tubes in order to test whether the growth stimulatory effect of filbert nut meal extract could be replaced by some members of the B complex.† It was observed that no significant change in growth over that in the blank occurred except in the cases of nicotinic acid and pyridoxine, where slight stimulation occurred. Moreover, when the vitamins in the medium were increased 5 to 10 times, no increase in growth was observed. This indicated the sufficiency of the basal medium in this respect, and the stimulatory effect of filbert nut meal was not due to the known B vitamins tested.

Effect of other known compounds. Since the growth rate of *L. fermenti* 36 could be increased by filbert meal extract, it appeared advisable to test the possible effect of an additional series of known compounds.‡ The results are summarized in Table I.

Since the sample for thiamine assay in this method is prepared by digesting the substance with a mixture of takadiastase and papain, it is likely that the starch will break down into dextrin and maltose. When these compounds were tested they were found to cause stimulation of *L. fermenti* 36 in restricted thiamine medium. An erroneous result of thiamine assay by this method would be expected if the material contains starch. Results in Tables II to IV demonstrate the stimulatory effects of maltose or takadiastase digested starch on the growth of *L. fermenti*

† 5 μg and 10μg of riboflavin, pantothenic acid, nicotinic acid, p-aminobenzoic acid, and pyridoxine, 0.1 μg and 0.2 μg of folic acid, 0.4 μg and 0.8 μg of biotin or 10 μg and 20μg of inositol.

‡ 4 to 8 mg of untreated Bacto-peptone or boiled alkali treated peptone, 0.8 mg of *L*-arginine, *L*-glutamic acid or *DL*-tryptophan, 0.4 mg of inositol, L-aspargine, succinic acid or maleic acid, 0.1 mg of xanthine and 4 mg of starch had no effect. However, yeast extract, liver extract, ascorbic acid, papain digested vitamin free casein and maltose are found to be stimulatory. Takadiastase and papain had slight thiamine activity and stimulatory effect.

TABLE II. Stimulatory Effects of Maltose or Takadiastase Digested Starch on Growth of *Lactobacillus fermenti* 36 at Various Thiamine Levels.

Thiamine levels, μg	Control, Klett units	Turbidity reading: Enzyme digd. starch added, 20 mg/tube, Klett units	Turbidity reading: Maltose 1 added, 20 mg/tube,* Klett units	Turbidity reading: Maltose 2 added, 20 mg/tube,* Klett units
0 (not inoc.)	0	5	0	0
0	60	71	80	78
.01	100	119	122	115
.02	124	145	135	137
.03	135	163	171	166
.04	144	183	186	184
.05	151	197	200	196
.10	175	246	240	244
.20	177	260	264	262

* Maltose 1 source unknown, Maltose 2 is Eastman Kodak Co. product.

TABLE III. Stimulatory Effects of Various Amounts of Maltose or Takadiastase Digested Starch on Growth of *Lactobacillus fermenti* 36 at .1 μg Thiamine Level.

Enzyme digested starch added per tube, mg	Thiamine level (Klett units): None	Thiamine level (Klett units): .1 μg	Maltose added per tube, mg	Thiamine level (Klett units): None	Thiamine level (Klett units): .1 μg
0	60	179	0	60	179
10	65	228	10	69	216
20	67	236	20	71	240
30	79	242	30	71	252
40	78	240	40	71	258

TABLE IV. Recovery of Thiamine in A Synthetic Sample Containing Takadiastase Digested Starch (each value is average of 4 tubes).

Amt added, ml	Synthetic sample: B_1 content per tube, μg	Synthetic sample: Enzyme digested starch per tube, mg	Recovery: Sarett & Chelelin's medium (1,2), %	Recovery: Same medium + 40 mg maltose per tube, %
1	.005	3.5	98.8	93
2	.010	7	124	93.2
3	.015	10.5	146	99.2
4	.020	14	156	102
5	.025	17.5	160	105
Avg			137	98.6

36 and the percent recovery of thiamine with this method if a sample contains enzyme digested starch. Since the thiamine assay medium contains Bacto-peptone which is known to be rich in strepogenin(5) it would seem that the stimulatory effect of maltose or enzyme digested starch on the growth of *L. fermenti* 36 is not due to this factor.

It is evident that neither maltose nor enzyme digested starch has a sparing action on thiamine but will greatly enhance the thiamine activity on the early growth of *L. fermenti* 36. In other experiments not reported in detail we have added both maltose and takadiastase digested starch in microbiological assay of other B vitamins. Stimulation was found in the growth of both *L. arabinosus* 17-5 in suboptimum amount of pantothenic acid when using Skeggs and Wright's(8) medium, and *Strep. faecalis R.* in suboptimum amount of pyridoxine when using Rabinowitz and Snell's(9) medium. The stimulation is increased with increasing amounts of maltose or enzyme digested starch in pantothenic acid assay. It was found that dextrins and fructose also have a stimulatory

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effect on the early growth of *L. fermenti* 36. In all cases, these substances do not affect the lactic acid production after 72 hours of incubation.

In the microbiological assay of B vitamins, it is frequently found that the growth of organisms may greatly be stimulated by the natural product. We are now inclined to believe that enzyme digested starch is one of the many stimulants which may be the decisive cause of erratic results obtained in assay of B vitamins of some natural products; thus addition of it to the assay medium is recommended.

Summary. (1) Maltose or takadiastase digested starch has been found to be one of the many growth stimulants for *L. fermenti* 36 in assay of thiamine. It is also stimulatory to *L. arabinosus* 17-5 and *Strep. faecalis R.* in assays of pantothenic acid and pyridoxine. (2) Evidence has been obtained to prove that a precaution should be made on thiamine assay of starchy substances using *L. fermenti* 36 as test organism. Addition of enzyme digested starch or maltose to the assay medium is recommended.

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Chemical Inhibition of Decapsulation in the Beta Hemolytic Streptococcus. (19105)

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The following study represents an attempt to explain the loss of hyaluronic acid capsular material (HA) from beta hemolytic streptococci which occurs spontaneously in culture or in washed suspensions of young organisms. The problem has already received considerable attention(1,2,3). In the pneumococcus the disintegration of the capsule has been shown to be an enzymatic process(4) and it is possible that a similar mechanism might be involved in the streptococci. Since hyaluronidase brings about rapid decapsulation, it was necessary to consider this enzyme as a possible factor which might be operating under natural conditions. Studies by McClean(2) and Pike(5) suggest that certain capsulated strains of streptococci may produce hyaluronidase under special conditions, although it is probable that this is a result of dissociation(6). In the course of our study, extensive attempts to demonstrate an agent in cultures of various ages or in mechanically disrupted organisms which would accelerate the normal rate of capsular disintegration were unsuccessful; we were unable to duplicate the results of Morison(1) who found small amounts of such an agent released by organisms. Possibly the examination of other strains would have been successful. In these attempts the measurement of the rate of capsular loss was the same as that to be described below in connection with the demonstration of chemical inhibition of a hypothetical decapsulating enzyme which comprises the main part of this report.

Material and methods. The beta hemolytic streptococcus used in this work, strain 4, was a group C strain isolated from guinea pigs during a fatal outbreak of lymphadenitis(3). This organism was grown in a pH 7.6, 2% proteose-peptone broth containing 10% sterile horse serum, 1% dextrose, and 0.5% NaCl. Ten ml of such neutralized cultures were in-

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