dynamics in man, about which little is known. The method is being included in an investigation of the effects of pulmonary and valvular heart disease upon the cardiac output, right heart pressures, arterial pressure and peripheral resistance. Similar measurements are being made in cases of peripheral circulatory failure. The technic is also being applied to study of the cardiac cycle and its pathological alterations in man and to investigation of the pharmacological actions of some cardiovascular drugs.

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Effect of Alanine on Response of *Lactobacillus casei* to Pyridoxine and Folic Acid.

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Previous work[1] has shown that in the presence of sufficient quantities of dl-alanine, *Streptococcus lactis* R did not require pyridoxine or "pseudopyridoxine"[2,3] while in the absence of these quantities of alanine, pyridoxine or compounds with pyridoxine activity were required for growth of this organism. From structural considerations, it appeared possible that alanine might serve as a precursor for the active substance derived from pyridoxine.[1] It was further found that *Lactobacillus casei*, an organism which also requires pyridoxine[4,5] (or pseudopyridoxine) for growth, could not dispense with this substance when excess alanine was present in the medium. It was observed, however, that when limited amounts of pyridoxine were present, growth of *L. casei* was considerably heavier when alanine was also added than it was in the absence of alanine. This effect of alanine has been investigated in connection with other growth-factor requirements of *L. casei*, with results presented below.

Experimental. The medium and technic used are the same as those previously described in detail[2] except that the medium was modified by the addition of 1 mg of asparagine, 3 γ of pyridoxine hydrochloride, and 1 γ of p-aminobenzoic acid per 10 cc. With these additions, this medium becomes, except for minor variations, almost identical with that proposed by Landy and Dicken[6] for estimation of 6 B vitamins by use of *L. casei*. Various vitamins were omitted in turn from this medium, and the quantitative growth response to additions of the vitamin determined in the presence and absence of dl-alanine. This was employed at a level of 2 mg per 10 cc. Results are given in Table I. Without exception, addition of dl-alanine improved response to the vitamin in the concentration range used for assay. This improvement, scarcely detectable with riboflavin, becomes slightly more marked with pantothenic acid and nicotinic acid. With pyridoxine and folic acid, the difference becomes so great as to alter considerably the nature of the dose-response curves, especially in that portion which would be used for assay. These differences, obtained turbidimetrically after 24 hours incubation, are also evident when acidimetric measurements are made after 72 hours incubation (Table II). In the pyridoxine

1 Snell, E. E., and Guirard, B. M., *Proc. Nat. Acad. Sci.*, 1943, **29**, 66.

2 Snell, E. E., Guirard, B. M., and Williams, R. J., *J. Biol. Chem.*, 1942, **143**, 519.

3 Snell, E. E., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 356.

4 Snell, E. E., and Peterson, W. H., *J. Bact.*, 1940, **39**, 273.

5 Bohonos, N., Hutchings, B. L., and Peterson, W. H., *J. Bact.*, 1942, **44**, 479.

6 Landy, M., and Dicken, D. M., *Lab. Clin. Med.*, 1942, **27**, 1086.

TABLE I.
Effect of dl-Alanine on Response of *Lactobacillus casei* to Various Vitamins.

Vitamin added	Amt. per 10 cc γ	Galvanometer reading* No alanine added	Galvanometer reading* dl-Alanine added
Riboflavin	.0	3.0	4.0
	.02	11.0	12.5
	.04	18.0	19.0
	.07	27.5	28.0
	.10	42.0	40.0
	.20	63.0	62.0
	.40	73.0	68.0
	1.0	76.0	72.0
Calcium pantothenate	.0	2.0	3.0
	.02	7.7	6.8
	.04	17.5	16.2
	.07	28.0	29.0
	.10	39.3	45.0
	.15	51.7	54.5
	.30	63.0	69.0
	1.0	69.0	76.0
Nicotinic acid	.0	11.0	13.0
	.02	20.0	21.0
	.04	26.3	28.0
	.07	35.0	38.0
	.10	44.0	47.0
	.20	60.0	66.0
	.40	69.0	70.0
Pyridoxine hydrochloride	.0	7.5	18.0
	.1	14.5	27.5
	.2	18.5	35.0
	.4	25.0	42.0
	.7	31.5	47.0
	1.0	38.5	49.0
	2.0	53.0	54.0
	3.0	62.0	55.0
	mg units		
Folic acid†	.0	8.0	9.0
	.03	16.5	21.5
	.07	24.0	34.0
	.10	29.0	39.0
	.15	36.5	47.0
	.20	41.0	51.0
	.30	55.0	55.0
	1.0	73.0	62.0

* Distilled water reads zero; a reading of 100 represents opacity. Cultures were read after 24 hours incubation at 37°C.
† "Potency" 3100.[10]

test, there is displacement of turbidity and acid production upward, but the response to added pyridoxine is not greatly sensitized. In the folic acid test, there is no upward displacement of the blank, but the response to a given amount of folic acid is considerably increased, so that the organism in effect becomes more sensitive to folic acid. In neither case is the maximum growth obtained increased by the presence of dl-alanine.

In view of the increased sensitivity of *L. casei* to folic acid evident in the presence of additional dl-alanine, an experiment was designed to determine whether the response of *S. lactis* R to folic acid[7] was similarly affected (Table III). No significant difference was produced with this organism by added alanine, either in the above medium, or in the medium used by Mitchell and Snell[7] for folic acid assay. As shown previously[1] no response to pyridoxine can be obtained with *S. lactis* R in the presence of these quantities of alanine, since alanine alone produces maximum growth.

TABLE II.
Effect of dl-Alanine on Response of *L. casei* to Pyridoxine and Folic Acid as Determined Acidimetrically.

Substance added	Amt per 10 cc γ	cc N/10 acid produced* No alanine added	cc N/10 acid produced* dl-Alanine added
Pyridoxine hydrochloride	.0	.84	3.29
	.1	1.23	3.30
	.2	1.71	3.36
	.4	2.42	3.96
	.7	3.63	4.84
	1.0	4.73	5.95
	2.0	8.42	8.70
	3.0	10.0	9.37
	mg units		
Folic acid†	.0	1.08	0.97
	.03	1.68	2.89
	.07	2.51	4.40
	.10	3.63	6.38
	.15	4.75	7.90
	.20	6.93	8.80
	.30	8.80	10.10
	1.0	10.30	10.1

* 3 days' incubation at 37°.
† Potency 3100.

TABLE III.
Effect of dl-Alanine on Response of *S. lactis* R to Folic Acid.

Mg units* per 10 cc	Galvanometer reading† No alanine added	Galvanometer reading† dl-Alanine added
0	7.0	7.0
.02	26.0	27.0
.04	36.5	37.0
.07	46.0	47.0
.10	52.0	52.0

* Potency 3100.
† Incubated 16 hours.

[7] Mitchell, H. K., and Snell, E. E., *Univ. of Texas Pub.*, 1941, **4137**, 36.

TABLE IV.
Turbidimetric Assays for Folic Acid with *S. lactis* and *L. casei* in Presence and Absence of Added Alanine.

Concentrate	Relative potency* for *S. lactis*		Relative potency for *L. casei*	
	No alanine added	dl-Alanine added	No alanine added	dl-Alanine added
I	3,860	3,890	3,230	2,900
II	5,420	5,400	3,630	3,660
III	37,400	37,300	20,400	26,500
IV	79,200	79,300	42,300	66,600

* Potencies in all cases were compared to that of an empirical standard of Liver Fraction B, assigned potency = 1.0.[10] *S. lactis* was incubated 16 hours, *L. casei* for 24 hours before culture turbidities were determined.

Various workers[8,9] have reported differences in the relative activities of concentrates or pure compounds in promoting growth of *S. lactis* and *L. casei* on folic acid-free media. The relative potencies of a number of concentrates derived from spinach by methods previously described[10] were determined with both organisms in the presence and absence of dl-alanine, to see if differences in sensitivity of *L. casei* to folic acid under these conditions would significantly alter the results. The data obtained are given in Table IV. With *S. lactis,* the potencies obtained in the presence and absence of alanine are identical within experimental error. The relative potencies of these same concentrates for *L. casei* is generally lower than for *S. lactis.* In 2 of 4 cases the apparent potency is significantly higher in the presence of alanine than in its absence; but the magnitude of change is not sufficient to explain the discrepancy in assay results obtained with the two organisms.

Discussion. The favorable effect of alanine on response of *L. casei* to various vitamins on the above medium is not readily explained. According to the data of Hutchings and Peterson,[11] presence of alanine in the basal medium is not required for growth of this organism; hence it probably is able to synthesize alanine. Furthermore, the 50 mg of hydrolyzed casein present in each tube of medium supplies about 0.9 mg of alanine, if present figures for the alanine content of casein are correct. All of the effects described in the present paper can be produced by addition of one milligram of alanine per tube. Similarly, on this medium, pyridoxine or compounds derived from pyridoxine are required for growth of *S. lactis;* an additional milligram of alanine eliminates this requirement.[1] Obviously, these effects of alanine are dependent upon a comparatively high concentration of it; and the concentration supplied with the hydrolyzed casein of the basal medium is insufficient to produce these effects.

Only in the pyridoxine test is growth in the blank tubes increased significantly by added alanine. This suggests that cells of the inoculum contain a substance or substances in the presence of which alanine can be utilized in place of pyridoxine by *L. casei,* as it is by *S. lactis.*[1]

The fact that the effect of alanine on growth is more pronounced in the case of the folic acid and pyridoxine assays than in tests for the other vitamins and is scarcely discernible in at least one assay (riboflavin), suggests a somewhat specific influence on metabolism of these substances, rather than an entirely general effect in increasing vigor of the test organism.

Summary. Addition of dl-alanine to a basal medium similar to that recommended for assay of 6 B vitamins with *Lactobacillus casei*[6] somewhat improves response to the vitamins when they are present in concentra-

[8] Stokstad, E. L. R., *J. Biol. Chem.*, 1943, **149**, 573.

[9] Keresteszy, J. C., Rickes, E. L., and Stokes, J. L., *Science*, 1943, **97**, 465.

[10] Mitchell, H. K., Snell, E. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1941, **63**, 2284; *J. Am. Chem. Soc.*, in press.

[11] Hutchings, B. L., and Peterson, W. H., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 36.

tions limiting growth. The effect varies in magnitude with different vitamins, and is especially pronounced with pyridoxine and with folic acid. The relative effectiveness of concentrates of the latter in promoting growth of *L. casei* may differ considerably when determined on 2 media which differ only in their alanine content.

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Effect of Sulfonamides on Coenzyme I-Linked Enzyme Systems.*

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At present the best theory for the mode of action of the sulfonamides postulates their inhibition of certain metabolic reactions due to the similarity in structure of the sulfonamides and the prosthetic groups of the respiratory enzymes. Evidence in support of this theory has been contributed by studies on the inhibition of the respiration and growth of bacteria, and also by the discovery that p-aminobenzoic acid has a counteracting effect on sulfonamides. Particular emphasis has been placed on the reactions involving coenzyme I, because its active group, nicotinamide, is chemically similar to the pyridine ring of sulfapyridine and to the isosteric ring of sulfathiazole. This work has been summarized by Dorfman and Koser[1] in a paper on the inhibitory effect of sulfapyridine and sulfathiazole on the nicotinamide stimulated respiration of the *dysentery bacillus*. Since all of the previous work has dealt with respiration in the whole cells of bacteria, an attempt was made to reproduce this effect in isolated enzyme systems.

For this purpose, reactions were employed in which coenzyme I was necessary for maximum activity, and where a quantitative relationship could possibly be traced between the amount of coenzyme I needed for stimulation, and the amount of sulfa drug needed for inhibition. The first reaction tried was that of the yeast fermentation method for the quantitative determination of coenzyme I.[2,3,4] The method used was essentially that of Axelrod and Elvehjem,[4] except that it was found necessary to add 0.1 mg muscle adenylic acid and 20 μg cocarboxylase to each respirometer flask for maximum carbon dioxide evolution. Coenzyme I was used at levels of 5 and 10 μg per flask.

Sulfapyridine or sulfathiazole in solution as its sodium salt (10 mg per cc) was added to the flasks with the rest of the constituents before the addition of the yeast preparation. Amounts added varied from 1-10 mg per flask.

With the final pH of the solution in the flask adjusted to 6.2-6.6 so that the normal fermentation of the yeast was not inhibited by the alkaline reaction of the sodium sulfonamides, no inhibition occurred even at the highest level of the drug. The use of the hexose diphosphate at twice the normal level, with the coenzyme I at 5 μg or 10 μg per flask did not alter the results.

Another approach to the problem was the use of enzymes present in normal rat liver which require coenzyme I in their hydrogen-transport systems. One such system—malic

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2 von Euler, H., *Ergebn. Physiol.*, 1936, **38**, 1.

3 Myrback, K., in Nord, F. F., and Weidenhagen, R., *Ergebnisse der Enzymforschung*, 1933, **2**, 139.

4 Axelrod, A. E., and Elvehjem, C. A., *J. Biol. Chem.*, 1939, **131**, 77.