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ISOLATION OF A NEW LACTOBACILLUS CASEI FACTOR

SNELL and Peterson¹ first presented evidence for the existence of a new growth factor (the "norite eluate" factor) for *Lactobacillus casei*. Mitchell, Snell and Williams² reported the concentration of a factor ("folic acid") from spinach. This factor was active for both *Streptococcus lactis* R and for *L. casei*. Hutchings, Bohonos and Peterson³ showed that purified concentrates of the "norite eluate" factor from liver stimulated the growth of *Lactobacillus helveticus*, *Lactobacillus delbrückii*, *Propionibacterium pentosaceum* and *Streptococcus lactis* R.

Since then a number of different compounds have been described which are active in stimulating the growth of *L. casei* or *S. lactis* R. Piffner⁴ *et al.* reported the isolation from liver of a crystalline compound which was active for *L. casei*. This substance which they designated vitamin B₆ was also active in preventing anemia and in promoting growth in chicks. Stokstad⁵ described two compounds; one was obtained from liver and the other from yeast. That obtained from liver was thought to be identical with the compound obtained by Piffner *et al.* The free acids of the compounds obtained from liver and yeast had equal potency for *L. casei*. However, when assayed with *S. lactis* R the preparation from yeast was only half as active as from liver. Both the factors from liver and yeast appear to be different from the growth factor for *S. lactis* R described by Keresztesy *et al.*⁶ Their preparation was approximately 2,500 times as active for *S. lactis* R as for *L. casei*.

In this communication we wish to report the isolation in crystalline form of a new compound which is active for *L. casei* and *S. lactis* R and is also active in the nutrition of the chick. This new compound was crystallized as the barium salt, as the free acid and as the methyl ester. The barium salt crystallized as needles, the free acid and the ester crystallized as small needles or threads. The absorption spectrum in 0.1 N NaOH was very similar to the compound iso-

¹ E. E. Snell and W. H. Peterson, *Jour. Bact.*, 39: 273, 1940.

² H. K. Mitchell, E. E. Snell and R. J. Williams, *Jour. Am. Chem. Soc.*, 63: 2284, 1941.

³ B. L. Hutchings, N. Bohonos and W. H. Peterson, *Jour. Biol. Chem.*, 141: 521, 1941.

⁴ J. J. Piffner, S. B. Binkley, E. S. Bloom, R. A. Brown, O. D. Bird, A. D. Emmett, A. G. Hogan and B. L. O'Dell, *SCIENCE*, 97: 404, 1943.

⁵ E. L. R. Stokstad, *Jour. Biol. Chem.*, 149: 573, 1943.

⁶ J. C. Keresztesy, E. L. Rickes and J. L. Stokes, *SCIENCE*, 97: 465, 1943.

TABLE 1
COMPARISON OF ABSORPTION SPECTRA

	New compound		Liver compound		Ratio	
	mμ	E ₁ ^{1 per cent.} 1 cm	mμ	E ₁ ^{1 per cent.} 1 cm	New compound E ₁ ^{1 per cent.} 1 cm	Liver compound E ₁ ^{1 per cent.} 1 cm
Maxima ...	259	317	255	440	0.72	
Minima ...	266	305	267	376	0.81	
Maxima ...	280	333	283	425	0.78	
Minima ...	332	92	331	103	0.89	
Maxima ...	365	130	365	151	0.86	

lated from liver (Table 1). It will be noted that the E₁^{1 per cent.} was less for the new compound, being only 86 per cent. as great at 365 mμ.

This new compound was 85 to 90 per cent. as active as that from liver when assayed with *L. casei*, but only 6 per cent. as active as the liver compound by *S. lactis* R assay. The amounts of the liver compound required for half-maximum growth were 0.000055 micrograms per ml for *L. casei* and 0.00025 micrograms per ml for *S. lactis* R. The new compound was required in amounts of 0.000061 micrograms per ml for *L. casei* and 0.0042 micrograms per ml for *S. lactis* R.

On the basis of their absorption spectra the three *L. casei* factors (present compound, liver and yeast factors) appear to be different from "folic acid." The E₁^{1 per cent.} for this new compound and the liver *L. casei* factor were determined at pH 11.0 and compared with the data at the same pH reported by Mitchell⁷ (Table 2). The wave-lengths chosen do not

TABLE 2
COMPARISON OF E₁^{1 per cent.} AT pH 11.0 OF THE *L. CASEI* FACTORS AND FOLIC ACID

mμ	New compound	Liver compound	Folic acid
	E ₁ ^{1 per cent.} 1 cm	E ₁ ^{1 per cent.} 1 cm	E ₁ ^{1 per cent.} 1 cm
260	296	404	338
280	336	410	190
300	245	334	102
380	121	125	92

represent maxima or minima but were used to correspond with the wave-lengths reported for folic acid.

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⁷ H. K. Mitchell, *Jour. Am. Chem. Soc.*, 66: 274, 1944.