antacid because of the insoluble and non-absorbed calcium compounds reformed in the intestinal tract after the passage of the chloride from the stomach.[11] It may therefore prove to be a safer antacid to administer repeatedly with penicillin than a soluble buffer salt such as sodium citrate, which is a systemic antacid and can lead to alkalosis. Dosage schedules for the maintenance of therapeutic blood levels by the oral administration of penicillin with calcium carbonate are being investigated.

VICTOR P. SEEBERG

CUTTER LABORATORIES,
BERKELEY, CALIF.

MORRIS F. COLLEN

PERMANENTE FOUNDATION HOSPITAL,
OAKLAND, CALIF.

SYNTHESIS OF A COMPOUND IDENTICAL WITH THE L. CASEI FACTOR ISOLATED FROM LIVER[1]

PREVIOUS work has indicated the existence of a new growth factor(s) essential for the growth of *Lactobacillus casei* and *S. faecalis* R and necessary for growth and hemoglobin formation in the chick. These fractions or compounds have been variously designated as the norite eluate factor,[2,3] folic acid,[4] vitamin Bc,[5] *L. casei* factor from liver[6] and *L. casei* factor from a fermentation residue.[7] We wish to report the synthesis of a compound which is identical with the *L. casei* factor isolated from liver. The synthetic compound is active for *L. casei*, *S. faecalis* R and is effective in promoting growth and hemoglobin formation in the chick.

The identity of the synthetic compound and the *L. casei* factor isolated from liver is based on the following observations. The ultraviolet absorption spectra of the synthetic and natural compounds are identical. The $E^{1\%}_{1\text{ cm}}$ values for the two compounds are shown in Table 1.

The infra-red spectra of the synthetic and natural compound were determined and compared by Dr. R. C. Gore, of the Stamford Research Laboratories, American Cyanamid Company. The per cent. transmission of the two compounds is given in Fig. 1. Dr. Gore states, "With correspondence in absorption exhibited at so many frequencies the probability is extremely high that the two molecules are identical."

TABLE 1

ULTRAVIOLET ABSORPTION SPECTRA OF NATURAL AND SYNTHETIC L. CASEI FACTOR

Solvent		mμ	*L. casei* factor from liver $E^{1\%}_{1\text{ cm}}$	Synthetic *L. casei* factor $E^{1\%}_{1\text{ cm}}$
0.1 N NaOH	Minima	235	287	290
	Maxima	256	565	570
	Minima	268	485	495
	Maxima	283	550	560
	Minima	332	133	135
	Maxima	365	195	199
0.1 N HCl	Minima	262	253	265
	Maxima	296	440	445

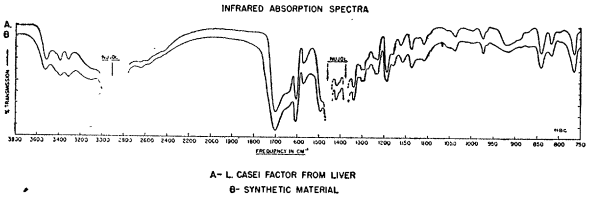

FIG. 1. Infrared absorption spectra of natural and synthetic *L. casei* factors.

Microscopical examination of the natural and synthetic compound was performed by Dr. A. F. Kirkpatrick, of the Stamford Research Laboratories, American Cyanamid Company. Dr. Kirkpatrick reported:

> The compounds which were crystallized as the free acids formed thin lenticular crystals, exhibiting birefringence and parallel extinction. The refractive index for light vibrating parallel to the length of the crystals was 1.559 ± 0.003 for the natural compound and 1.559 ± 0.003 for the synthetic compound; the refractive index for light vibrating parallel to the width was 1.744 ± 0.003 for the natural product and 1.744 ± 0.003 for the synthetic compound.

The natural and synthetic compounds were equally active when assayed by *L. casei* or *S. faecalis* R. The amount required per ml of medium for half-maximum growth of *L. casei* was 0.00007 micrograms for the natural and 0.00007 micrograms for the synthetic compound. For *S. faecalis* R the amount required per ml for half-maximum growth was 0.0003 micrograms for the natural and 0.0003 micrograms for the synthetic compound. The amounts required for half-maximum growth are slightly greater than previously reported, but the amount required to produce half-maximum growth varies somewhat with different experiments.

[11] L. Goodman and A. Gilman, "The Pharmacological Basis of Therapeutics," The Macmillan Company, 1941.

[1] The announcement of the synthesis of this compound and its availability for experimental use was made at Gibson Island, Maryland, July 18, 1945.

[2] E. E. Snell and W. H. Peterson, *Jour. Bact.*, 39: 273, 1940.

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

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

[5] J. J. Pfiffner, 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.

[6] E. L. R. Stokstad, *Jour. Biol. Chem.*, 149: 573, 1943.

[7] B. L. Hutchings, E. L. R. Stokstad, N. Bohonos and N. H. Slobodkin, SCIENCE, 99: 371, 1944.

The synthetic compound was assayed with chicks and found to be active. Day-old New Hampshire Red chicks in groups of ten were placed on a purified ration[8] supplemented with varying amounts of the synthetic compound. The positive control diet consisted of commercial chick-starting mash fortified with 3 per cent. cerophyl, 3 per cent. dried liver cake and 2 per cent. yeast. The responses are tabulated in Table 2. These results show that the synthetic compound is active in promoting growth and hemoglobin formation in the chick. The growth obtained with the purified diet supplemented with the *L. casei* factor was about the same as that obtained on a fortified stock diet.

TABLE 2

EFFECT OF SYNTHETIC L. CASEI FACTOR ON GROWTH AND HEMOGLOBIN FORMATION IN THE CHICK

Supplements per kg ration	Average weight and number alive (–) at 28 days	Average hemoglobin gm per cent. at 28 days
None	93 (5)	4.40
0.5 mg synthetic compound	306 (9)	7.80
1.0 mg synthetic compound	304 (9)	7.36
Fortified stock diet	319 (10)	6.44

From the data presented it is evident that the synthetic compound is identical with the natural *L. casei* factor isolated from liver.

Details of the isolation, degradation and synthesis will be subjects of subsequent communications.

ROBERT B. ANGIER
JAMES H. BOOTHE
BRIAN L. HUTCHINGS
JOHN H. MOWAT
JOSEPH SEMB
E. L. R. STOKSTAD
Y. SUBBAROW
COY W. WALLER

LEDERLE LABORATORIES, INC.,
PEARL RIVER, NEW YORK

DONNA B. COSULICH
M. J. FAHRENBACH
M. E. HULTQUIST
ERWIN KUH
E. H. NORTHEY
DORIS R. SEEGER
J. P. SICKELS
JAMES M. SMITH, JR.

CALCO CHEMICAL DIVISION,
AMERICAN CYANAMID COMPANY,
BOUND BROOK, NEW JERSEY

[8] B. L. Hutchings, E. L. R. Stokstad, N. Bohonos, J. J. Oleson and L. W. McElroy. Paper presented April 5, 1944, at the Cleveland meetings of the American Chemical Society.

ISOLATION OF AN ANTIANEMIA FACTOR (VITAMIN Bc CONJUGATE) IN CRYSTALLINE FORM FROM YEAST

THE constituents of yeast which are active in preventing nutritional anemia have been of particular interest since Wills[1] in 1931 demonstrated the curative effect of yeast extract in the macrocytic anemia of pregnancy which occurs commonly in India. She and Bilimoria[2] found that monkeys developed an anemia, leucopenia and granulocytopenia on a diet comparable to that consumed by the natives. These and other symptoms were relieved by yeast extract. Day and his co-workers[3] devised a different type of diet for monkeys on which the animals developed a similar syndrome. They were able to correct or prevent the deficiency state with yeast and suggested that the monkey factor be called vitamin M.[4] According to Day[5] it required about a year to evaluate the potency of a material by studies on monkeys. Later Hogan and Parrott[6] observed that chicks develop an anemia under defined dietary conditions which could be cured with liver extracts and they named the chick factor vitamin Bc. Their observations on chicks made available a convenient animal assay method for at least one antianemia factor and we initiated studies on the concentration of the chick antianemia factor in yeast in 1941. It was apparent early in our work that the chick antianemia factor in yeast differed chemically from vitamin Bc in liver extracts. Meanwhile Mills *et al.*[7] suggested that the microbiological growth factor in liver ("norite eluate factor" of Snell and Peterson[8]) and the chick antianemia factor in liver extracts may be identical. Use of both microbiological and chick assay methods soon led us to the isolation from liver of a crystalline compound having both activities. This compound we called tentatively vitamin Bc.[9] However, concentrates from yeast rich in chick antianemia activity were found to have very little microbiological growth activity for *L. casei* or *S. faecalis,* and we showed that crystalline vitamin Bc

[1] L. Wills, *Brit. Med. Jour.*, 1: 1059, 1931.
[2] L. Wills and H. S. Bilimoria, *Indian Jour. Med. Research*, 20: 391, 1932.
[3] P. L. Day, W. C. Langston and C. F. Shukers, *Jour. Nutrition*, 9: 637, 1935.
[4] P. L. Day, W. C. Langston and W. J. Darby, *Proc. Soc. Expt. Biol. and Med.*, 38: 860, 1938.
[5] P. L. Day, in "Vitamins and Hormones," edited by R. S. Harris and K. V. Thimann, Acad. Press Inc., New York, 1944, Vol. 2, p. 99.
[6] A. G. Hogan and E. M. Parrott, *Jour. Biol. Chem.*, 132: 507, 1940; 128: Proc. xlvi, 1939.
[7] R. C. Mills, G. M. Briggs, Jr., C. A. Elvehjem and E. B. Hart, *Proc. Soc. Exp. Biol. and Med.*, 49: 186, 1942.
[8] E. E. Snell and W. H. Peterson, *Jour. Bact.*, 39: 273, 1940.
[9] J. J. Pfiffner, 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.