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.

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[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.
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[6] A. G. Hogan and E. M. Parrott, *Jour. Biol. Chem.*, 132: 507, 1940; 128: Proc. xlvi, 1939.
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[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.

could be isolated from such concentrates following digestion with suitable enzyme preparations.[10] In view of these results we referred to the chick antianemia factor in yeast as vitamin Bc conjugate and the enzyme which formed vitamin Bc from it, vitamin Bc conjugase.[11] On the basis of these observations we devised an enzymatic-microbiological method of assay for vitamin Bc conjugate.[12]

Using the enzymatic-microbiological assay method in conjunction with chick anemia methods of assay we have recently isolated a crystalline compound from yeast which by its properties we judge to be the substance to which we referred earlier as vitamin Bc conjugate. The compound crystallizes from 5 per cent. sodium chloride solution in the form of yellow birefringent microcrystalline spherulites and on repeated recrystallization as rosettes of microcrystalline needles. It has no melting point. When heated on the hot stage it begins to darken from about 200° C. and partially melts at 230–260° C., but the charred mass never becomes entirely molten up to 360° C. A specimen containing 2.56 per cent. ash (sodium chloride) had the following corrected elementary composition: C 49.61 per cent.; H 5.36 per cent.; N 14.79 per cent. Phosphorus and sulfur were absent. The compound yields a crystalline methyl ester which melts with decomposition at 212–215° C. (corr.). The melting range of the methyl ester is reproducible but depends to a considerable extent on the size of the crystalline aggregate being observed and the rate of heating. The following analytical values were obtained on an ash-free sample of the crystalline ester: C 50.94, 50.91 per cent.; H 6.04, 6.10 per cent.; N 14.20 per cent.

The specific ultraviolet absorption properties of the compound are compared with those of vitamin Bc in Fig. 1.[13] The shapes of the curves are almost identical, differing only in the $E^{1\%}_{1\,cm}$ values indicating that both substances have the same chromophoric groups. It appears from these data that the molecular size of this yeast antianemia factor is 2.8 times that of vitamin Bc. From the elementary composition it follows that the non-vitamin Bc portion of the molecule contains nitrogen.

The new crystalline product is only slightly stimulating to the growth of *L. casei* or *S. faecalis*. One microgram is equivalent to .003–.006 micrograms of vitamin Bc when measured with *L. casei* and .002 micrograms when using *S. faecalis*. These microbiological growth data as well as the ultraviolet absorption constants clearly differentiate this compound from the three *Lactobacillus casei factors* described by Stokstad[14] and Hutchings *et al.*[15] They report that all three of their compounds, including their *new Lactobacillus casei factor* obtained from an undisclosed source, are powerful growth factors for *L. casei*.[15]

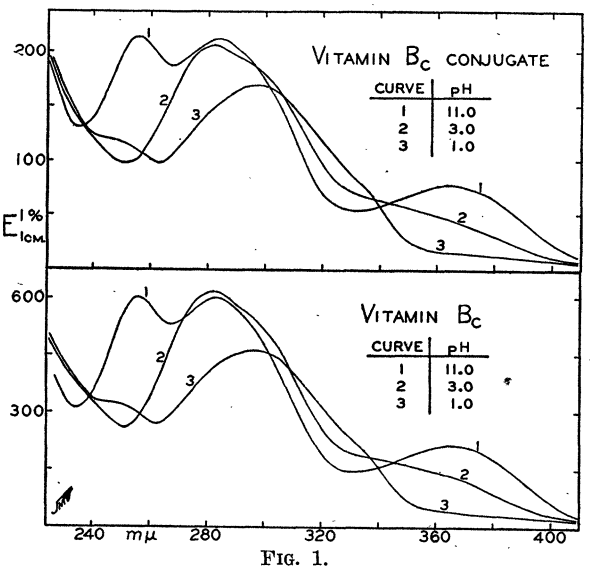

FIG. 1.

Vitamin Bc conjugase from hog kidney releases an amount of vitamin Bc from the crystalline compound approximating that present in conjugated form as calculated from the ultraviolet absorption data in Fig. 1. The amount of vitamin Bc in the digestion mixtures was estimated by microbiological assay using *L. casei* and *S. faecalis*. It should be pointed out that the enzymic degradation of the conjugate is not an entirely reliable reaction. The endpoint under presumably identical conditions may vary appreciably. The enzyme does not attack the methyl ester but degrades the regenerated parent compound. Hence there must be at least one free carboxyl group in the conjugate molecule in order for the compound to serve as a substrate for vitamin Bc conjugase.

The antianemic and growth effects of the new crystalline compound were compared in the chick with those of crystalline vitamin Bc. The results are summarized in Table 1. The data demonstrate that the vitamin Bc present in the conjugate molecule as calculated from ultraviolet absorption data and as found by enzymatic microbiological assay is available to the chick.

[10] S. B. Binkley, O. D. Bird, E. S. Bloom, R. A. Brown, D. G. Calkins, C. J. Campbell, A. D. Emmett and J. J. Pfiffner, SCIENCE, 100: 36, 1944.

[11] O. D. Bird, S. B. Binkley, E. S. Bloom, A. D. Emmett and J. J. Pfiffner, *Jour. Biol. Chem.*, 157: 413, 1945.

[12] O. D. Bird, Betty Bressler, R. A. Brown, C. J. Campbell and A. D. Emmett, *Jour. Biol. Chem.* In press.

[13] We wish to thank Dr. J. M. Vandenbelt for the ultraviolet absorption measurements and the preparation of the chart.

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

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

All the foregoing results support our working hypothesis that vitamin Bc is an integral part of the molecule. Until chemical facts are available which will allow of a more suitable terminology we propose to refer to this crystalline antianemia factor as vitamin Bc conjugate.

Workers in several laboratories have recognized a nutritional factor in liver or yeast extracts having vitamin Bc activity in animals but having little microbiological growth activity. Thus Elvehjem and associates[16] have prepared liver fractions having antianemia activity in the chick but having little microbiological growth activity for *L. casei* or *S. faecalis*. They postulated the existence of two new vitamins in these fractions, vitamins B_{10} and B_{11}. Day and his coworkers[17] have recognized the existence in yeast of a "potential *S. lactis* R stimulating factor" which could be converted to an "*S. lactis* R stimulating factor" by an enzyme in liver and other organs. They found that extracts rich in vitamin M activity had a high content of "potential *S. lactis* R stimulating factor" and recently suggested the possible identity of vitamin M, vitamin Bc conjugate and the "potential *S. lactis* R stimulating factor."[18] Welch and Wright[19] and Wright *et al.*[20] recognized a "potential folic acid" in milk powder, and Sebrell[21] noted the existence of a factor in yeast which had little "folic acid" activity but which was active in correcting the anemia of rats on a succinylsulfathiazole diet. Hill, Norris and Heuser[22] have recently found their yeast factors R and S to be associated with antianemia activity in the chick but not with microbiological growth activity. Factor R was thought to be identical with the chick growth factor U of Stokstad and Manning.[23] It appears probable that further study will demonstrate the identity of a number of these factors, including the yeast human nutritional factor of Wills with the crystalline compound described herein.

TABLE 1

THE RESPONSE OF CHICKS TO CRYSTALLINE VITAMIN Bc CONJUGATE

Group No.†	Vitamin Bc per gm of ration	Vitamin Bc conjugate per gm of ration	No. of chicks		Four-week findings		
			Started	Survived	Body weight (gm)	Hematocrit	Hemoglobin gm per 100 cc
	(mcg.)	(mcg.)					
1	0	0	10	5	126	15.4	3.73
2	0.05*	0.14	10	7	122	17.1	4.58
3	0.15*	0.42	9	9	166	24.0	7.01
4	0.25*	0.70	9	8	198	27.0	8.34
5	2.00*	5.60	5	5	202	30.8	9.53
6	0.05	0	9	7	134	15.6	4.53
7	0.15	0	10	10	175	28.4	8.27
8	0.25	0	10	10	240	29.3	8.78
9	2.00	0	5	5	221	30.8	9.45

† The conditions for the prophylactic assay were those described by C. J. Campbell, R. A. Brown and A. D. Emmett, *Jour. Biol. Chem.*, 152: 483, 1944.
* As estimated from the specific ultraviolet absorption properties of crystalline vitamin Bc conjugate.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE EFFECT OF SPECIFIC AMINO ACIDS ON THE YIELD OF PENICILLIN IN SUBMERGED CULTURE[1]

IN the course of a survey of media to substitute for corn-steep liquor in the production of penicillin by deep culture the basic nutrition of *Penicillium notatum No. 832* was investigated. When it was found that wheat extracts or "stillage" from alcoholic fermentation of wheat could serve as suitable media, these were fractionated and the fractions tested to identify the nature of the stimulus to growth of the mold and the production of penicillin. At the same time fractions of tryptic or acid hydrolysates of pure

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[1] With financial assistance from the National Research Council of Canada.