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 (mcg.)	Vitamin Bc conjugate per gm of ration (mcg.)	No. of chicks: Started	No. of chicks: Survived	Four-week findings: Body weight (gm)	Four-week findings: Hematocrit	Four-week findings: Hemoglobin gm per 100 cc
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.

proteins were also tested in deep culture and found to be active.

In each instance stimulation of growth and production of penicillin was obtained with the butyl alcohol soluble fraction. This was attributed to proline or hydroxyproline, and tests in deep culture with continuous shaking showed that pure proline was active but hydroxyproline was inactive.

A straight line relationship existed between the concentration of proline and the yield of mycelium in a basal media containing Czapek-Dox minerals and 4 per cent. lactose. The penicillin assays increased with the yield of mycelium but the yields of penicillin were low as compared to corn-steep liquor.

Other amino acids were investigated separately and in combination with proline. Glutamic acid with proline increased the yield of penicillin, *e.g.*, Czapek-Dox salt solution plus 4 per cent. lactose, 0.1 per cent. proline and 0.1 per cent. glutamic acid, inoculated with spores of the mold and agitated in a shaker, gave a yield of 35 Oxford units of penicillin per cc. Aspartic acid could substitute for glutamic acid. Histidine, methionine, tryptophane, hydroxyproline and phenylalanine had no effect. The addition of arginine to proline had a slight stimulatory effect. A summary of the more significant results is given in Table 1.

TABLE 1

	Age of culture (days)	Wt. of mycelium (gm)	Yield of penicillin (Oxford units per cc)*
Czapek-Dox + 4% lactose	7	0.019	0
	10	....	0–0.1
Czapek-Dox + 4% lactose, + 0.2% proline	7	0.290	4–6
	10	....	9–11
Czapek-Dox + 4% lactose, + 0.2% proline + 0.2% glutamic acid	7	0.358	17–21
	10	....	32–36

* Range of values in triplicate tests. Yields confirmed by extracting the penicillin from the acidified crude medium with amyl acetate and reextracting from amyl acetate into a buffer solution at pH 7 which was then re-essayed.

It is interesting to note that proline and glutamic acid are the amino acids present in the highest concentration in wheat and corn proteins. Details of this research will be published elsewhere.

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AUTOMATIC ELECTRIC SWITCH FOR CONSTANT AIR PRESSURE

In the measurement of such physiological processes as respiration, photosynthesis and transpiration, by methods involving the continuous flow of air around the plant material, it is often desirable to have the rate of air movement remain uniform during each experiment. A constant air pressure gradient facilitates this end. To accomplish this purpose, under the stress of material shortages and priorities, the writer has devised a pressure switch for the automatic control of an electrically driven air pump, which is thus caused to maintain a fairly constant partial vacuum in an air reservoir.

The general plan of the apparatus and a detailed drawing of the pressure switch are shown in Fig. 1. The air reservoir and pump are drawn on a much

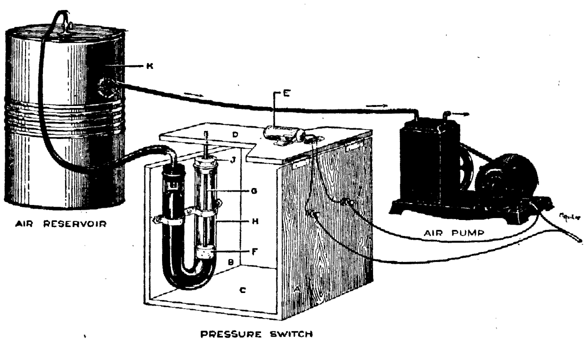

Fig. 1. Automatic pressure switch. Description in text.

smaller scale than the pressure switch. The pressure switch is designed to operate a rocker-type mercury electric switch (E) of suitable capacity. A switch (Central Scientific Co., number 84350C), having a rating of 220V and 1000W, has been quite satisfactory. This mercury switch will make and break contact when tilted through an angle of 3.5 degrees. It is mounted upon a light weight board (D) which is further trimmed to reduce its weight. This board is fastened at one end by hinges to the vertical board (A). The free end of the board (D) rests upon the upper end of a light bamboo rod (G) which passes through a loose-fitting hole in a cork stopper (J) into a cork float (F). The float rides upon the surface of the mercury in one arm of the glass U-tube (H). The bore of the U-tube is 3 cm, but this dimension may be varied, as required, to accommodate a float of sufficient buoyancy to support board (D). The arms of the U-tube are 6 inches in length, which allows for a maximum of about 5 inches, in mercury, of air pressure deficit (suction) in the air reservoir. Values less than 5 inches may be obtained by shortening rod (G). To obtain a larger air pressure deficit would only necessitate using a U-tube with longer arms and increasing the lengths of the vertical supports (A, B) and the bamboo rod (G) proportionately. The length of the bamboo rod is adjusted, by cutting off the top