there it seemed to be: the luminous point appeared to dance wildly and irregularly. Was it only that the pencil which I held as a marker trembled in my hand? I waited for many more minutes to be sure that the record did not miss any of the shocks that might follow the first. Then finally the film was taken off and developed. By that time I had almost convinced myself that I must have been mistaken and that what I saw was the motion of my own hand rather than the signal from the first hydrogen bomb. Then the trace appeared on the photographic plate. It was clear and big and unmistakable. It had been made by the wave of compression that had traveled for thousands of miles and brought the positive assurance that "Mike" was a success.

### What Next?

I believe that everyone who has worked on the hydrogen bomb was appalled by the success and by its possible consequences. I also believe that everyone who was closely or distantly connected with the effort and who made any contribution, great or small, had a clear feeling that the work was necessary in the interest of the safety of our country. To that extent I feel that all of us had an equal sense of satisfaction in the final success on 1 November 1952 at Eniwetok in the Marshall Islands.

In the whole development I want to claim credit in one respect only. I believed and continued to believe in the possibility and the necessity of developing the thermonuclear bomb. I feel that it was a great privilege that I could stay with it until a time at which the successful conclusion was in sight.

At the present time I find myself unhappily in a situation of being given certainly too much credit and perhaps too much blame for what has happened. Yet, I feel that the development of the hydrogen bomb should not divide those who in the past have argued about it but rather should unite all of us who in a close or distant way, by work or by criticism, have contributed toward its completion. Disunity of the scientists is one of the greatest dangers for our country.

The very size of our progress has opened up other dangers. We may be led to think that this accomplishment is something ultimate. I do not believe that this is so. Where the next steps will lead, I do not know. It is not likely that it will be just bigger bombs again. The world is full of surprises, and great developments rarely go along straight lines. But the skills and the knowledge that developed the A-bomb and the H-bomb can undoubtedly be turned to new directions, and we shall fail if we rest upon our accomplishments.

The greatest and most obvious danger of the hydrogen bomb is its destructive power. Some may think that it would have been better never to develop this instrument. I respect their opinion and I understand their feelings. There can be nothing more strong and definite than our desire for peace and I am sure that those who were most closely connected with the development of the new destructive weapons feel this at least as strongly as anyone else. But I also believe that we would be unfaithful to the tradition of Western civilization if we were to shy away from exploring the limits of human achievement. It is our specific duty as scientists to explore and to explain. Beyond that our responsibilities cannot be any greater than those of any other citizen of our democratic society.

It is clear and it is true that atomic bombs and hydrogen bombs are terrible and unprecedented, but so have been many other developments that past generations have faced. The construction of the thermonuclear weapon was a great challenge to the technical people of this country. To be in possession of this instrument is an even greater challenge to the free community in which we live. I am confident that, whatever the scientists are able to discover or invent, the people will be good enough and wise enough to control it for the ultimate benefit of everyone.

# A Labile Precursor of Citrovorum Factor

Charles A. Nichol,* Aaron H. Anton,† Sigmund F. Zakrzewski

*Department of Pharmacology, School of Medicine, Yale University, New Haven, Connecticut*

THE 4-amino antagonists of pteroylglutamic acid (PGA), such as Aminopterin and A-methopterin, apparently exert their effect by blocking the formation of derivatives of folic acid concerned as coenzymes with the transfer of single carbon units, and thus with the synthesis of several components of proteins and nucleic acids (*1*). Aminopterin was the first agent to show striking effectiveness in the treatment of acute leukemia of children (*2*). The availability of an organism, *Leuconostoc citrovorum*, ATCC 8081 (recently reclassified as a typical strain of *Pediococcus cerevisiae*, *3*), which requires a reduced derivative of PGA, has made possible the observations that PGA is reduced metabolically and that the formation of citrovorum factor (CF, $N^5$-formyl-5,6,7,8-tetrahydro-PGA) derived from PGA by liver preparations and by suspensions of bacterial or leukemic cells, is blocked effectively by Aminopterin (*1, 4*).

Although evidence was available that CF itself is

Table 1. Effect of heat and exposure to nitrogen and oxygen on a product derived from pteroylglutamic acid by cells of *S. faecalis*/A.

| Exposure time for supernatant* (min) | Folic acid activity (*S. faecalis* 8043) | | Citrovorum factor activity (*L. citrovorum* 8081) | |
|---|---|---|---|---|
| | Unheated (μg/ml of supernatant fluid) | Heated | Unheated (μg/ml of supernatant fluid) | Heated |
| 0 | 54.0 | 55.0 | 4.10 | 19.4 |
| *Atmosphere of nitrogen at* 37°C | | | | |
| 30 | 54.1 | 56.4 | 4.65 | 18.6 |
| 120 | 56.8 | 57.1 | 8.48 | 20.2 |
| 240 | 58.5 | 57.6 | 12.3 | 20.6 |
| *Atmosphere of oxygen at* 37°C | | | | |
| 30 | 57.6 | 57.6 | 4.34 | 11.9 |
| 120 | 58.1 | 58.3 | 4.96 | 5.05 |
| 240 | 60.1 | 55.4 | 3.88 | 4.11 |

* Cells of *S. faecalis*/A [2.1 mg (dry wt.)/ml of incubation mixture] were suspended in phosphate buffer (0.2*M*, *p*H 6.5) containing glucose (0.02*M*), sodium ascorbate (0.01*M*), and sodium formate (0.01*M*). The cells were incubated anaerobically for 1 hr in plastic centrifuge cups, which were then capped and spun in a refrigerated centrifuge. Portions of the supernatant fluid were returned to the incubator under atmospheres of nitrogen and oxygen and were agitated constantly. Duplicate aliquots were removed at intervals and were frozen immediately. One of each pair of aliquots was heated at 120°C for 30 min. The samples were assayed for their activity for *S. faecalis* 8043 and *L. citrovorum* 8081 (*10*), using PGA and CF, respectively, as standards of comparison.

not a coenzyme (*5–8*), its close relationship to the functional cofactor(s) has been regarded favorably. In fact, recent investigations have suggested that the functional forms of the vitamin may be derivatives of tetrahydro-PGA carrying specific one-carbon substituents at the formate or formaldehyde levels of oxidation (*5–8*). Unfortunately, direct methods for the study of the effect of the antagonists on the formation of the coenzymes, as distinct from CF, have been lacking.

We have observed recently that following the incubation of either preparations of pigeon liver or suspensions of an A-methopterin-resistant strain of *S. faecalis* (*9, 10*) with PGA, the appearance of CF results from the nonenzymatic degradation of a more labile compound and that the formation of CF is inhibited by Aminopterin only indirectly. The lability of the product of the enzymatic reactions under aerobic conditions, and the conditions that favor its conversion to CF are features that require careful consideration in the interpretation of metabolic studies of the nature and activity of derivatives of PGA.

The product formed by the anaerobic incubation of PGA with cells of *S. faecalis*/A was exposed to atmospheres of nitrogen and oxygen with the results shown in Table 1. The supernatant fluid that was obtained after the removal of the cells in a refrigerated centrifuge did not have the ability to alter added PGA. Comparison of the heated and unheated samples of the supernatant fraction derived from the incubation of the cells with PGA indicated the presence, not only of CF, but also of a derivative of PGA that was converted to CF by heating in the manner described. Incubation in the presence of oxygen led to the disappearance of the heat-labile precursor of CF. However, in each case, the total activity of the solution for *S. faecalis* remained the same.

Under anaerobic conditions at 37°C the compound was converted gradually to CF, a compound that is stable upon exposure to heat or oxygen, under the conditions described. Since the formation of the labile compound by this anaerobic system required the presence of ascorbate, which stabilizes the oxidizable product, it is probable that the true rate of oxidation of the labile precursor of CF is greater than that indicated by this experiment. The presence of cysteine or glutathione did not duplicate the effect of ascorbate in liver preparations; however, in this bacterial system an effect of these compounds, similar to that of ascorbic, could be demonstrated.

The rate of formation of CF from its precursor (formed by the incubation of PGA with homogenates of pigeon liver) was more rapid at 120°C than at 100°C. Also, after the elimination of enzymatic activity by removal of the bacterial cells, the rate of formation of CF in the supernatant fluid derived from *S. faecalis*/A incubated with PGA was approximately doubled for each 10-deg increase in temperature from 40° to 80°C.

The evidence that CF can be derived from a precursor nonenzymatically implies that CF may very well not be a direct intermediate in the formation of the functional form of the vitamin derived from PGA. The occurrence of separate pathways for the formation of the cofactor from PGA and CF could account for the observation that an A-methopterin-resistant strain of *S. faecalis* required less PGA than CF for half-maximal growth (*10, 11*). We have found that Aminopterin exerts an effect that is observed as an inhibition of the formation of CF, but this is an *indirect* effect. Aminopterin effectively blocked the formation of the precursor but was without effect upon the subsequent formation of CF, per se. Blakley (*6*) observed that Aminopterin inhibited the serine-glycine interconversion in the presence of PGA and yeast extract, but not in the presence of tetrahydro-PGA.

Isolation of the CF formed by heating the filtrates of *S. faecalis*/A incubated with PGA yielded a material indistinguishable from synthetic CF (folinic acid-SF; leucovorin) with respect to absorption spectra, $R_f$ values on paper chromatography, and stability in acid and alkali (*12*); like the CF isolated from liver (*13*), however, it was twice as active as the synthetic compound in promoting the growth of *S. faecalis* (*12*). Bioautograms using *S. faecalis* showed that in addition to PGA and CF, two folic acid-like compounds were present in the heated supernatant fluid. Fractions of the supernatant fluid obtained by the use of Dowex-1 columns contained $N^{10}$-formyl-PGA and

a compound that was degraded to $N^{10}$-formyl-PGA in neutral solutions (*12*).

The properties and chemistry of synthetic compounds related to CF have been described in detail (*14, 15*). The lability of the $N^{10}$-formyl derivative of tetrahydro-PGA may be contrasted sharply with the rather marked stability of the $N^5$-formyl derivative (CF). Thus, $N^{10}$-formyl-tetrahydro-PGA can be converted to CF by an anaerobic process involving heating. Such a conversion probably involves the intermediate formation of a compound having a one-carbon bridge linking the $N^5$- and $N^{10}$-positions. In neutral or mildly alkaline solutions such imidazoline compounds can yield CF under anaerobic conditions, or $N^{10}$-formyl-PGA under aerobic conditions. Upon exposure to oxygen, $N^{10}$-formyl-tetrahydro-PGA is readily oxidized to $N^{10}$-formyl-PGA, and the formyl substituent is readily removed by hydrolysis under mildly alkaline conditions. Recently Jukes (*16*) has directed attention to the instability of compounds in the folic acid series.

The labile compound formed from PGA in these studies would appear to be a derivative of PGA at the tetrahydro level of reduction, since it yields CF upon heating and is labile upon exposure to oxygen. We refrain from adding to the complex terminology related to folic acid-like compounds (*17*) by naming this labile derivative of PGA prior to a clarification of its chemical nature. For the sake of brevity, as well as utility, it is designated as CFX.

The results of investigations on the nature and activity of folic acid-like compounds occurring naturally in tissues or synthesized metabolically from *p*-aminobenzoic acid (PABA) or PGA are compatible with the tentative interrelationship between such derivatives shown in Fig. 1. We suggest that a distinction between the enzymatic and the nonenzymatic phases of the formation of these folic acid-like compounds is important to the interpretation of the experimental observations.

Certain microorganisms that require PABA synthesize from it compounds which appear to function as coenzymes and from which CF or PGA can be derived; the formation of such compounds by these organisms can be inhibited by sulfonamides. Although PGA and CF have been regarded as derived metabolically from PABA, there is substantial evidence to show that PABA is more efficient than either PGA or CF in promoting the growth of these organisms and in counteracting the inhibition of their growth by sulfonamides (*18–20*). Woods (*19*) proposed that PGA and CF are not direct intermediates between PABA and the coenzyme but that they could be converted to such intermediates with varying efficiency by some organisms and not at all by others. Since PGA and CF may represent relatively stable products formed by the degradation of a labile CF-like compound formed enzymatically from PABA, the activity or inactivity of PGA or CF would be related to the occurrence of enzymes capable of forming the labile cofactor-precursor from these stable derivatives. PGA and CF ordinarily do not counteract the chemotherapeutic activitity of sulfonamides, even though these compounds obviously are converted to functional cofactors in mammals, including man. This may be because PABA plays other roles of critical importance besides serving as a precursor of CF-like coenzymes. Consideration must also be given, how-

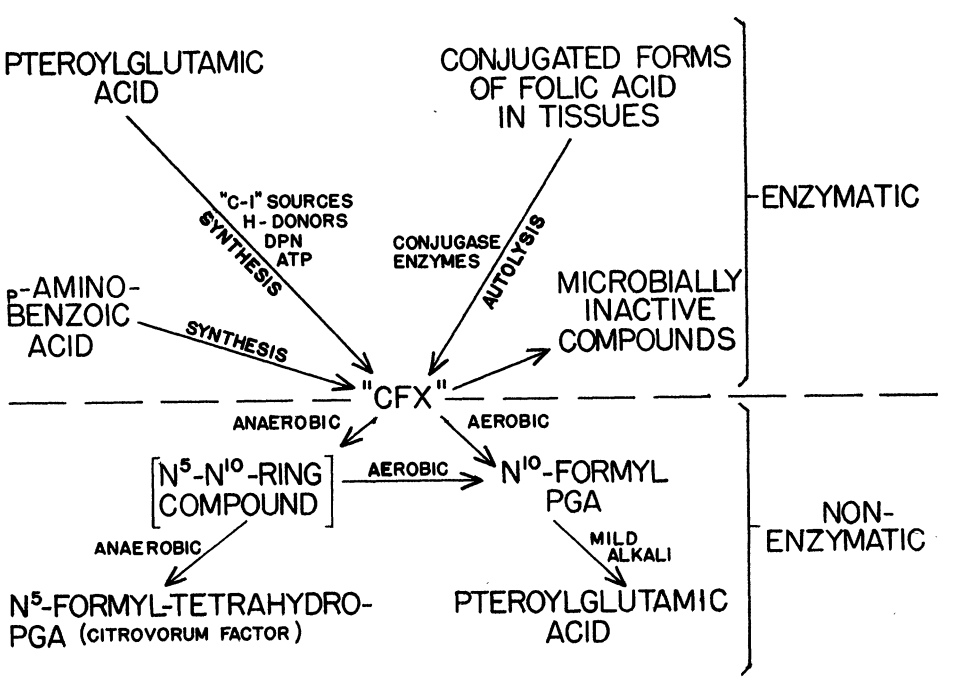


Fig. 1. Tentative interrelationships of folic acid-like compounds.

ever, to the possibility that parasitic organisms inhibited by sulfonamides may be unable to obtain benefit from the tissue coenzymes that are formed in animals from ingested folic acid-like materials.

Compounds having folic acid-like activity can be released by autolysis from forms occurring naturally in tissues, presumably by the action of the conjugase enzymes that degrade the polyglutamate compounds to forms that are available to the usual assay organisms, but the activity and role of these enzymes under physiological conditions is quite unknown. Keresztesy and Silverman (*13*) isolated CF from autolyzed horse liver and demonstrated its similarity to the active isomer of the synthetic form of CF (*21*). When liver homogenates were incubated under anaerobic conditions, these workers observed that after a brief autolysis the CF activity was a small proportion of the total folic acid-like activity, whereas upon continued autolysis the proportion of folic acid-like activity measurable as CF increased greatly (*22*). An attempt to separate the precursor of CF resulted in the isolation of a compound that was converted to PGA on alkaline hydrolysis and that had properties similar to those of $N^{10}$-formyl-PGA (*23*). It would appear that partial autolysis of liver resulted in the release of a substance that could yield folic acid-like compounds without activity for *L. citrovorum,* whereas upon continued incubation under anaerobic conditions the stable product formed was apparently CF.

In metabolic systems the apparent amount or activity of compounds in the folic acid series may involve the interconversions indicated in Fig. 1 and also the inactivation of these compounds. The enzymatic cleavage of PGA and CF has been reported (*24, 25*). In 1949 Welch *et al.* (*26*) suggested that xanthopterin, which, when incubated aerobically with homogenates of liver, apparently increases their folic acid-like activity (*27*), may function as an inhibitor of the enzymatic inactivation of precursor compounds. More recently, Silverman and Keresztesy (*23*) observed that the precursor of CF in autolyzing liver could be converted to a microbially inactive product by a DPN-dependent reaction and that this inactivation was blocked by sodium arsenite, Antabuse or xanthopterin.

The metabolic alteration of PGA to a functional form requires the availability of suitable donors of carbon, hydrogen, and, for reactions involving the utilization of formate, a source of energy. Greenberg (*7*) reported that a compound that activated the transfer of formate to an acceptor compound (4-amino-5-imidazole carboxamide ribotide) in the synthesis of inosinic acid, can be derived enzymatically from dihydro-PGA with the mediation of ATP, DPN, and formate. During an attempted isolation, however, a compound that was identified as $N^{10}$-formyl-PGA was obtained. Nichol (*28*) has observed that the formation of CF from PGA by "sonic" extracts of *S. faecalis*/A requires the presence of ATP and DPN in addition to formate or serine. Kisliuk and Sakami (*8*) have noted an important distinction between formaldehyde and formate as sources of carbon for the formation of the β-carbon of serine. In the presence of tetrahydro-PGA, ATP was required for the utilization of formate in this system but not for the utilization of formaldehyde. The role of homocysteine in these systems has not been elucidated.

The relationship of CFX to the active cofactor is under investigation. By analogy with other cofactors, the functional form of the vitamin may be a nucleotide or dinucleotide. A pentose substituent at the 8-position of the reduced pteridine, as suggested by Welch and Heinle (*29*), would tend to stabilize the structure and this would make lability to oxygen less likely. By analogy with the structure of coenzyme A, the possible attachment of a phosphate substituent through the essential hydroxyl group at the 4-position should also be considered. CFX itself may be a derivative of the cofactor, since it was found in high concentration in the medium suspending the intact cells, while the enzymatic capacity to alter PGA was retained within the cells. The ability of PGA, CF, anhydroleucovorin, or tetrahydro-PGA to activate certain metabolic systems (*5–8, 30*) may reflect degrees of refinement of such systems with respect to the capacity to form the functional derivative. The relationship of the polyglutamate pteridines to the active forms of the vitamin remains to be clarified. It is probable that the CF-series of compounds will be extended to include a group of polyglutamates that could include CFX or more complex forms.

Our present studies (*31*) represent, in part, attempts to contribute to an explanation of the mechanism of resistance to folic acid antagonists in leukemia. It is apparent that experiments designed to investigate the manner by which a cell or organism circumvents the action of these toxic compounds must be concerned with the true product of the reactions blocked by these antagonists. The evidence that relatively stable CF and other folic acid-like compounds result from the nonenzymatic degradation of the metabolically formed product of the reactions susceptible to inhibition directs attention to the limitations of present techniques and alters the design of future experiments. The interrelationships indicated in Fig. 1 represent our current working hypothesis.

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* Scholar in cancer research of the American Cancer Society.

† Predoctoral fellow of the American Foundation for Pharmaceutical Education.

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# Frederick D. Heald, Dynamic Plant Pathologist

FREDERICK DEFOREST HEALD, plant pathologist, mycologist, botanist, and in all a scientist, died in Spokane, Washington, 24 April 1954, at the age of 82. He had been professor emeritus since his retirement in 1941 from the positions of professor and head of the department of plant pathology in the College of Agriculture, and plant pathologist and head of the division of plant pathology in the Agricultural Experiment Station at the State College of Washington. Although he was not very active after his retirement, he certainly retired with an enviable record of professional achievement that had long since won him international renown.

Dr. Heald was born on 23 July 1872 at Midland City, Mich., the son of Henry Francis Heald and Hettie Charles Heald. After attending preparatory school, he entered the University of Wisconsin, where he received his B.S. and M.S. degrees. Following an additional year of intensive graduate study in plant physiology and plant pathology at the University of Leipzig, he received his Ph.D. degree in 1897.

His first professional position was professor of biology at Parsons College, Fairfield, Iowa, which he held from 1897 to 1903. In 1903 he went to the University of Nebraska, where he served successively as "adjutant professor" of plant physiology, as associate professor of botany and botanist of the Agricultural Experiment Station, and finally as professor of agricultural botany. In 1908 Dr. Heald moved to the University of Texas as head of the School of Botany, where he remained until 1912 when he went to work for the state of Pennsylvania and the U.S. Department of Agriculture as plant pathologist, investigating biological factors involved in the serious chestnut blight epiphytotic. His contributions to the knowledge of the life-history of the pathogen and the means of spread of the disease gained him a reputation as a very capable and dynamic plant pathologist and undoubtedly led to his appointment in 1915 as professor of plant pathology at the State College of Washington and plant pathologist in the Washington Agricultural Experiment Station.

By this time Dr. Heald was thoroughly convinced of the importance of plant pathology and was so enthusiastic about its bright future that he was able to persuade the college administration to create a separate department (from botany). This was accomplished in 1918, and it is entirely logical that this tremendously energetic and courageous man was promptly installed as head of the new department.

In 1899 Dr. Heald married Nellie Townley. Much of his outstanding professional development can be attributed to the constructive and balancing influence of this brilliant woman who was as ambitious for her husband's professional development as he was himself. From this marriage came three children, Doris (Mrs. A. H. Tonge), Henry and Marion (Mrs. Emil Shebesta). Mrs. Heald died in 1939. Her death left a great void in Dr. Heald's life. In 1942 he married Charlotte Chamberlin. She and all three children survive him.

Dr. Heald's professional influence has been pronounced through accomplishments in research, teaching, and scholarly writing. In addition, the personality and drive of the man himself has had no small influence on his students and associates.

Although Dr. Heald is best known for his contributions to plant pathology, his first love was expressed in plant physiology and in the biology and taxonomy of mosses. His earlier papers dealt with such diverse subjects as the histology of pulvini, regeneration in mosses, electric conductivity of plant juices, biology wall charts, and analytic keys to North American mosses. His first contribution in plant pathology, "Methods of investigating plant diseases," came in 1905. From this point on, his papers dealt almost exclusively with plant pathology and mycology. Following his appointment at the State College