desoxypyridoxine is probably that of a metabolic competitor to the active B_6 vitamin and, in itself, does not activate an alarm reaction. The demonstration that the hematologic abnormalities occur in pyridoxine-deficient, adrenalectomized mice proves conclusively that the mechanism is not that of an alarm reaction mediated through the adrenal. Thus, the mechanism for the production of these abnormalities remains to be elucidated. It may conceivably involve interference with some enzyme activity in nucleoprotein metabolism not understood at this time.

Since simultaneous pyridoxine deficiency and cortisone administration produced greater changes than either alone, the possibility should be considered that cortisone actually accelerated the production of pyridoxine deficiency. Stoerk(3) considered this when he observed that the combination was more deadly in rats than either one alone. He offered the hypothesis that cortisone, by increasing the utilization of amino acids, increased the body's demand for vitamin B_6 and thereby further aggravated the pyridoxine deficiency. Against this hypothesis is our finding that enormous amounts of pyridoxine, pyridoxal, and pyridoxamine in no way modified the hematologic effect of cortisone. Since the hematologic changes are so similar in these two states, the possibility exists, however, that the ultimate mechanism may be the same. Thus, the effect might be on the "end-organ" whether this be nucleoprotein synthesis or some other unknown metabolic reaction. This would adequately explain all the results of these experiments.

Summary. 1. The production of pyridoxine deficiency in adrenalectomized mice calls forth the same hematologic response, neutrophilia and lymphopenia, as in intact animals. This is positive evidence that these blood changes occurring in pyridoxine deficiency are not the result of an alarm reaction mediated through the adrenal gland. (2) Pyridoxine deficiency and cortisone administration produce similar hematologic responses. When combined, a synergistic effect results. This may be evidence that the mechanism of the production of the hematologic changes is different in the 2 conditions. (3) Further evidence that the mechanisms may differ is the fact that the cortisone effect cannot be blocked by giving large amounts of pyridoxine, pyridoxal, and pyridoxamine, although the possibility exists that cortisone has a role at another site in the B_6 cycle.

Pyridoxine, pyridoxal, pyridoxamine, and desoxypyridoxine were supplied by Merck and Co.

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Received May 17, 1951. P.S.E.B.M., 1951, v77.

Interrelationships of Folic Acid and Ascorbic Acid in Rat Liver.* (18763)

J. N. WILLIAMS, JR. (Introduced by C. A. Elvehjem.)

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

Nichol and Welch(1,2) reported that one probable role of ascorbic acid in animal tissues is to aid in the conversion of folic acid to the *Leuconostoc citrovorum* factor (LCF). Recently we have reported that the choline oxidase system of rat tissues is intimately related to folic acid, ascorbic acid, LCF, and vit. B_{12}(3,4) as demonstrated by *in vitro*

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

1. Nichol, C. A., and Welch, A. D., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 52.

2. Nichol, C. A., and Welch, A. D., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 398.

3. Williams, Jr., J. N., *J. Biol. Chem.*, 1951, v190, in press.

experiments. When rats are made folic acid "deficient" by feeding aminopterin, the choline oxidase activity of the liver is decreased considerably. The activity may be increased to values approaching normal by the incubation *in vitro* of the factors just named with the rat liver homogenate prior to addition of the substrate. Some of the factors, *i.e.*, ascorbic acid and folic acid, also stimulate normal rat liver choline oxidase, though not as markedly as the liver enzyme from aminopterin-fed rats. Since the most active factor for stimulating choline oxidase activity *in vitro* was ascorbic acid(4), it appeared possible that one of the reasons for the lowered choline oxidase activity in aminopterin-fed rats was a decreased synthesis of ascorbic acid by these rats, as well as a blocking of the conversion of folic acid to the *L. citrovorum* factor.

Experimental. Adult male rats of the Holtzman strain weighing from 200 to 250 g were used as experimental animals. They were divided into 2 groups of 7 animals each. One group received a purified ration containing 18% casein as previously described(1), and the other group was fed the same ration with 4 mg aminopterin† per kilo ration. The animals fed aminopterin developed marked deficiency symptoms within about 10 days. They were used in the analytical or enzyme studies immediately after the first noticeable symptoms developed. The analyses for ascorbic acid content of the livers of both groups of rats were carried out by titration with 2,6-dichlorophenolendophenol reagent. In several cases a check on this method was made using the colorimetric method of Roe and Oesterling(5) as modified by Bolomey and Kemmerer(6) and Bolin and Book(7). In the latter determinations values for reduced, oxidized, and total ascorbic acid were obtained. In the titration method 1 part of liver

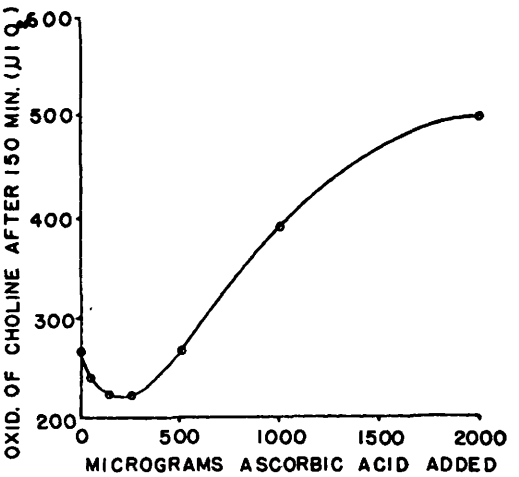

FIG. 1. Effect of ascorbic acid added *in vitro* to a normal rat liver homogenate upon oxidation of choline in the presence of homocysteine.

was homogenized in 5 parts of water, and 3 ml of 30% trichloroacetic acid were added to 10 ml of the homogenate. After centrifuging, 4 ml of the supernatant extract was titrated with 0.025% 2,6-dichlorophenolendophenol reagent. In the colorimetric method 1 g of liver was homogenized in 4 ml mixed acid(7) and diluted to 20 ml with mixed acid. After centrifuging and filtering, the ascorbic acid of the extract (total, reduced, and oxidized) was determined according to the references listed above.

In the enzyme determinations the effect of various levels of ascorbic acid added *in vitro* to the liver homogenates of normal rats was studied. The enzyme system and method of assay have been described previously(4). In the present experiments the levels of neutralized ascorbic acid given in Fig. 1 were added to one side-arm of a series of double side-armed flasks at the expense of water. After incubating the ascorbic acid with the liver homogenate, the choline and homocysteine were added from the second side-arm, and readings were taken at 10-minute intervals for 150 minutes. Oxygen uptake due to oxidation of choline was obtained by subtracting the endogenous oxygen uptake in the flask without added choline from the flask containing choline.

4. Williams, Jr., J. N., *J. Biol. Chem.*, 1951, in press.

† The author wishes to thank Dr. J. J. Oleson of Lederle Laboratories, Pearl River, N. Y., for the generous supply of aminopterin used in these studies.

5. Roe, J. H., and Oesterling, M. J., *J. Biol. Chem.*, 1944, v152, 511.

6. Bolomey, R. A., and Kemmerer, A. R., *J. Biol. Chem.*, 1946, v165, 377.

7. Bolin, D. W., and Book, Lucille, *Science*, 1947, v106, 451.

Results. The ascorbic acid determinations with the 2,6-dichlorophenolendophenol reagent in the livers of the 2 groups of rats gave the following results: Rats fed the complete basal, 403 ± 27‡ γ per g liver; rats fed the complete basal + aminopterin, 189 ± 20‡ γ per g liver. The colorimetric results checked very well with the titration results and in no instance was any measurable oxidized ascorbic acid found in the livers of either groups of rats. From the results it can be observed that the livers of the rats fed aminopterin exhibit a considerably lower ascorbic acid concentration than the livers of rats fed the same ration without aminopterin. Therefore, it appears that aminopterin not only blocks the utilization of folic acid but also blocks the endogenous synthesis of ascorbic acid in the rat and that folic acid (or *L. citrovorum* factor) is probably involved in the synthesis of ascorbic acid as well as in other functions. If the utilization of folic acid is inhibited by aminopterin, the synthesis of ascorbic acid, which incidentally is also involved in the utilization of folic acid itself(1,2), is inhibited.

In Fig. 1 are presented the results of the effect of various levels of ascorbic acid on choline oxidase activity in the presence of homocysteine. It should be emphasized here that these effects and those presented in an earlier paper(4) can be demonstrated best in the presence of homocysteine, though less amplified effects can be observed without homocysteine(3). The results in the figure are presented as total oxidation after 150 minutes due to added choline (μl of O_2) versus the level of ascorbic acid (γ) added to the flasks. The results in the figure depict a typical experiment with a normal rat fed the complete synthetic ration. It appears that low levels of ascorbic acid added *in vitro* actually inhibit the oxidation of choline in the system employed while higher levels (over 500 γ per flask) stimulate the system markedly. This unique situation is difficult to explain from present knowledge. It may, however, be related to the decreased choline oxidase activity observed in tissues of animals fed aminopterin. Since as shown above the concentration of liver ascorbic acid is markedly decreased by dietary aminopterin and since low levels of ascorbic acid inhibit choline oxidase rather than stimulate it, the low liver ascorbic acid concentrations of rats fed aminopterin may help to explain the low choline oxidase activity of livers of those rats.

The fact that all of the ascorbic acid present in the livers of both groups of animals is reduced indicates that the action of the aminopterin is to block the actual synthesis of the ascorbic acid molecule rather than merely to inhibit maintenance of the reduced form. Further studies need to be made to discover the points in ascorbic acid synthesis which are blocked by aminopterin or in which folic acid is involved.

Summary. 1. Rats fed aminopterin exhibit a concentration of liver ascorbic acid less than one-half normal, indicating that folic acid is probably involved in ascorbic acid synthesis in the rat. 2. Enzyme studies indicate that low ascorbic acid levels added *in vitro* reduce liver choline oxidase activity in the presence of homocysteine whereas higher levels stimulate activity of the enzyme system. Implications of this phenomenon with depression of choline oxidase activity by dietary aminopterin have been discussed.

‡ Standard error of the mean.

Received May 14, 1951. P.S.E.B.M., 1951, v77.