oxidation of choline and betaine aldehyde is only one-half the theoretical. (3) Betaine aldehyde appears to be metabolized by 2 pathways, one an aerobic oxidation and the other an anaerobic conversion to betaine. (4) Choline, betaine aldehyde, and betaine are equivalent methyl group donors to homocysteine under aerobic conditions. Anaerobically choline is almost inactive in furnishing methyl groups to homocysteine. Betaine, however, is a more active methyl donor anaerobically than under aerobic conditions.

Received August 13, 1951. P.S.E.B.M., 1951, v78.

Effect of Aminopterin on Choline, Betaine Aldehyde, and Betaine Metabolism.* (19022)

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In several recent publications(1-3) it has been reported that dietary aminopterin causes a marked decrease in tissue choline oxidase activity. This decrease in activity has been reversed by incubating the liver homogenates with combinations of several of the presumed precursors of the *Leuconostoc citrovorum* (LCF) factor, namely, ascorbic acid, folic acid, vit. B_{12}, and LCF itself(1,2). Moreover, it has been shown from this laboratory that dietary aminopterin induces a fault in the ability of rats to synthesize ascorbic acid(4). The sum of the preceding work, therefore, indicates that folic acid and ascorbic acid metabolism are disturbed by dietary aminopterin, and that this may be the basic cause of the decrease in choline oxidase activity. Since the choline oxidase(5) and betaine aldehyde oxidase(6) systems are believed to be intimately connected with the transmethylation reactions of homocysteine, and since a deficiency of dietary folic acid has been shown to disturb this transmethylation reaction in chickens(7), the present investigation was undertaken to determine the extent of the disturbance of the transmethylation reactions involving choline, betaine aldehyde and betaine by dietary aminopterin in rats.

In a preceding publication(6), a study of the mechanism of the metabolism of choline and betaine aldehyde has shown that the latter metabolite can be converted to betaine either aerobically or anaerobically, while choline oxidase is entirely an oxygen-requiring system. Because of the close relationships of these metabolites with transmethylation, it appeared desirable to study the effects of dietary aminopterin on the different aspects of choline, betaine aldehyde, betaine, and methionine metabolism uncovered in the preceding investigation.

Experimental and results. Adult male rats of the Holtzman strain were employed as experimental animals. One group of rats was fed a normal synthetic ration containing 18% casein as previously described(1). Other animals were fed the same ration except that 4 mg of aminopterin† were included per kilo

* 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.

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† 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.

TABLE I. Effect of Dietary Aminopterin on Aerobic Oxidation of Choline and Betaine Aldehyde, Anaerobic Dehydrogenation of Betaine Aldehyde, and Formation of Methionine in Rat Liver Homogenates.

	Substrate	Microliters of gas exchanged (μl O_2)		Methionine formed per 210 min per g liver (μg)	
		I*	II*	I*	II*
Aerobic results	Choline	255	172	354	40†
	Betaine aldehyde	107	72	354	80†
	Betaine	0	0	354	112
		(μl CO_2)		(μg)	
Anaerobic results	Choline	0	0	90†	9†
	Betaine aldehyde	39	27	320	167
	Betaine	0	0	770	530

* I = Avg of 10 rats fed normal ration; II = avg of 8 rats fed normal ration + aminopterin.
† These figures must be considered negligible amounts since they are below the range of accuracy of the method.

of ration. After about 10 days deficiency symptoms suddenly became marked in the latter animals and death resulted within a few hours after the onset of these symptoms. For this reason several groups of aminopterin-fed rats had to be set up in order to obtain enough animals before death for the enzyme studies. When the effects of the aminopterin became strongly evident, the animals were sacrificed for determination of the effect of the anti-vitamin upon the oxidation of choline and betaine aldehyde, the anaerobic conversion of betaine aldehyde to betaine, and the aerobic and anaerobic formation of methionine from choline, betaine aldehyde, and betaine. The experimental methods are the same as those indicated in the preceding paper(6).

The average results of the aerobic oxidation of choline and betaine aldehyde, the anaerobic dehydrogenation of betaine aldehyde, and the aerobic and anaerobic formation of methionine in homogenates of the 2 groups of rats are presented in Table I. The results have been included in a single table for easier study of the data as a whole. There was no overlapping of the corresponding results for the 2 groups of animals.

From the results it can be seen that the oxidation of choline is depressed by dietary aminopterin as has been previously reported (1-3). Both the aerobic oxidation and anaerobic dehydrogenation of betaine aldehyde are also depressed by the anti-vitamin. This raises the question of whether the first enzyme, *i.e.*, choline oxidase, or the second, betaine aldehyde oxidase, is responsible for the decrease in oxygen uptake by aminopterin when choline is the substrate. The results reported in Table I for choline oxidase activity probably partially reflect the lowered betaine aldehyde oxidase activity since the figures reported are the total oxygen uptakes for the 210-minute period. However, choline oxidase activity, when measured at the end of the first 10 minutes after addition of choline, is not influenced to an appreciable extent by the slower second reaction (betaine aldehyde oxidase) as has been pointed out in earlier work(6). Also the depression of choline oxidase activity by dietary aminopterin can be demonstrated by data taken from the first 10 minutes of the choline oxidase assay. Therefore, even though betaine aldehyde oxidase is decreased by aminopterin, the similar effect on choline oxidase does not appear to be an artifact.

It is also interesting to observe that the anaerobic conversion of betaine aldehyde to betaine is decreased by dietary aminopterin. Therefore, it appears that the entire mechanism for converting choline or betaine aldehyde to the actual methyl donor, betaine, is disturbed by interfering with folic acid metabolism.

The data representing the formation of methionine under the various conditions presented in the table indicate that the transmethylating mechanism is also depressed by dietary aminopterin. In some cases, *i.e.*, in the aerobic transmethylation from choline and betaine aldehyde and anaerobic transmethylation from choline, the formation of methionine

in the aminopterin-fed rats was decreased so low it could not be measured.

It is interesting to note that in most cases the gas exchanges and formation of methionine for the rats fed aminopterin appear to be decreased in a constant ratio with respect to the normal controls. The ratios of the results for the aminopterin-fed rats to those for the normal rats in the oxidation of choline and betaine aldehyde, the anaerobic conversion of the aldehyde to betaine, and the anaerobic formation of methionine tend to approach a common value of about 0.6. Therefore, it appears that a factor common to each of these enzyme systems is disturbed by dietary aminopterin. It remains to be seen what the common factor is, although it is undoubtedly concerned with the metabolism of folic acid.

Summary. 1. The effects of dietary aminopterin upon the aerobic and anaerobic metabolism of choline, betaine aldehyde, and betaine have been investigated. 2. Oxidation of choline and betaine aldehyde, anaerobic conversion of betaine aldehyde to betaine, and both aerobic and anaerobic transmethylation to homocysteine are decreased significantly by dietary aminopterin.

Received August 13, 1951. P.S.E.B.M., 1951, v78.

System for Measuring and Designating Antigenic Components of Influenza Viruses with Analyses of Recently Isolated Strains. (19023)

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Previous investigations(1,2) in this laboratory to examine the antigenic composition of influenza viruses have been extended, in the current study, to include the A prime agents which were present during 1950-51, a number of WS- and PR8-like (type A) strains isolated in recent years, and the influenza C viruses. A system for measuring and designating certain antigenic components of influenza viruses has been developed on the basis of these findings and is presented here. The new system promises to be especially useful for those investigations in the fields of epidemiology and preventive medicine in which definitive information on the antigenic relationships of newly isolated strains of influenza is required quickly.

Materials and methods. Virus strains. Pertinent information concerning the history of certain of the viruses used in the present study is given in Table I. The Syr II(3), Robert and Saunders strains were obtained from Dr. Bernice Eddy. Bur 99 and the Ba, Nou, Chantal and Fa viruses(4) were furnished by Dr. P. Lepine. The HSC-18, HSC-19 and HSC-20 agents, isolated from infants at Toronto, Canada, were supplied by Dr. C. E. van Rooyen. Dr. F. P. Nagler sent the Bent I and Bent 2 strains and Dr. C. H. Andrewes supplied the London-1-51, England-1-51 and Eire-1-51 viruses. Strains Ann Arbor-1-51 and Ann Arbor-2-51 were obtained from Dr. T. Francis, Jr., and Dr. G. Meikeljohn furnished the Be-1-51 virus. Strain Bonoan, isolated at the Fourth Army Area Medical Laboratory, Fort Sam Houston, Texas, was obtained from Major L. V. Murphy, V.C., U.S.A., and the SWE-3-50 virus was sent by Dr. A. Isaacs. The Greenland-3-51, FM-10-51, FM-111-51 and Dion agents were isolated in this laboratory. The Benson strain was obtained from Dr. J. Salk. The remaining A-type viruses as well as the B strains em-

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