found to be distinctly decreased in syphilitic testes.

The author wishes to thank Dr. Wilton E. Vannier for photographing the amino acid chromatogram, and Mr. John W. Clark, Jr. and Miss Martha Gaston for technical assistance.

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Received July 16, 1954. P.S.E.B.M., 1954, v86.

Effect of Aminopterin on Incorporation of C^{14} Formate into the 2 and 8 Carbons of Guanine.* (21249)

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Whether formate enters the 2 and 8 position of the purine ring(1) via the same immediate precursor or cofactor is not known. Its entry into the 2 position appears to require its prior incorporation into a reduced folic acid derivative in the presence of ATP (2). When aminopterin is injected into an animal this step may be partially blocked and it is possible that C^{14} formate incorporation might be so altered that the specific activity of the 2 carbon of purine would be different from that of the 8 carbon. This might occur if the immediate C^{14} precursors of the 2 and 8 carbons were different and the antimetabolite changed their pool sizes, or if a purine intermediate such as 5-amino-4-imidazolecarboxamide ribotide accumulated. However, in this study the C-2/C-8 ratio of C^{14} formate incorporation into rat nucleic acid guanine was consistently very close to 1 and did not change in the presence of aminopterin.

Methods. Guanine degraded in the following experiments was obtained from the nucleic acids of adult rats used in studies previously reported(3). All animals received C^{14} sodium formate,† 3.6 x 10^7 cts/min/kg by intraperitoneal route, while some of the animals received simultaneously aminopterin, 50 mg/kg. All animals were sacrificed after 24 hours, and the sodium nucleate from different tissues obtained by hot 10% sodium chloride extraction and ethanol precipitation. In control experiments the following organs were employed: 1, total viscera; 2, total viscera plus added small intestine. In the aminopterin experiments total viscera plus intestine were used in No. 3, while small intestine was used in No. 4. 750 mg samples of sodium nucleate were refluxed in 50 ml of 0.5 N H_2SO_4 for 2 hours, filtered, and to the filtrate 50 ml of 10% $AgNO_3$ was added. The purine silver salt was washed, decomposed with HCl at 100° and treated with norite. After filtering, the volume was reduced to 7 ml and guanine crystallized out on neutralization. This was recrystallized from 2 N H_2SO_4. 10 mg of guanine sulfate in 1 ml of 4 N H_2SO_4 was converted to xanthine by addition of 30 mg of $NaNO_2$ in 0.7 ml of water at 75° over a 10-minute period. After cooling at 2°C overnight, the xanthine which crystallized was collected, and converted to uric acid by means of xanthine oxidase(4). The conversion was estimated spectrophotometrically(5). 84 mg of carrier uric acid was added as the lithium salt and uric acid was precipitated on acidification. This was repeated, and the resulting

* The radioactive isotope used in these studies was obtained on allocation from the Atomic Energy Commission.

† Sodium formate 0.28 mM/mc. dissolved in 0.775 ml of dilute alkali.

TABLE I. Effect of Aminopterin on Incorporation of C^{14} Formate into the 2 and 8 Positions of the Guanine. Four experiments. Cts/min./mM carbon.

Control			Aminopterin		
C-2	C-8	Ratio 2/8	C-2	C-8	Ratio 2/8
5140	5260	.98	5400	5640	.96
31400	32500	.97	19200	19400	.99

precipitate extracted twice with hot ethanol and dried with ether. The degradation of the uric acid described in detail elsewhere(1,6), involved a perchlorate oxidation of the purine ring to urea (C-8) and alloxan; the latter was reduced with H_2S to alloxantin and this then oxidized with PbO_2 to urea (C-2). Both samples of urea were degraded with urease and the CO_2 evolved was trapped in NaOH and plated as $BaCO_3$. Samples were counted with an end window Geiger-Muller tube to a statistical error of 2%.

Results. Because samples taken for different experiments (Table I) were not comparable, no conclusions can be drawn from this data regarding the effect of aminopterin on the total C^{14} formate incorporation into guanine. An extensive study of this effect has been reported elsewhere(3). However, the results do indicate that under these conditions, aminopterin has no effect on the ratio of incorporation of C^{14}-formate into the 2 and 8 positions of guanine. This confirms the conclusions of Drysdale *et al.*(6) drawn from data which showed some variation in the ratio of C-2 to C-8 activity. It is impossible to conclude from the present study that the formate derivative which is the precursor for C-2 is the same as that for C-8. However, if the precursors differ, they must have attained the same specific activity from C^{14}-formate, not only in the different tissue samples but also in the presence or absence of aminopterin.

Further evidence to support the concept that the incorporation of formate into the 2 and 8 carbons is via the same pathway has been obtained recently by *in vitro* experiments on the synthesis of a formylated glycine amide ribotide(7). The cofactor present in boiled extract which is necessary for the synthesis of this compound can be replaced by citrovorum factor(8), under conditions comparable to those required for the introduction of formate into the 2 position(2).

Summary. The ratios of specific activity of carbon 2 vs. carbon 8 of rat nucleic acid guanine, labeled *in vivo* with C^{14} formate, remains constant in aminopterin treated animals compared to controls.

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Received July 21, 1954. P.S.E.B.M., 1954, v86.