EFFECTS OF A FOLIC ACID ANTAGONIST ON NUCLEIC ACID METABOLISM*

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Microbiological evidence (1) first implicated folic acid in nucleic acid synthesis, and several lines of investigation suggest that citrovorum factor, or another derivative of folic acid, may function in some manner as a coenzyme in the metabolism of a 1-carbon moiety. The function of the formyl group in rhizopterin (2) and citrovorum factor (3) has not been clarified. The incorporation of formate into carbon atoms 2 and 8 of purines (4) and the production by bacteria of an incomplete purine skeleton (4-amino-5-imidazolecarboxamide (5)) in the presence of a sulfonamide (6) or a folic acid antagonist (7) led to the demonstration in vitro that citrovorum factor was necessary for the production of the purine ring from formate and the carboxamide ribotide (8). This was substantiated by the requirement of citrovorum factor in a system in vitro in which the purine skeleton could be synthesized from small fragments (9). Experiments in vivo have shown a decrease in formate incorporation into the purines of folic acid-deficient bacteria (10) and into the serine of folic acid-deficient rats (11), while the conversion of serine to glycine in the deficient rat was also depressed (12). In mice treated with a folic acid antagonist, the incorporation of formate into the nucleic acids was decreased (13). It was also demonstrated that nitrogen mustards, urethane, cortisone, and x-rays produce a decrease in formate incorporation into nucleic acids (14). However, because the folic acid antagonist produced a more marked decrease in formate incorporation than that observed when bicarbonate was employed as a precursor, it was concluded that the folic acid antagonist had a specific effect on formate incorporation.

The experiments described here involved the simultaneous administration of C14-formate and N15-adenine as precursors of nucleic acid. It was demonstrated that the ratio of incorporation of formate to that of adenine was decreased when aminopterin was also given. The degree of formate

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AMINOPTERIN AND NUCLEIC ACIDS

incorporation into the desoxypentose nucleic acids (DNA) of various organs was shown to parallel the degree of pathological change due to the drug.

Materials and Methods

Sherman strain rats weighing about 200 gm. were used in these experiments. They were kept in a constant temperature room in separate cages and fed a Purina chow diet. From the original isotopic formate solution, approximately 0.2 cc. was diluted to 50 cc. with water, and 1 drop of 10 N NaOH was added. The dose was 5 cc. per kilo or \( 3.6 \times 10^7 \) c.p.m. per kilo, determined in an internal flow counter. Administration was by the intraperitoneal route. The adenine was labeled in the 1 and 3 positions (15) and contained 4.83 atom per cent excess N\(^{15}\). Adenine \( \cdot \frac{1}{2}H_2O \cdot \frac{1}{2}H_2SO_4 \) (193 mg.) was dissolved in 50 cc. of water to which 0.18 cc. of 85 per cent lactic acid was added. The dose was 10 cc. or 0.2 mM of adenine per kilo and was given intraperitoneally. Aminopterin (250 mg.) was suspended in 11.4 cc. of 0.1 \( \text{m} \) NaHCO\(_3\) and made up to 50 cc. with water. A dose of 50 mg. per kilo was administered by intraperitoneal injection unless specified otherwise.

Isolation of Whole Nucleic Acids—Immediately after sacrifice, intestine from 2 kilos of rat was placed in a dry ice-alcohol mixture, homogenized in a Waring blender, filtered, rehomogenized twice with alcohol and once with ether, and dried (yield 32 gm.). 8 gm. of this dry tissue were then suspended in 20 volumes of 10 per cent NaCl and stirred for 6 hours at 85°. After hot filtration, sodium nucleate in the filtrate was precipitated with 3 volumes of alcohol. After it was cooled overnight, the sodium nucleate was collected and was washed with alcohol and ether; 775 mg. were obtained. Other organs were worked up similarly.

For total nucleic acid, 300 mg. of sodium nucleate were suspended in 15 cc. of water, stirred on a steam bath for 10 minutes, chilled, and then acidified to pH 2 with 6 N HCl. The precipitated free nucleic acid was washed twice each with water, alcohol, and ether.

Isolation of Bases—The bases were isolated by a procedure which will be described in detail elsewhere.\(^2\) In brief, this consists of a separation of the isolated sodium nucleates by alkaline hydrolysis of the pentose nucleic acid (PNA), followed by acid precipitation of DNA. The PNA nucleotides were recovered from the supernatant as the barium salts. Each fraction was subjected to perchloric acid hydrolysis, and the bases, obtained as silver salts, were liberated with hydrochloric acid. Chromatographic separation of bases was effected on a Dowex 1 column, with varying concentrations

\(^1\) Obtained from the Oak Ridge National Laboratory, sodium formate 0.28 mm per mc. of C\(^{14}\) dissolved in 0.775 cc. of dilute alkali.

\(^2\) Roll, P. M., in preparation.
of sodium chloride and sodium hydroxide. The fractions containing each base were concentrated on Dowex 1 columns and eluted with dilute hydrochloric acid.

**Determination of Isotope**—When Cl⁴ activity of total nucleic acid was determined, 90 mg. of the dried, free nucleic acid preparation were finely powdered and were placed in an aluminum planchet, 2 cm. in diameter, and the activity was determined by the use of an end window Geiger-Müller tube.

The Cl⁴ activity of the bases isolated chromatographically was determined as follows: The HCl in a portion of the eluate was removed by repeated concentrations to dryness in *vacuo*, the residue was taken up in water, and infinitely thin samples were prepared, the activities of which were determined in an internal Geiger-Müller flow counter (Radiation Counter Laboratories, mark 12, model 1, helium-isobutane gas). Simultaneously, two or three 0.5 cc. aliquots were diluted to 25 cc. with 0.01 N HCl for quantitative determination by the spectra. Adequate separation of the bases on ion exchange columns and ratios of optical density at wavelengths 250, 260, 280, 290 mμ corresponding to pure bases served as criteria of purity. Estimation of purine nitrogen by micro-Kjeldahl analysis as well as from spectrophotometric data, however, differed by 9 per cent.

All samples with Cl⁴ activity greater than 30 c.p.m. per μμ were counted to within 2 per cent error, while those with less activity were counted to within 10 per cent error.

For N¹⁵ determinations, an aliquot of the HCl eluate employed for micro-Kjeldahl analysis was converted to N₂. The atom per cent excess N¹⁵ was then measured with a Consolidated-Nier mass spectrometer, model 21-201; probable error ±0.001; tank nitrogen was used as a standard.

**EXPERIMENTAL AND RESULTS**

**Decrease of Formate Incorporation Due to Aminopterin**—At varying times after one injection of aminopterin, Cl⁴-formate was given to rats which were sacrificed 24 hours later. The dose of aminopterin (50 mg. per kilo or about 10 × LD₅₀, killing all animals in 3 to 7 days (16)) was chosen to provide a more uniform response than could be expected from chronic administration. Two animals received isotopic formate simultaneously with aminopterin, one animal received the formate 24 hours later, and two 48 hours later. The nucleic acids of the total viscera were isolated. The results (Table I) indicate that the incorporation of formate into the mixed nucleic acids of total viscera was initially decreased, but by the 3rd day after injection of aminopterin the incorporation of isotope had returned towards the control value. This return several days prior to death indicates that the initial decrease was due to drug action *per se* and not the
poor general condition of the animals. The increase in incorporation with increasing time probably represents both a recovery from the drug effect by some tissues and a loss in other tissues of total nucleic acid, thus giving less of a dilution effect. In the intestine, for example, the recovery of sodium nucleate from control rats was 2.0 gm. per kilo, while in the aminopterin-treated group it was 0.86 gm. per kilo.

To determine whether the result from the simultaneous intraperitoneal administration of C\textsuperscript{14}-formate and aminopterin might have been due to

**TABLE I**

*Incorporation of Formate Administered at Varying Times after Single Dose of Aminopterin*

<table>
<thead>
<tr>
<th>Time of administration</th>
<th>C.p.m.</th>
<th>Per cent of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>670</td>
<td>100</td>
</tr>
<tr>
<td>Formate simultaneously</td>
<td>204</td>
<td>31</td>
</tr>
<tr>
<td>&quot; 24 hrs. later</td>
<td>234</td>
<td>35</td>
</tr>
<tr>
<td>&quot; 48 &quot;</td>
<td>476</td>
<td>71</td>
</tr>
</tbody>
</table>

* Total nucleic acids; animals sacrificed 24 hours after administration of formate. † Infinitely thick (28 mg. per sq. cm.).

**TABLE II**

*Effect of Varying Doses of Adenine on Incorporation of Fixed Dose of C\textsuperscript{14}-Formate into Nucleic Acids*

<table>
<thead>
<tr>
<th>Dose of adenine (mM)</th>
<th>C.p.m.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>538</td>
</tr>
<tr>
<td>0.10</td>
<td>508</td>
</tr>
<tr>
<td>0.20</td>
<td>290</td>
</tr>
</tbody>
</table>

* Infinitely thick (28 mg. per sq. cm.).

chemical interaction of these two substances, one rat was given aminopterin by mouth and isotopic formate intraperitoneally. The activity of the whole nucleic acids isolated was similar to that when both substances were administered intraperitoneally.

**Decrease of Formate Incorporation by Adenine**—The effect of adenine on the incorporation of formate was investigated by the administration of varying doses of unlabeled adenine (0.02, 0.10, or 0.20 mM per kilo) to pairs of rats which received simultaneously the above standard dose of C\textsuperscript{14}-formate. The animals were sacrificed 24 hours after injection of isotope and the activity of the nucleic acids of total viscera was determined. The results (Table II) indicate that with a sufficiently large dose of adenine the
incorporation of C\textsuperscript{14}-formate into nucleic acids is decreased. This could represent either an inhibitory effect of adenine on nucleic acid synthesis or a sparing action of adenine on the need for 
\textit{de novo} synthesis from formate. Two facts support the latter interpretation. When adenine is administered with formate, the formate incorporation into purine relative to thymine is decreased with respect to that observed in a similar experiment without adenine.\textsuperscript{3} The ratio of C\textsuperscript{14} activity of the adenine and thymine of DNA of

\textbf{Table III}

\textit{Effect of Aminopterin on Incorporation of C\textsuperscript{14}-Formate and N\textsuperscript{15}-Adenine into Nucleic Acids of Intestine and Liver}

<table>
<thead>
<tr>
<th></th>
<th>C\textsuperscript{14} activity, c.p.m. per (\mu\text{M})</th>
<th>Atom per cent excess N\textsuperscript{15}</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Aminopterin</td>
<td>Per cent change</td>
</tr>
<tr>
<td><strong>Gastrointestinal tract</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenine</td>
<td>189</td>
<td>11.8</td>
<td>-94</td>
</tr>
<tr>
<td>Guanine</td>
<td>291</td>
<td>18.3</td>
<td>-94</td>
</tr>
<tr>
<td>Thymine</td>
<td>251</td>
<td>21.4</td>
<td>-92</td>
</tr>
<tr>
<td>DNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenine</td>
<td>254</td>
<td>81</td>
<td>-68</td>
</tr>
<tr>
<td>Guanine</td>
<td>277</td>
<td>79</td>
<td>-71</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenine</td>
<td>7.1</td>
<td>2.1</td>
<td>-50% Ca.*</td>
</tr>
<tr>
<td>Guanine</td>
<td>7.0</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Thymine</td>
<td>7.8</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenine</td>
<td>63</td>
<td>39</td>
<td>-38</td>
</tr>
<tr>
<td>Guanine</td>
<td>43</td>
<td>27</td>
<td>-36</td>
</tr>
</tbody>
</table>

* A more accurate estimation of the decrease is not justified in view of the low activities measured.

control intestine (Table III) is 0.75, while in this tissue when no adenine is employed this ratio approached 2.0. Also there is an appreciable incorporation of N\textsuperscript{15}-adenine into the adenine isolated from the DNA of control intestine and spleen (Table V). A similar sparing action of preformed purines has been demonstrated in \textit{Lactobacillus casei} (17) and in yeast (18).

\textit{Effect of Aminopterin on C\textsuperscript{14}-Formate and N\textsuperscript{15}-Adenine Incorporation. Experiment 1a—}Both formate and adenine were administered simultaneously with and without aminopterin to ascertain whether a differential effect of the drug on either of these precursors could be detected. A control group of ten rats received simultaneously N\textsuperscript{15}-adenine (0.2 mm per kilo) and C\textsuperscript{14}-

\textsuperscript{3} Goldthwait, D. A., unpublished observations.
formate (standard dose), while a second similar group received, in addition, one injection of aminopterin (50 mg. per kilo) 15 minutes before the isotopic compounds. All animals were sacrificed 24 hours later. The gastrointestinal tracts, livers, spleens, kidneys, and testes were pooled separately and, from these, nucleic acid bases were isolated. The results of the isotope analyses on the PNA and DNA of the gastrointestinal tract and liver are presented in Table III. The aminopterin produced an extreme decrease in the incorporation of formate, which was greatest in the DNA fraction of intestine. In all fractions, the decrease in the incorporation of formate was not accompanied by a corresponding decrease in the incorporation of adenine. In fact, the incorporation of $N^{15}$-adenine in the aminopterin-treated animals was greater than the control values in all samples except intestinal DNA adenine and liver PNA adenine. The apparent increase in the incorporation of $N^{15}$-adenine into PNA of intestine, following aminopterin administration, may be related to the breakdown of cells and the decrease in total nucleic acid of this organ. This would dilute the isotope to a smaller extent.

Aminopterin appears to affect the amount of guanine derived from adenine. The ratio of incorporation of the administered $N^{15}$-adenine into the adenine and into the guanine of each of the PNA and DNA fractions from the controls was about 4 (liver PNA, 6), but in all cases it was nearer 2 in the aminopterin-treated animals. The obvious absolute increase in the $N^{15}$ content of the guanine of PNA of intestine and liver of the aminopterin-treated animals (Table III) can thus be correlated with a relative increase in the proportion of guanine derived from adenine in all other fractions. Earlier results, showing a greater incorporation of glycine (19, 20) and formate (21) into guanine than into adenine, suggested that there must be an anabolic pathway leading to guanine derivatives which does not involve adenine as an intermediate. If aminopterin decreases formate availability, a greater proportion of polynucleotide guanine might be derived from the preformed $N^{15}$-adenine. This interpretation is compatible with the consistent decrease, following aminopterin, in the ratio of $N^{15}$ in the adenines compared to the guanines.

The results in Table III indicate that incorporation of both adenine and formate is affected more in the DNA than in the PNA fractions. The reason for this is not apparent, but it is pertinent that both x-rays (22) and nitrogen mustard3 also have this differential effect on intestinal tissue.

The incorporation of $C^{14}$-formate into pyrimidines other than thymine was negligible. The activity of cytosine of the control intestinal DNA was 11 c.p.m. per $\mu$M, while that of the cytosine and uracil of the control intestinal PNA was 9 c.p.m. per $\mu$M.

Experiment 1b—To extend the above results, a comparable experiment
was carried out. Two groups of five animals were similarly injected and, on sacrifice, the gastrointestinal tracts were divided into two portions, the small intestine (duodenum, jejunum, and ileum) and large intestine plus stomach. The bases of the DNA were isolated and the results are presented in Table IV. Again the differential effect on formate incorporation, with respect to adenine, is apparent. The higher formate activity in the small intestine and the more extreme depression of this activity by aminopterin supplement the results with the whole gastrointestinal tract (Table III).

Experiment 1c—To determine whether or not there is a correlation between the pathological changes noted in different organs (16, 23) and the incorporation of formate, the DNA bases were isolated from pooled spleens, kidneys, and testes of the two groups of animals from Experiment 1a. These results plus those from Experiment 1b are presented in Table V. The biological significance of these results will be discussed in the following section. It is noteworthy that there is no constant ratio of incorporation of formate into adenine, guanine, and thymine of the DNA of different tissues. The reported constancy of amount (24) and composition (25) of DNA among different organs in one species might lead one to expect, under proper experimental conditions, more nearly equal ratios of incorporation.

**DISCUSSION**

The differential effect of aminopterin on the incorporation into nucleic acids of formate compared to adenine at first suggests that the drug exerts a specific effect on formate. However, other possibilities exist which have not been ruled out in this experiment. Were aminopterin to produce an increase in the size of the formate pool, the resulting greater dilution of

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**Table IV**

|                      | **C14 Activity, C.p.m. per µm** | **Atom per cent excess N15** |
|----------------------|---------------------------------|-----------------------------
|                      | **Control** | **Aminopterin** | **Per cent change** | **Control** | **Aminopterin** | **Per cent change** |
| Small intestine      |            |                |                     |            |                |                     |
| Adenine              | 213        | 6.6            | -97                | 0.079      | 0.043          | -46                 |
| Guanine              | 354        | 26             | -93                | 0.021      | 0.021          |                     |
| Thymine              | 390        | 24             | -94                |            |                |                     |
| Large intestine and  |            |                |                     |            |                |                     |
| stomach              |            |                |                     |            |                |                     |
| Adenine              | 89         | 22             | -75                | 0.047      | 0.036          | -23                 |
| Guanine              | 103        | 35             | -66                | 0.011      | 0.017          |                     |
| Thymine              | 142        | 15             | -89                |            |                |                     |
isotopic precursor would give results similar to those presented. It has been demonstrated that one reaction contributing to the 1-carbon pool, namely the conversion of serine to glycine, is decreased in the folic acid-deficient rat to one-half its normal rate (12). This evidence indicates a decrease rather than an increase in the formate pool. If aminopterin decreased the concentration of both adenine and formate equally, then enzyme saturation would become an important consideration. If the first enzyme concerned with adenine incorporation was saturated (adenine was administered at 0.2 mM per kilo), while the first enzyme concerned with formate incorporation was not saturated (formate was administered at approximately 0.007 mM per kilo), an equal decrease in concentration of both precursors would result in a more marked decrease of formate incorporation. It has been observed that nitrogen mustard decreases about equally the incorporation of somewhat similar levels of both precursors into intestinal DNA, but this is not a good control for the differential effect of aminopterin. That aminopterin by some obscure mechanism might increase the level of adenine or a related intermediate and thereby produce a sparing effect on formate incorporation is unlikely, since the incorporation of formate is depressed by aminopterin when no adenine is administered (Table I) and the formate incorporation into thymine is also depressed by the drug even when adenine is administered (Table III). If the above possibilities could be ruled out, it would be reasonable to assume that the drug affects some step in the purine synthetic chain leading to nucleic acid. The few facts regarding synthesis of the purine skeleton indicate that ribose or its phosphate is attached to a precursor of the 4-amino-5-imidazolecarboxa-

**Table V**

*Effect of Aminopterin on Incorporation of C₁⁴-Formate into DNA of Different Organs*

<table>
<thead>
<tr>
<th>Activity in counts per minute per micromole.</th>
<th>Control</th>
<th>Aminopterin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adenine</td>
<td>Guanine</td>
</tr>
<tr>
<td>Small intestine</td>
<td>213</td>
<td>354</td>
</tr>
<tr>
<td>Large &quot; and stomach</td>
<td>89</td>
<td>103</td>
</tr>
<tr>
<td>Spleen</td>
<td>39</td>
<td>44</td>
</tr>
<tr>
<td>Testes</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Liver</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Kidney</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

* Data from Experiments 1b and 1c. The incorporation of the N¹⁵-adenine into the DNA adenine of the control tissues resulted in the following N¹⁵ values: small intestine 0.079, large intestine and stomach 0.047, spleen 0.022, liver 0.009.
mide before the purine skeleton is formed (8, 9). Since the adenine ring probably does not open during its incorporation into nucleic acids, adenine cannot be directly on the path described, but may enter as a ribose derivative at a later step. In this synthetic scheme, aminopterin could affect any step from the small precursors up to the possible linkage of ribotides and produce the differential effect on formate noted. However, one fact in the data points to the locus of drug action on a 1-carbon moiety. There is a remarkable parallel between the decrease in formate incorporation into thymine and into purines produced by aminopterin. This suggests that this drug affects a 1-carbon fragment before it is incorporated into either purine or pyrimidine skeleton. Also, because of this parallel decrease of incorporation, it seems unlikely that the drug affects some other nucleic acid precursor.

It is possible to correlate the relative incorporation data with the varying degrees of pathological change noted in different tissues (16, 23) after the administration of aminopterin. In Table V the tissues are listed in the order of formate incorporation into DNA, but this listing could well be based upon the extent of histological damage due to aminopterin. The small intestine, particularly the duodenum, suffers the most severe changes, while the large intestine shows moderate damage and the stomach only slight damage. The myeloid tissue, comprising 15 to 20 per cent of the rat spleen, is severely affected, while the lymphoid tissue shows little if any change (in the adrenalectomized animal, aminopterin has no effect on lymphoid tissue (26)). Testes show no obvious damage and the kidney and liver are unaffected. There is thus a parallel between the degree of tissue damage and the extent of incorporation of formate into the DNA of those tissues in the normal animal.

There is also a parallel between the extent of cell division and the incorporation of formate into the DNA of those tissues. In the jejunum of the mouse and the duodenum of the rat the intermitotic times of crypt epithelium are 43 (27) and 38 (28) hours respectively. This rapid mitotic rate in the small intestine correlates well with the high degree of formate incorporation into DNA of this tissue. By contrast, the intermitotic time of lymphoid tissues in the mouse is estimated to be 24 days (27). In a human with lymphatic leucemia the peak of incorporation of one injection of P32 into white cells occurred at 23 days. The fact that thymine activity in the spleen is approximately one-tenth that of the intestine (Table V) agrees with the observation regarding a long intermitotic time for the

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5 Balis, M. E., unpublished observations.
6 Buckley, J. J., personal communication.
7 Hellman, L., personal communication.
lymphoid tissues. This is not in line with data on the life span of the lymphocyte (29, 30). In the testes of the rat, the spermatogenic cycle is calculated as 16 days (31), while, following irradiation, 2 to 3 weeks elapse before undifferentiated cells reappear (32). The mitotic rate in the liver (33) and the kidney is extremely low, as is the incorporation of formate into the DNA bases of those organs. It is apparent from the data on both formate and adenine that the tissues showing the highest mitotic rate synthesized the most DNA per unit time. From the data presented it is not valid to conclude that DNA is synthesized solely in premitotic or mitotic cells; however, any DNA synthesis in resting cells must be minimal compared to that in mitotic cells. The correlation between mitotic activity and formate incorporation makes it tempting to hypothesize that the action of the drug in decreasing the availability of formate results in a deficiency which evidences itself first in those tissues which utilize the most formate per unit time for DNA synthesis.

A point of interest in the interrelationship of the simultaneously administered adenine and formate is the different extent of utilization of adenine compared to formate for the synthesis of PNA adenine in different tissues. Thus, in liver, adenine is used for PNA synthesis more extensively, relative to formate, than it is in intestine, and these results confirm the separate observations made on the relative incorporation of adenine (34) and of formate* into PNA of liver and intestine.

Conflicting evidence has accumulated from different laboratories regarding the relative renewals of PNA and DNA of the liver. The ratio of incorporation of glycine (19, 20) into the PNA and DNA purines was approximately 3:1, and with formate this ratio approaches that of glycine in the liver of rats.9 However, with adenine (34), this ratio was greater than 50:1, and simultaneous administration of adenine and glycine (35) confirmed these differences. The ratio for adenine reported here (Table III, corrected by −0.005 for the difference between biological and “tank” nitrogen (36)) is about 50:1, while that for formate is 9:1, and thus the results for adenine and formate are quite similar to those found for adenine and glycine. An explanation for the different results with the various precursors may involve the preferential utilization of adenine for liver PNA. This again emphasizes the conclusion (37, 38) that the renewal of the nucleic acids may be dependent upon the nature of the isotopically labeled precursor.

The authors wish to thank Dr. George Bosworth Brown for advice and support throughout this work, Dr. Frederick S. Phillips for many helpful

9 Bendich, A., unpublished observations.
discussions, Mr. Frederick Breslof for valuable technical assistance, and Mr. Roscoe C. Funk, Jr., for the microanalytical determinations.

SUMMARY

The effect of aminopterin on the incorporation of C\textsuperscript{14}-formate and N\textsuperscript{15}-adenine into nucleic acids of the rat has been investigated.

The incorporation of formate into purines was depressed by aminopterin to a greater extent than was the incorporation of adenine. This effect was noted in PNA and DNA of intestine and PNA of liver. The effect of aminopterin on the incorporation of formate into thymine was similar to its effect on the incorporation of formate into purines. The decrease of formate incorporation was more marked in the DNA than in the PNA of intestine.

A "sparing" action of adenine on formate utilization for nucleic acid synthesis has been demonstrated in the rat.

Investigation of C\textsuperscript{14}-formate incorporation into the bases of DNA revealed the highest incorporation in small intestine. Other organs, in diminishing order, were large intestine plus stomach, spleen, and finally testes, kidney, and liver.

The extent of incorporation of formate into DNA of these organs appears to be correlated with the rate of mitosis. The incorporation and the extent of its depression by aminopterin were directly related to the degree of tissue damage caused by the drug.

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