The Clinical and Experimental Studies on the Metabolism of Folic Acid Using Tritiated Folic Acid

II. The Experimental Studies on the Absorption Site and Mechanism of Tritiated Folic Acid in Rats

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1. The site and mechanism of folic acid (FA) absorption were studied in the rat, using ³H-FA.
2. After intragastric administration of 40 μ g of ³H-FA per kg of body weight, 79.3 per cent of the administered dose was absorbed from the upper gastrointestinal tract in 6 hours.
3. From the jejunal loop, 91.7 per cent of the injected dose was absorbed in 6 hours, and the absorption from the ileum was about 50 per cent of that from the jejunum.
4. With the increase of administered doses, the rate of absorption was decreased, while the absolute amount of the absorbed FA increased almost linearly.
5. The absorption of FA was not affected by the following conditions: simultaneous administration of Amethopterin or dinitrophenol, pretreatment with Amethopterin or FA, and deficiency of FA.
6. The site and mechanism of FA absorption were discussed and a passive transport for FA was suggested.

The pathologic physiology of the anemia encountered in digestive diseases was experimentally studied with special reference to the absorption mechanism of tritiated folic acid (³H-FA). The site and mechanism of folic acid (pteroylglutamic acid, FA) absorption have not been well established, while the site of vitamin B₁₂ (B₁₂) absorption is proved to be the ileum (1) and intrinsic factor is necessary for its absorption (2). FA has been thought to be absorbed from the upper gut (3, 4), but defective FA absorption was observed in some patients with diseases of the distal ileum (5, 6). On the other hand, concerning the mechanism of FA absorption, it has been suggested that active transport system may play a main role (7, 8), but it is not established yet and is still controversial.

In the present study, experimental observations were made to elucidate the site and mechanism of FA absorption using male rats of Wistar strain.

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MATERIALS AND METHODS

1. Radioactive Materials

The specification of ^3H -FA was described in the previous paper (9).

2. Measurement of ^3H -FA Absorption after Intragastric Administration

Male rats of the Wistar strain weighting about 200 g were kept fasting for 24 hours and then anesthetized with ether gas. After fixing, the abdomen was opened by a small incision. ^3H -FA in a dose of $40\text{ }\mu\text{g}$ per kg of body weight was injected into the stomach and the abdomen was closed. The rat was sacrificed 30 minutes, 3 and 6 hours after the injection, respectively and gastrointestinal tract was ligated at the distal ends of the esophagus and of the rectum. The gastrointestinal tract was removed and divided into six portions as follows: the stomach, duodenum, small intestine 1, small intestine 2, small intestine 3 and colon. The content of each portion was washed out in a test tube with 20 ml of 0.01 M sodium phosphate buffer and filtered. One and five-tenth ml of the filtrate was taken in a counting vial for radioactive assay. The amount of FA absorbed was calculated from the residual activity in the entire gastrointestinal tract.

The liver and kidneys were also removed for radioactive assay. Two g of the liver was put into a glass blender (Potter type) and homogenized for one minute at 4° . Water was then added to bring the total volume to 10 ml. One ml of the homogenate was taken to a counting vial and applied to assay. Whole kidneys were also put into the glass blender and measured.

Radioactive assay was carried out as follows. The counting vial containing each sample was brought to dryness in the oven at 80° . After adding 0.4 ml of hyamine 10-X, the vial was allowed to stand until solution was completed. Then 10 ml of scintillator solution containing 600 mg 2,5-diphenyloxazole (PPO) and 20 mg 1,4-bis-2-(5-phenyloxazolyl) benzene (POPOP) in 100 ml toluene (10) was added and tritium concentration was counted in a Packard Tri-Carb liquid scintillation spectrometer. Then the radioactivity was recounted after adding tritiated toluene as an internal standard and the amount of radioactivity was calculated. These methods are illustrated in Fig. 1.

3. Measurement of ^3H -FA Absorption from the Jejunum and Ileum

The intestines of anesthetized rats were ligated to form isolated loops of proximal or distal ileum,

FIG. 1 The Method of the Intestinal Absorption of Tritiated Folic Acid after Its Intragastric Administration

each about 10 cm long. ^3H -FA in the dose of $40\text{ }\mu\text{g}$ per kg of body weight was injected into these loops. They were removed 30 minutes, 3 and 6 hours after the injection and the contents were collected for radioactive assays. The liver and kidneys were also assayed.

Further experimental observations were made on the following groups. In these observations, the absorption of ^3H -FA was determined in 3 hours after the administration into the jejunal loop.

(a) ^3H -FA absorption after administration of various amounts: The labeled FA in doses of 400 and $4000\text{ }\mu\text{g}$ per kg of body weight were injected into the loops, and the absorption and the uptake to the liver and kidneys were estimated.

(b) ^3H -FA absorption after simultaneous administration with Amethopterin: The labeled FA in the dose of $40\text{ }\mu\text{g}$ per kg was injected into the loop simultaneously with Amethopterin of the same dose.

(c) ^3H -FA absorption in the rats pretreated *in vivo* with Amethopterin: Amethopterin in a dose of 1.5 mg per kg of body weight was injected intramuscularly for 3 days. Twenty four hours after the final injection of Amethopterin, the loop was made ^3H -FA in the dose of $40\text{ }\mu\text{g}$ per kg was injected as described above.

(d) ^3H -FA absorption in the rat pretreated with FA: Non-radioactive FA in a dose of 1.5 mg per kg of body weight was injected intramuscularly for 3 days. Experimental observation was performed 24 hours after the final injection.

(e) ^3H -FA absorption in the rat with simultaneous administration of dinitrophenol: The labeled FA in the dose of $40\text{ }\mu\text{g}$ per kg of body weight was injected into the loop simultaneously with 0.3 ml of 1 mM dinitrophenol solution.

(f) ^3H -FA absorption in the rat with FA deficiency: Deficiency of FA was produced by feeding the rats on a diet deficient in FA for 13 weeks. During the last 2 weeks, 1 g of sulfa drug was added to 1 kg of the deficient diet.

RESULTS

1. Recoveries in ^3H -FA Measurement of Tissues

Known amounts of ^3H -FA were added to homogenized liver and kidney that did not contain the isotope. Their radioactivities were estimated and the recoveries were calculated.

TABLE I
Recoveries of Tritiated Folic Acid Added to Liver and Kidney

	^3H -FA added	^3H -FA counted	After correction with quenching	Original ^3H -FA	Recovery
	<i>dpm</i>	<i>cpm</i>	<i>cpm</i>	<i>dpm</i>	
Liver	222,000	11,074	17,942	216,690	97.6
	222,000	10,553	18,995	229,400	103.3
	222,000	10,304	18,248	220,380	99.3
					100.1
Kidney	222,000	10,262	19,806	218,360	98.4
	222,000	10,650	21,174	233,670	105.3
	222,000	10,574	19,879	219,170	98.7
					100.8

The recovery from the liver ranged from 97.6 to 103.3 per cent, with a mean of 100.1 per cent. The recovery from the kidney ranged from 98.4 to 105.3 per cent and a mean value was 100.8 per cent as shown in Table 1.

2. Absorption of ^3H -FA After Its Intragastric Administration

The fractionated residual amount of radioactivity in each portion is shown in Table 2. Thirty minutes after administration, 44.1 per cent was found in the stomach, 7.6 per cent in the duodenum and 7.4 per cent in the proximal portion of the small intestine, but very little radioactivity was found in other portions. After 3 hours, the residual amount of radioactivity in the stomach was reduced to 16.3 per cent and only 1.8 per cent was found in the duodenum, while 19.0 per cent was found in the proximal portion of the small intestine. In one out of three rats, 1.46 per cent of the injected dose was found in the middle portion of the small intestine, but the radioactivity was scarcely found in the distal portion of the small intestine and colon. Six hours after the administration, the radioactivity in the stomach was 11.5 per cent and that in the proximal portion of the small intestine was decreased. In some cases, considerable radioactivity was found in the middle portion of the small intestine, but little was found in the distal portion of the small intestine and the colon.

TABLE 2
Residual Amounts in Gastrointestinal Tract of ^3H -Folic Acid After Intragastric Administration

Administered dose of ^3H -FA, 40 μg per kg.

	Stomach	Duodenum	Small int. 1.	Small int. 2.	Small int. 3.	Colon	Total
	<i>per cent</i>						
30 min	31.4	13.3	15.0	0.10	0.06	0.05	59.9
	47.4	6.62	6.52	0.05	0.05	0.03	60.7
	53.6	2.96	0.52	0.80	0.03	0.00	57.9
Mean	44.1	7.63	7.35	0.62	0.05	0.03	59.5
3 hr	19.9	1.25	15.3	0.07	0.05	0.07	36.6
	16.4	2.29	16.3	0.04	0.07	0.04	35.1
	12.7	1.86	25.3	1.46	0.06	0.02	41.4
Mean	16.3	1.80	19.0	0.52	0.06	0.04	37.7
6 hr	15.1	0.76	3.26	2.08	0.04	0.02	21.3
	14.8	0.44	8.75	0.10	0.02	0.00	24.1
	4.50	0.51	1.81	9.79	0.19	0.03	16.8
Mean	11.5	0.57	4.61	3.99	0.08	0.02	20.7

The absorption of FA after its intragastric administration is shown in Table 3 and Figs. 2 and 3. After the administration of ^3H -FA in a dose of 40 μg per kg of body weight into the stomach, its mean absorption from the gastrointestinal tract was 40.5, 62.3 and 79.3 per cent of the administered dose at 30 minutes, 3 and 6 hours, respectively. The hepatic uptake was 1.27, 4.66 and 8.00 per cent of the administered dose in each time. The radioactivity of the kidneys was 0.66, 2.29 and 2.58 per cent of the injected dose at 30 minutes, 3 and 6 hours, respectively. The FA absorption after intragastric administration was increased according

TABLE 3
Absorption and Organ Uptake of ³H-Folic Acid After Intragastric Administration
Administered dose of ³H-FA, 40 μg per kg.

	Amounts absorbed	Hepatic uptake	Renal uptake
		<i>per cent</i>	
30 min	40.1	1.46	0.67
	39.3	1.04	0.53
	42.1	1.32	0.77
Mean	40.5	1.27	0.66
3 hr	63.4	5.41	2.44
	64.9	5.84	2.08
	58.6	2.72	2.35
Mean	62.3	4.66	2.29
6 hr	78.7	6.91	2.62
	75.9	6.90	2.82
	83.2	10.2	2.30
Mean	79.3	8.00	2.58

FIG. 2 *The Absorption of Tritiated Folic Acid After Its Intragastric Administration*
Administered dose of ³H-FA, 40 μg per kg.
A, stomach; B, duodenum; C, small int. 1; D, small int. 2; E, small int. 3; F, large int. ■, absorbed amounts; □, residual amounts.

FIG. 3 *The Organ Uptakes of Tritiated Folic Acid After Its Intragastric Administration.*
—, liver; ---, kidneys.

FIG. 4 The Method of the Intestinal Absorption After its Administration in the Jejunum or the Ileum

to the course of time.

As described above, 79.3 per cent of the dose was absorbed by 6 hours after its intragastric administration and during the period of absorption, little radioactivity was found in the distal portion of the small intestine and the colon.

3. ³H-FA Absorption from the Jejunum and Ileum

The results of ³H-FA absorption from the jejunum or ileum are shown in Tables 4 and 5 and Fig. 5, and the hepatic and renal uptakes are shown in Tables 4 and 5 and Figs. 6 and 7.

When the labeled FA in the dose of 40 µg per kg of body weight was administered into the loop of the proximal jejunum, the amount of FA absorbed was 32.4 per cent, the hepatic uptake 4.26 per cent and the renal uptake 1.79 per cent at 30 minutes. Three hours after the

TABLE 4
Absorption and Organ Uptake of ³H-Folic Acid After Administration in Jejunum

Administered dose of ³H-FA, 40 µg per kg.

	Amounts absorbed	Hepatic uptake	Renal uptake
		per cent	
30 min	35.5	4.28	2.07
	24.0	5.07	1.59
	37.7	3.44	1.72
Mean	32.4	4.26	1.79
3 hr	68.7	6.62	6.14
	87.1	4.48	4.54
	88.0	6.64	4.45
	67.7	9.98	6.85
	88.4	10.1	7.71
	79.4	8.89	5.35
	87.3	9.00	6.99
Mean	80.9 ± 9.1	7.95 ± 2.09	6.00 ± 1.26
6 hr	90.9	10.0	5.52
	91.3	15.8	5.65
	92.9	17.9	6.47
Mean	91.7	14.6	5.88

TABLE 5
Intestinal Absorption and Organ Uptake of ^3H -Folic Acid After its Administration in Ileum
 Administered dose of ^3H -FA, 40 μg per kg.

	Amounts absorbed	Hepatic uptake	Renal uptake
		<i>per cent</i>	
30 min	20.8	2.98	1.08
	13.2	1.79	1.00
	11.8	2.12	0.98
Mean	15.3	2.40	1.02
3 hr	33.5	2.09	1.08
	34.4	3.70	2.69
	26.9	2.10	1.37
Mean	31.6	2.63	1.71
6 hr	49.7	2.77	1.51
	53.4	3.77	1.30
	53.4	4.29	2.27
Mean	52.2	3.61	1.69

dose, the mean absorption was 80.9 ± 9.1 per cent, with a range of 67.7 to 88.4 per cent. The hepatic uptake in this time ranged from 4.48 to 10.1 per cent with a mean of 7.95 ± 2.09 per cent and the renal uptake from 4.45 to 7.71 per cent, with a mean of 6.00 ± 1.26 per cent. At 6 hours after the administration, FA absorption was 91.7 per cent, the hepatic uptake 14.6 per cent and the renal uptake 5.88 per

FIG. 5 *The Absorption of Tritiated Folic Acid After Its Intestinal Administration*
 —, given in jejunum; ---, given in ileum.

FIG. 6 *The Hepatic Uptake of Tritiated Folic Acid After Its Intestinal Administration*
 —, given in jejunum; ---, given in ileum.

FIG. 7 The Renal Uptakes of Tritiated Folic Acid After Its Intestinal Administration —, given in jejunum; ---, given in ileum.

cent at 30 minutes, 3 and 6 hours, respectively. The hepatic and renal uptake when given into the ileum were apparently lower than that from the jejunum.

4. ^3H -FA Absorption After Administration of Various Doses in the Jejunum

The observations were made in 3 hours after the administration of ^3H -FA into

TABLE 6
Intestinal Absorption and Organ Uptake of ^3H -Folic Acid in 3 Hours After Administration of Various Doses in Jejunum

Dose of ^3H -FA admin.	Amounts absorbed	Hepatic uptake	Renal uptake
		per cent	
40 μg per kg	80.9 $\pm$ 9.1	7.95 $\pm$ 2.09	6.00 $\pm$ 1.26
400 μg per kg	44.2	4.10	1.44
	46.8	2.82	1.23
	50.3	3.79	1.26
Mean	47.1	3.57	1.31
4,000 μg per kg	19.8	1.60	0.63
	31.9	1.09	0.31
	19.8	1.49	0.45
Mean	23.8	1.39	0.46
Dose	Amounts absorbed	Hepatic uptake	Renal uptake
		$\mu\text{g}/\text{kg}$ body weight	
40	32.4	3.18	2.40
400	188.0	14.3	5.24
4,000	952.0	55.6	18.4

cent, respectively.

When ^3H -FA in the dose of 40 μg per kg of body weight was injected into the loop of the distal ileum, its absorption was 15.3 per cent in 30 minutes. Three hours after the administration, the absorption of FA ranged from 26.9 to 34.4 per cent, with a mean of 31.6 per cent. This value was apparently lower than that of the absorption from the jejunum. The labeled FA was absorbed 52.2 per cent in 6 hours after the administration. Generally speaking, it was found that the amount of absorption from the ileum was approximately 50 per cent of that from the jejunum. Concerning the hepatic uptake, 2.40 per cent was taken up in 30 minutes after the administration, 2.63 per cent in 3 hours and 3.61 per cent in 6 hours. The renal uptake was 1.02, 1.71 and 1.69 per

FIG. 8 *The Intestinal Absorption of Tritiated Folic Acid Three Hours After Administration of Its Various Amounts in the Jejunum*

—, % of dose; ---, absolute amount.

FIG. 9 *The Hepatic Uptake of Tritiated Folic Acid Three Hours After Administration of Its Various Amounts in the Jejunum*

—, % of dose; ---, absolute amount.

FIG. 10 *The Renal Uptake of Tritiated Folic Acid Three Hours After Administration of Its Various Amounts in the Jejunum*

—, % of dose; ---, absolute amount.

the jejunum in the following experiments.

The results of the FA absorption, and the hepatic and renal uptakes are shown in Table 6, and Figs. 8 to 10. When the labeled FA in the dose of 400 μg per kg of body weight was injected into the loop of the jejunum, its absorption was diminished to 47.1 per cent of the administered dose, while the absolute amount of absorbed FA increased to 188 μg per kg. When the dose was increased to 4,000 μg per kg of body weight, the FA absorption rate was extremely decreased and the mean value was 23.8 per cent, while the absolute amount of absorbed FA rose to 952 μg per kg of body weight. Thus, it was found that the absolute amounts of absorbed FA increased, as the injected dose increased. The hepatic and renal uptakes showed similar tendencies as the absorption. The hepatic uptake was 3.57 per cent at the dose of 400 μg per kg and 1.39 per cent at the dose of 4,000 μg per kg of body weight. But the absolute amounts of FA increased to 14.3 and 55.6 μg per kg, respectively. The renal uptake diminished to 1.31 and 0.46 per cent of the administered dose and the absolute amounts increased to 5.24 and 18.4 μg per kg, respectively.

5. ^3H -FA Absorption under Various Conditions

The results of these experimental observations are shown in Table 7 and Figs.

TABLE 7
Intestinal Absorption and Organ Uptake of ^3H -Folic Acid Under Various Conditions

Observations were made 3 hr after jejunal administration of 40 μg per kg.

	Amounts absorbed	Hepatic uptake	Renal uptake
		<i>per cent</i>	
Control	80.9 $\pm$ 9.1	7.95 $\pm$ 2.09	6.00 $\pm$ 1.26
Simultaneous administration of Amethopterin and FA.	90.0	6.31	7.63
	75.0	6.01	6.68
	86.1	5.80	5.49
	88.8	6.99	7.36
Mean	85.0	6.28	6.77
Saturation with Amethopterin	68.6	3.38	4.29
	63.8	4.49	6.32
	71.3	2.98	5.25
	82.1	7.96	4.73
Mean	71.5	4.83	5.15
Saturation with folic acid	84.0	4.50	3.17
	66.6	7.18	3.99
	91.3	7.61	6.35
	92.0	9.90	4.60
Mean	83.5	7.30	4.53
Simultaneous administration of dinitrophenol and FA	84.2	6.84	8.57
	92.6	6.01	8.07
	94.9	7.47	—
	70.9	9.28	5.42
Mean	85.7	7.40	7.35
Folic acid deficiency	79.5	12.2	6.04
	90.8	12.9	5.72
Mean	85.2	12.6	5.88

11 to 13. When ^3H -FA in the dose of $40\text{ }\mu\text{g}$ per kg of body weight was administered into the jejunal loop simultaneously with the same amount of Amethopterin, its absorption from the jejunum was 85.0 per cent, the hepatic uptake 6.28 per cent and the renal uptake 6.77 per cent. Comparing with the control value, FA absorption was not decreased by the presence of Amethopterin at the concentration tested.

In the rat pretreated with Amethopterin, FA absorption was 71.5 per cent. The intestine at the time of sacrifice was edematous and petechiae were observed on the mucosal surface. The hepatic uptake was diminished in some cases, while the renal uptake was normal in all cases.

After the rat was pretreated with FA, the absorption of ^3H -FA from the jejunal loop was not significantly changed. The liver uptake was 7.30 per cent and the renal one 4.53 per cent. The renal uptake was lower than that of the control animals.

FIG. 11 *The Intestinal Absorption of Tritiated Folic Acid under Various Conditions*

It was measured three hours after administration of $40\text{ }\mu\text{g}$ per kg of body weight in the jejunum.

FIG. 12 *The Hepatic Uptake of Tritiated Folic Acid under Various Conditions*

It was measured three hours after administration of $40\text{ }\mu\text{g}$ per kg of body weight in the jejunum.

FIG. 13 *The Renal Uptake of Tritiated Folic Acid under Various Conditions*

It was measured three hours after administration of $40 \mu\text{g}$ per kg of body weight in the jejunum.

Dinitrophenol was used as a metabolic inhibitor and 0.3 ml of 1 mM solution was injected into the loop with ^3H -FA in the dose of $40 \mu\text{g}$ per kg of body weight. In 3 hours 85.7 per cent of the administered radioactivity was absorbed from the jejunal loop. The hepatic and renal uptakes were normal. The absorption of FA was not disturbed under this circumstance.

In the FA deficient rat, the absorption of ^3H -FA was 85.2 per cent. In spite of the deficiency of FA, the absorption of the vitamin was not increased. On the other hand, the hepatic uptake rose to 12.6 per cent, while the renal uptake was normal.

DISCUSSION

In the previous report (9), it was observed that the majority of FA was absorbed rapidly from the gastrointestinal tract after an oral administration of FA in man. The purpose of the present paper is to study the site and mechanism of absorption of FA. ^3H -FA in a dose of $40 \mu\text{g}$ per kg of body weight, the same dose as used in clinical absorption studies, was injected into the stomach to observe the FA absorption in the rat under the similar conditions in man. The FA absorption of the rat showed the similar tendency to the absorption test in man; approximately 80 per cent was absorbed for the first 6 hours after intragastric administration. This fact suggests that FA is absorbed very rapidly as shown in the absorption study in man (9).

Although the rapidity of movement of fluid from the stomach through the intestine was not studied in this experiment, it is of interest that 40.5 per cent of the dose was absorbed in 30 minutes. During 30 minutes to 3 hours, 27.8 per cent of the dose was moved from the stomach through the intestine and approximately the same per cent was absorbed, while little radioactivity was found in the middle and distal portion of the small intestine and the colon. From these observations, it appears possible to conclude that the majority of FA given is absorbed mainly from the upper intestine and the lower intestine may not be concerned with FA absorption in the rat under these conditions.

Concerning the site of FA absorption, further observations were made using the loops of the jejunum and ileum. Isolated loops were made in the proximal jejunum and the distal ileum, each about 10 cm long. After the administrations of ^3H -FA into these loops, the absorption and the hepatic and renal uptakes were estimated. Approximately 90 per cent of the dose given into the jejunal loop was absorbed in 6 hours. This fact indicates that the jejunum has great ability to absorb FA, and almost all the FA in the dose of $40\text{ }\mu\text{g}$ per kg was absorbed from the jejunum rapidly. The rapid and complete absorption of FA is thought to be possibly due to passive transport system for FA. There might be another possibility, however, that an active transport system on FA absorption may exist, although its capacity is supposed to be considerably high.

FA absorption from the loop of the ileum was approximately 50 per cent of that from the jejunal loop. Burgen and Goldberg (7) observed similar result and the rate of absorption from the ileum was about 60 per cent of that in duodeno-jejunum. The difference between the absorption from the jejunum and that from the ileum could not be explained certainly. It was clearly demonstrated that the absorption of FA from the jejunum was almost twice as much as that from the ileum. These data suggest that FA is absorbed mainly in the upper portion of the gastrointestinal tract in the physiological states.

Dietary intake of FA in man is thought to be 1 to $2\text{ }\mu\text{g}$ per kg per day (11-14) and the usual dose of clinical FA absorption test is $40\text{ }\mu\text{g}$ per kg (5, 6, 10, 15). Further observations were made to detect the maximum limit of FA absorption in terms of an active transport system. According to the increases of the administered doses from 40 to $4,000\text{ }\mu\text{g}$ per kg of body weight (from 10^{-5} to $10^{-3}\text{ }M$ per kg), the rate of absorption was decreased from 80 to 24 per cent of the dose, while the absolute amount of absorbed FA rose from 32 to $952\text{ }\mu\text{g}$ per kg of body weight. Thus, the rate of absorption was decreased considerably after administration of $4,000\text{ }\mu\text{g}$ per kg, but the absolute amount of absorbed FA was increased almost linearly. If an active transport system for FA absorption exists, its maximum capacity must be considerably high.

Burgen and Goldberg (7) reported that FA absorption was not diminished, when FA was perfused into the duodenojejunal segment with Amethopterin. In the present study, FA absorption was not disturbed by simultaneous administration of Amethopterin. This fact suggests the evidence that dihydrofolic acid reductase may not be concerned with the transport of FA across the intestinal wall and the competition on the intestinal mucosa was not proved between FA and Amethopterin.

The absorption of FA was not disturbed significantly in rats pretreated with Amethopterin for successive three days. Under this condition, binding capacity of dihydrofolic acid reductase was thought to be saturated with Amethopterin. The hepatic uptake was lower than the control value indicating decreased binding capacity for FA. The result ascertained in such experimental condition also supports the idea that dihydrofolic acid reductase may not be concerned with FA absorption.

The absorption of ^3H -FA was not disturbed by FA saturation. The absorption test was carried out 24 hours after the last injection of non-radioactive FA. As the hepatic uptake was not decreased in this experiment, the interval of 24 hours was thought to be too long.

On the other hand, increased hepatic uptake was observed in the rat fed on a diet deficient in FA, while the absorption of FA was normal. Thus, an adaptive increase in transport capacity was not observed in spite of FA deficiency. Burgen and Goldberg (7) reported normal FA absorption in animals deficient in FA.

Dinitrophenol has been used as a metabolic inhibitor. If energy is necessary for the process of FA transport, the absorption must be decreased by simultaneous administration of dinitrophenol. In the present study, the absorption of FA was not decreased by the presence of dinitrophenol.

From the experimental observations of this series, it will be difficult to deny completely the possible existence of an active transport system for FA absorption, but if an active transport system exists, its maximum capacity will be considerably high. Burgen and Goldberg (7) observed that mucosal to serosal flux of FA was extremely higher than serosal to mucosal flux indicating an active transport system, but the percentage absorption against a raised blood FA level was not significantly different from that in control series. On the other hand, Turner *et al.* (16) reported that everted sacs of rat intestine transferred FA and other B-group vitamins from mucosal to serosal side and in the reverse direction at approximately equal rates.

It was clearly demonstrated in the present study that FA reductase has nothing to do with FA transport across the intestinal wall and the transport of FA in a dose of 40 μ g per kg was not disturbed by a metabolic inhibitor. With the concentration of FA used, the presence of an active transport system for FA absorption was not proved. These results, however, appear to give the evidences to support the passive transfer of FA across the intestinal wall, though it is not conclusive.

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