Inability of Thymine to Substitute for Folic Acid in the Rat

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Pteroyl glutamic acid or vitamin B_c (folic acid or liver *L. casei* factor) has been shown to be a growth factor for microorganisms and an essential nutritional factor in preventing and curing "folic acid" deficiency in the rat, chick, guinea pig, and monkey. This compound has also been shown to have therapeutic value in correcting the blood dyscrasias of Addisonian pernicious anemia and other macrocytic anemias.

Thymine (5-methyluracil) has also been shown by Stokes (*6*) to be a substitute for pteroyl glutamic acid in the nutrition of certain lactic acid bacteria when used in an amount 3,300 times the folic acid requirement. This microbiological work has been confirmed and extended to include other pyrimidines by Hitchings, Falco, and Sherwood (*1*) and by Krueger and Peterson (*2*). Even though the mechanism of this action of thymine was obscure and probably not identical with that of folic acid, its action in human anemias was tried on the basis of these microbiological data. Spies and his associates (*5*) have shown that large doses of thymine (4.5 grams or more daily) will cause remission from Addisonian pernicious anemia or other macrocytic anemias, thus indicating that in humans as well as in microorganisms thymine may be involved in folic acid nutrition in some obscure way.

The lack of any reports on the action of thymine in alleviating folic acid deficiency in experimental animals prompts us to report our inability to find it a useful substitute for folic acid in the prevention or cure of folic acid deficiency in the rat.

METHOD

The experimental procedures were essentially those used by Mallory, *et al.* (*3*) and Spicer, *et al.* (*4*). Twenty-eight-day-old rats of random sex having an average weight of 45–50 grams were fed ad libitum a purified diet containing 2 per cent succinyl sulfathiazole (sulfasuxidine). The basal diet consisted of 71 per cent sucrose, 18 per cent casein (Labco vitamin free), 3 per cent cottonseed oil, 2 per cent cod-liver oil, 4 per cent salts (Sure, 7), 2 per cent sulfasuxidine, 0.1 per cent choline chloride, 0.1 per cent inositol, and 200 γ per cent vitamin K (dissolved in the cod-liver oil). Daily supplements were made in a cup as follows: thiamine hydrochloride, 200 γ; riboflavin, 100 γ; pyridoxine hydrochloride, 100 γ; calcium d-pantothenate, 200 γ; nicotinamide, 500 γ; and biotin, 1 γ.

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The data of our experiments are summarized in Table 1. In Experiment 1, the supplements were given between the 28th and 42nd days, the first four weeks being the depletion period marked by definite growth plateau. In Experiments 2 and 3 the supplements were given from the first day. The indicated supplements were fed along with the crystalline B vitamins. Conventional hematological techniques were used in all blood analyses.

Only in Experiment 2 was a positive control employed, namely, 3 γ of crystalline folic acid[2] supplied daily to each rat in the group. The response in growth and in white blood cell and granulocyte counts in this experiment is typical of our experience with folic acid, supplied either as crystalline product or as yeast or liver concentrates. In curative experi-

TABLE 1

EFFECT OF THYMINE SUPPLEMENTATION ON "FOLIC ACID" DEFICIENCY IN RATS FED PURIFIED DIET PLUS SUCCINYL SULFATHIAZOLE

Supplement	Thymine daily (mg.)	Folic acid equivalent (γ)	Experimental day	Gain in weight (grams)	R. B. C. (cells/mm.^{3} × 10^{-6})	Hb (grams/100 ml. blood)	HMCT volume (%)	W. B. C. (cells/mm.^{3} × 10^{-3})	Granulocytes (cells/mm.^{3} × 10^{-3})	Survival
Exp. 1										
Negative.....	—	—	28	46.3	—	—	—	—	—	—
			42	40.7	—	—	—	4.9	0.074	8/9
Thymine	15	4.2	28	34.0	—	—	—	—	—	—
			42	35.5	—	—	—	6.52	0.163	2/5
Thymine	7.5	2.1	28	35.8	—	—	—	—	—	—
			42	22.4	—	—	—	3.80	0.057	4/5
Exp. 2										
Negative	—	—	36	46	6.42	12.3	36.8	5.63	0.113	10/10
Thymine	15	4.2	36	43	7.19	11.5	36.2	6.57	0.131	9/10
Thymine	25	7.0	36	45	6.22	11.4	35.3	7.71	0.231	9/10
Pteroyl glutamic acid	—	3.0	36	92	7.97	13.9	42.1	13.75	1.650	10/10
Exp. 3										
Negative	—	—	35	40.7	7.40	14.1	40.4	5.50	0.055	10/10
Thymine	12	3.5	35	34.6	7.59	14.8	43.8	4.51	0.018	10/10
Thymine + PABA (2 mg.)	12	3.5	35	53.5	7.65	13.9	40.7	5.80	0.087	10/10
PABA (2 mg.)	—	—	35	47.0	7.40	13.5	41.8	4.26	0.043	7/10

[2] Supplied by E. L. R. Stokstad, of Lederle Laboratories, Inc.

ments similar to Experiment 1, we have found that 3 γ of folic acid given daily between the 35th and 49th days will allow a change in growth from about 93 grams to 140 grams, an increase in white cell count from about 4,000 to 10,000, and a change in the granulocyte count from about 300 to 1,000.

P-aminobenzoic acid has been previously tried for its effect on the syndrome produced with sulfasuxidine in rats fed a purified diet, but with little success. However, it was included in Experiment 3 to determine whether it might allow some subtle effect of thymine to be shown. This was not the case under our conditions.

Results and Discussion

Our data indicate that thymine has no curative or preventive effect on the course of folic acid deficiency in the rat with respect to either growth or any part of the blood picture, when it is fed daily in amounts equivalent to 2.1, 3.5, 4.2, and 7.2 γ of folic acid as determined microbiologically in our laboratory.[3]

In view of the striking observations reported by Spies, *et al.* in human patients (*5*), these results on rats are interesting. They point to the fact that thymine is not a true substitute for folic acid and indicate that when folic acid-like activity is shown by thymine, pyrimidines, or other preparations, some indirect mode of action must be operating. Such a viewpoint is in harmony with the fact that thymine is chemically very unlike folic acid.

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Experimental Diabetes and Diet

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The influence of different diets on sensitiveness to alloxan and experimental pancreatic diabetes in the white rat has been investigated.

Two hundred twenty-three female rats weighing between 90 and 120 grams were divided into several groups and fed different diets for a period of one month. At the end of the month and 12 hours after the last meal they were injected intraperitoneally with 160 mg./kg. of alloxan. The mortality rate was considered during one week after the injection, and in all cases high hyperglycemia preceded death.

[3] The thymine used in this work was chemically pure and found to have 280 γ of folic acid activity/gram.

The results were as follows (see Table 1):

(1) In the group given a high carbohydrate diet the mortality due to alloxan was 40 per cent.

(2) In the group given a low protein diet (10 per cent casein content) there was 90 per cent mortality. Increasing the protein content from 20 to 45 per cent increased the resistance to alloxan (40 and 33 per cent mortality).

TABLE 1

Diets	Wheat flour (%)	Corn flour (%)	Casein (%)	Fat (%)	Yeast (%)	Cod liver oil (%)	Salt mixture (McCollum Davis) (%)	No. of rats used	Mortality (%)
(a) High carbohydrate	35	34	20	0	5	5	1	15	40
(b) High protein	10	34	45	0	5	5	1	15	33
(c) Low protein	40	39	10	0	5	5	1	15	90
(d) High lard	10	25	20	34	5	5	1	20	100
(e) High lard and high protein	14	15	30	30	5	5	1	15	100
(f) High ox fat	10	25	20	34	5	5	1	14	86
(g) High lard, and methionine (200 mg./kg./day)	10	25	20	34	5	5	1	10	30
(h) High lard, and sulfanilamide (250 mg./kg./day)	10	25	20	34	5	5	1	10	90
(i) High lard, and choline (300 mg /kg./day)	10	25	20	34	5	5	1	15	100
(j) High lard, and thiouracil (200 mg./kg./day)	10	25	20	34	5	5	1	10	10
(k) High olive oil	10	25	20	34	5	5	1	15	40
(l) High butter	10	25	20	34	5	5	1	15	33
(m) High oleomargarine	10	25	20	34	5	5	1	15	21
(n) High corn oil	10	25	20	34	5	5	1	15	13
(o) High coconut oil	10	25	20	34	5	5	1	14	0
(p) High lard (30%) and coconut oil (10%)	9	20	20	40	5	5	1	10	20

(3) High lard and high ox fat diets decreased strongly the resistance to alloxan (100 and 86 per cent mortality, respectively). This effect was not modified in the former either by increasing the casein content from 20 to 30 per cent (100 per cent mortality) or by treatment with choline or sulfanilamide. In contrast, the treatment with methionine or thiouracil corrected the hypersensitiveness (30 and 10 per cent mortality, respectively).

(4) The sensitiveness to alloxan was not modified when high olive oil or high butter diets were administered (40 and 33 per cent mortality, respectively), but there was an increased resistance to alloxan in the rats fed either a high oleomargarine diet (21 per cent mortality) or in those fed a high corn oil diet (13 per cent mortality).

(5) Complete protection to alloxan in rats fed a high coconut oil diet (0 per cent mortality) was observed.

(6) In rats fed a high lard diet modified by the addition of 10 per cent coconut oil, a partial protection to alloxan was observed (20 per cent mortality).

The role of the quality of diets and the amount of food fed on the initiation and evolution of experimental pancreatic diabetes in rats has also been studied.

Fifty-one male rats weighing about 130 grams were subtotally pancreatectomized (95 per cent of the pancreas) and