FOLIC ACID DEFICIENCY AND URINARY FORMIMINOGLUTAMIC ACID EXCRETION IN THE RAT

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(*Accepted for publication 7th August, 1961.*)

SUMMARY.

Rats fed on a folic acid-deficient diet regularly excrete formiminoglutamic acid in the urine. The vitamin B_{12} and folic acid status of such rats was estimated by assay of liver tissue for these vitamins. Formiminoglutamic acid excretion occurs in the presence of normal liver vitamin B_{12} concentrations. There appears to be a critical level of liver folic acid below which there is excretion of formiminoglutamic acid.

INTRODUCTION.

Formiminoglutamic acid is an intermediate product in the breakdown of histidine and its further metabolism requires tetrahydrofolic acid (Tabor and Wyngarden, 1958). It appears in the urine of rats made folic acid deficient (Rabinowitz and Tabor, 1958). The present investigation was designed to assess any correlation between the degree of folic acid deficiency and the quantitative excretion of formiminoglutamic acid in the rat.

METHODS.

Three groups of 10 Wistar rats were fed on controlled diets from the time of weaning. Group 1 was given a standard cubed diet (see appendix) which is considered to be nutritionally adequate for normal growth and development. Group 2 was fed a synthetic diet deficient in folic acid (see Appendix) but supplemented with folic acid (20 mg. weekly) added to the drinking water. Group 3 received the folic acid-deficient diet without the folic acid supplement. Animals in all three groups received vitamin B_{12} (2 μg. weekly) added to the drinking water. When aged 9 months each rat was held in a metabolic cage for 48 hours and the urine collected. The rats were then killed and the livers assayed for vitamin B_{12} and folic acid.

All specimens of urine were adjusted to pH 4·0 and stored at 4° C. until required. Urinary formiminoglutamic acid was assayed quantitatively by a microbiological method using *L. arabinosus* as test organism (Davis and Onesti, 1960). Liver folic acid was estimated after papain digestion (Pitney and Onesti, 1961) using a mutant strain of *Streptococcus lactis R* (ATCC 8043). Liver vitamin B_{12} was assayed with the Z strain of *Euglena gracilis* (Hutner, Bach and Ross, 1956).

Austral. J. exp. Biol. (1961), 39, pp. 397-402.

RESULTS.

Two rats in Group 3 died during the course of the experiment and have been excluded from the results. Both died from bacterial infection. Table 1 shows the results obtained from the 10 rats in Group 1 fed a normal diet with vitamin B_{12} supplement. The weight of these rats at 9 months ranged from 116 gm. to 270 gm. (mean 206 gm.). The total vitamin B_{12} in the livers ranged from 0·38 μg. to 0·73 μg. (mean 0·56 μg.) and total folic acid from 7·24 μg. to 36·01 μg. (mean 17·53 μg.). Values are expressed as total amounts in the organ rather than as concentrations to obviate the influence of varying liver weights. One animal in the control group excreted a trace of formiminoglutamic acid in the urine. This was insufficient for quantitative assessment.

Table 2 shows the results in the 10 rats in Group 2 (folic acid-deficient diet with folic acid and vitamin B_{12} supplements). Total body weights ranged from 114 gm. to 203 gm. (mean 150 gm.). Total liver B_{12} ranged from 0·37 μg. to 0·84 μg. (mean 0·66 μg.) and total folic acid from 6·70 μg. to 27·77 μg. (mean 14·05 μg.). No animal excreted formiminoglutamic acid in the urine.

Table 3 shows the results in the eight surviving Group 3 rats (folic acid-deficient diet with vitamin B_{12} supplement). Total body weights ranged from 126 gm. to 212 gm. (mean 164 gm.). Total liver B_{12} ranged from 0·61 μg. to 1·07 μg. (mean 0·77 μg.) and total folic acid from 2·08 μg. to 4·50 μg. (mean 3·20 μg.). Seven rats excreted measurable amounts of formiminoglutamic acid in the urine. These values are given as concentrations rather than as 48-hour total excretions as it was not found possible to collect all urine passed by these animals.

TABLE 1.

Rats fed a normal diet with vitamin B_{12} supplement (Group 1).

Key	Body weight (gm.)	Liver weight (gm.)	Total liver B_{12} (μg.)	Total liver folic acid (μg.)	FGA* excretion
A1	116	4·17	0·38	21·14	0
A2	258	8·15	0·57	12·39	0
A3	147	5·24	0·73	7·65	0
A4	211	5·94	0·53	7·24	0
A5	154	4·33	0·61	17·15	0
A6	270	8·13	0·65	20·49	0
A7	232	8·53	0·60	13·90	trace
A8	246	7·94	0·48	10·88	0
A9	191	5·99	0·48	28·45	0
A10	240	5·78	0·52	36·01	0

* Formiminoglutamic acid.

TABLE 2.

Rats fed a folic acid-deficient diet with folic acid and vitamin B_{12} supplement (Group 2).

Key	Body weight (gm.)	Liver weight (gm.)	Total liver B_{12} (μg.)	Total liver folic acid (μg.)	FGA excretion
B1	134	5·36	0·75	6·70	0
B2	148	6·58	0·66	8·69	0
B3	114	4·54	0·68	12·67	0
B4	124	4·88	0·68	11·18	0
B5	150	6·63	0·66	11·54	0
B6	193	8·02	0·56	10·83	0
B7	134	4·48	0·72	19·58	0
B8	176	5·25	0·84	27·77	0
B9	128	5·30	0·37	13·30	0
B10	203	8·55	0·68	18·21	0

TABLE 3·

Rats fed a folic acid-deficient diet with vitamin B_{12} supplement (Group 3).

Key	Body weight (gm.)	Liver weight (gm.)	Total liver B_{12} (μg.)	Total liver folic acid (μg.)	FGA excretion (μg./ml.)
C1	126	7·65	1·07	3·44	110
C2	162	6·61	0·66	2·25	250
C3	212	7·19	0·86	4·10	0
C4	140	6·11	0·79	2·08	160
C5	210	8·29	0·75	4·50	75
C6	176	6·94	0·63	3·50	300
C7	138	5·44	0·76	2·90	310
C8	148	6·11	0·61	2·90	98

DISCUSSION.

These results confirm previous observations that folic acid-deficient rats regularly excrete formiminoglutamic acid in the urine. In the present experiment folic acid deficiency was achieved without the administration of succinylsulphathiazole with the folic acid-deficient diet. Sulphonamide administration, by altering the bacterial population in the gut, may produce a more profound degree of folic acid deficiency but such animals tend to develop diarrhoea and mortality is high. Sulphonamide administration is undesirable in experiments involving microbiological assays of tissues and urine since growth inhibition of the test organism may be produced unless p-aminobenzoic acid is added to culture media.

The mean body weights of Group 2 and Group 3 rats were similar and were less than that of Group 1 rats. This observation suggests that the folic acid-deficient diet was not entirely adequate in other respects since Group 2 received folic acid supplementation. It suggests, also, that growth failure on a folic acid-deficient diet may be due to deficiencies other than folic acid.

Total liver B_{12} content was similar in all groups of animals and the values in each group fell into a rather narrow range. This observation suggests that folic acid deficiency does not influence the liver B_{12} content provided that B_{12} intake is adequate.

Liver folic acid values were scattered over a wide range in Group 1 and Group 2 animals. Mean values were comparable, however (17·53 μg. and 14·05 μg. respectively). Both values are considerably higher than the mean value of 3·20 μg. obtained with Group 3 (folic acid-deficient) animals. The presence of demonstrable liver folic acid in Group 3 suggests that formiminoglutamic acid excretion occurs before there is complete depletion of folic acid in the liver. An alternative explanation is that some material assayed as folic acid may not have biological importance in the further metabolism of formiminoglutamic acid.

The results show a correlation between liver folic acid content and the appearance of urinary formiminoglutamic acid. In Groups 1 and 2, the lowest liver folic acid values were 7·24 μg. and 6·70 μg. respectively. These animals did not excrete formiminoglutamic acid. The highest liver folic acid value in Group 3 was 4·50 μg. and seven out of eight rats in this group excreted formiminoglutamic acid. Furthermore, the two highest readings in this group were associated with the least amounts of formiminoglutamic acid (rats C3 and C5).

Acknowledgments. The author wishes to thank Dr. W. R. Pitney for advice and criticism, Dr. Barbara J. Arnold for help with the folic acid-deficient diets, and Mr. A. Kelly for the vitamin B_{12} and folic acid assays.

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APPENDIX.

Formula of standard cubed diet (obtainable from Wesfarmers Pty. Ltd., Perth, W.A.):

Ground wheat	50 p.c.	Brewers' yeast	5 p.c.
Barley meal	12½ p.c.	Dried skim milk	5 p.c.
Lucerne meal	5 p.c.	Oyster flour	½ p.c.
Whale meal	16 p.c.	Molasses	3 p.c.
Bone meal	2 p.c.	Sodium chloride	½ p.c.

Riboflavin ...	3 mg. per Kg.
Vitamin A ...	8,000 I.U. per Kg.
Vitamin D ...	1,000 I.U. per Kg.

Formula of folic acid-deficient diet:

Sucrose	710	gm.
Lactic casein	180	gm.
Peanut oil	50	gm.
Ferric citrate $5H_2O$ ...	10	gm.
Choline chloride	2	gm.
Copper sulphate	0·4	gm.
ABDEC (Parke Davis) ...	10	ml.
Biotin	0·01	mg.
Vitamin K	5	mg.
Salt mixture	40	gm.

Salt mixture (all anhydrous salts):

Calcium carbonate ...	134·8	gm.
Magnesium carbonate ...	24·2	gm.
Sodium carbonate	34·2	gm.
Potassium carbonate ...	141·3	gm.
Phosphoric acid	103·2	gm.
Hydrochloric acid	53·4	gm.
Sulphuric acid	9·2	gm.
Citric acid	111·1	gm.
Potassium iodide	0·020	gm.
Manganese sulphate ...	0·079	gm.
Sodium fluoride	0·248	gm.
Potassium aluminium sulphate	0·245	gm.

Mix acids and add carbonates. Trace elements are added as solutions of known concentrations. Evaporate to dryness at 90°-100° C. and grind to fine powder.