weight during 10 days following hypophysectomy in the pigeon, and also cytological evidence of stimulation in their cortical cells, are found to agree satisfactorily with Moon's method of assay of adrenocorticotropin in 21-day rats. As tested by these 3 methods the ability of pituitary extracts to stimulate cortical tissue is independent of their prolactin, FSH and thyrotropin potencies. Gamone stimulates both cortical and medullary tissue in both normal and hypophysectomized pigeons. Estrone stimulates cortical tissue at least in intact birds. Thyroxine, plus vitamin supplements, gave evidence of ability to stimulate cortical tissue in hypophysectomized pigeons.

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Metabolic Interdependence of Vitamin B_1 and Manganese. Reciprocal Neutralization of Their Toxic Effects.*

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We observed that rats fed our standard adequate, varied diet, supplemented with 100 gamma of vitamin B_1 daily, either in the form of yeast, or as synthetic vitamin B_1 (parenterally administered), demonstrated after one generation interference with lactation, loss of the maternal instinct, cannibalism and progressive loss of fertility.[1] With reduction in the amount of vitamin B_1 to 40 gamma or the elimination of the supplements of vitamin B_1 for short periods, normal lactation and normal interest in the young was restored. When the vitamin B_1 content was again increased the same toxic effects were observed.† Further study completely confirmed our earlier findings. With daily supplements of 60 gamma of vitamin B_1, progressive decrease in fertility also occurred, with a moderate incidence of loss of litters due to cannibalism. After four generations breeding decreased.

* A preliminary note of this work appeared in *Science*, 1939, **89**, 2302. Read before the American Society of Experimental Pathology, April, 1939, at Toronto.

1 Perla, D., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 169.

† Interference with lactation and reproduction could not have been due to the absence of vitamins L_1 and L_2 recently postulated by Nakahara Inukai and Ugami (*Science*, 1938, **87**, 372) since yeast is a rich source of these factors.

The data of these earlier preliminary experiments are given in detail in Table I.

Our normal diet consists of the following: 15 g per rat per day of a basic mixture of hominy 100 parts, rolled oats, 25 parts, fine meat and bone 25 parts, salt 1 1-2 parts and dried skimmed milk 16 parts, to which are added a few drops of cod liver oil, 0.3 mg wheat germ and 0.3 g of crude Fleischmann's brewer's yeast per rat. The yeast product contained 6 I.U. of vitamin B_1 per gram. This is equivalent to about 2 to 3 I.U. (7 gamma) per rat per day. In addition the animals received fresh greens twice a week and fresh whole milk daily. On this complete diet it has been our experience that the rats maintain a good growth curve, reproduce well and rear their young without loss of any members of their litters.

In view of the fact that Williams[2] stated that as much as from 160 to 1000 gamma of vitamin B_1 daily could be given without any toxic effects when rats were fed a Sherman breeding diet (one-third whole milk and two-thirds whole wheat), it seemed probable to us that interference with some other factor in the diet might have induced the manifestations observed in our experiments.

It is known that deficiency of manganese in the diet presents similarly toxic effects on the maternal instinct and reproduction.[3] It was reasoned that perhaps manganese is essential as an oxidative catalyst in the utilization of vitamin B_1 in the tissues. If this were so the available manganese in the tissues might be exhausted by an excess of vitamin B_1 and manifestations would occur analogous to those observed in manganese deficiency. Furthermore, it was observed by Hamamoto[4] that large amounts of both vitamin B_1 and manganese are found together in nature in such sources as wheat products and the like.‡

To test our hypothesis we added small amounts of manganese (2 mg per rat per day as $MnCl_2$) to the diet. Rats which had shown loss of maternal instinct and cannibalism now bred and raised normal litters (Table I). The studies were then extended. Rats were raised on the normal diet and given parenterally 400 gamma of vitamin B_1 daily. Others were given the same diet and vitamin B_1 but the diet was supplemented with 2 mg of manganese as $MnCl_2$ per day per rat. The results completely confirmed our hypothesis. In those receiving the vitamin B_1 alone, cannibalism

2 Williams, R. R., and Spies, T. D., *Vitamin B_1 and Its Use in Medicine*, Macmillan Co., New York City, 1938, p. 286.

3 Orent, E. R., and McCollum, E. V., *J. Biol. Chem.*, 1931, **92**, 651.

4 Hamamoto, E., *Orient. J. Dis. Infants*, 1935, **18**, 21, 57.

‡ He observed a decrease in manganese in the tissues of beri-beri birds.

TABLE I.

Toxic Effects of Excess of Vitamin B_1 on Maternal Instinct and Reproduction.

Group	Yeast Excess									Betaxin Excess							Controls				
Quantity of B_1 (gamma)	100	100	50	50	0	100	100 + MN	50	50	100	100	40	0	100 + MN	50	50	Normal Diet Alone (7 gamma B_1)				
Generation	P	6w F_1	F_1	6w F_2	6w F_2	F_2	F_2	F_3	F_4	P	F_1	6w F_2	6w F_2	F_2	F_3	F_4	P	F_1	F_2	F_3	F_4
No. females breeding	8	7		13			6	6	4	6	6	6			6	6	9	8	8	8	8
No. litters	7	6	3	3	5	2	3	1	2	7	3	2	6	3	4	3	17	16	18	15	19
No. offspring	54	32	21	32	48	12	19	8	15	39	21	12	43	20	20	29	139	128	168	118	150
No. litters abandoned or eaten	2	6	0	3	1	8	0	0	2	4	2	2	4	0	6	0	0	0	0	0	0
No. offspring abandoned or eaten	16	30	0	30	10	2	0	0	12	10	13	12	21	0	2	0	0	0	0	0	0

TABLE II.

The Preventive Action of Supplements of Manganese to the Diet on the Effects of an Excess of Vitamin B_1 in Rats Fed Complete Diets.*

Group	Betaxin (400 gamma daily)				Betaxin (400 gamma) + MN (2 mg daily)				MN (2 mg daily)			Controls			
Generation	P	F_1	F_2	Total	P	F_1	F_2	Total	P	F_1	Total	P	F_1	F_2	Total
Total No. females breeding	6	8	8	22	6	8	8	22	6	8	14	6	8	8	22
Total No. litters	13	10	13	36	13	14	18	45	7	10	17	15	21	18	54
Total No. offspring	74	74	108	256	89	94	90	273	43	74	117	105	140	127	372
No. litters neglected or eaten	7	7	12	26	0.3	0.2	0.1	0.6	0.3	7	7.3	0	0	0	0
	54%	70%	92%	72%	2%	1%	.8%	1.3%	4%	70%	43%				
Total No. offspring neglected or eaten	34	33	76	143	3	2	1	6	2	33	35	0	0	0	0
	46%	44%	70%	56%	3%	2%	1%	2%	4%	44%	30%				

* The vitamin B_1 was given parenterally from the age of 3 weeks in amounts of 400 gamma per day but the young in each generation were not disturbed for 2 to 3 weeks. Cannibalism and deaths from neglect occurred in more than one litter in any given female in 11 instances. It frequently occurred in the 2d or 3d litter and not in the first. In only 2 instances did it occur only in a first litter. In all cases the loss of young occurred in the first 4 or 5 days.

and interference with lactation occurred in a high percentage in the P, F_1 and F_2 generation in successive litters. In those receiving supplements of manganese in the diet, none of these toxic symptoms was apparent. Normal lactation and the normal maternal instinct were preserved (Table II).

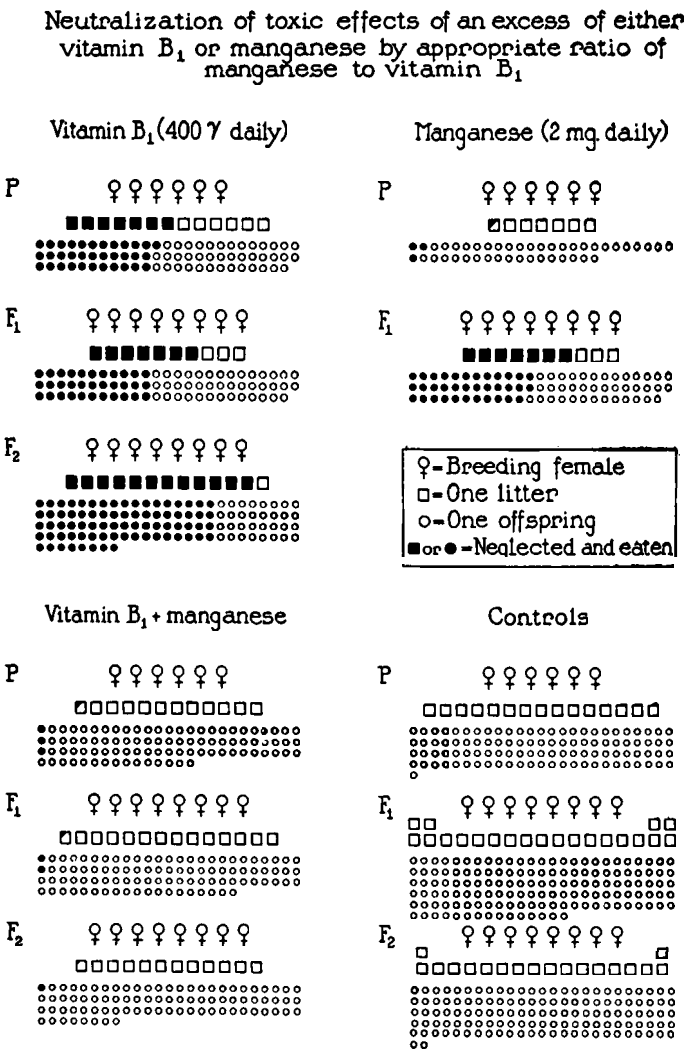

FIG. 1.

Breeding chart illustrating the neutralization of the toxic effects of an excess of either vitamin B_1 or manganese by appropriate ratio of manganese to vitamin B_1.

It is extremely significant that the animals receiving the normal diet to which was added a supplement of manganese alone in amounts of 2 mg a day, likewise showed a disturbance in lactation and in maternal instinct, which was slight in the first generation (P), but pronounced in the second generation. In spite of this fact, the rats receiving both excess of vitamin B_1 and excess of manganese (each in themselves capable of inducing these toxic effects) in practically every instance reared their young normally.

In work now in progress it has been found that manganese in amounts of about ½ mg a day per rat is more effective in neutralizing the toxic effects of an excess of vitamin B_1 (400 gamma), and in itself is less toxic than 2 mg.

These results demonstrate that manganese is essential in the utilization of vitamin B_1 in the tissues and is intimately bound up with the rôle of vitamin B_1 in the physiology of the organism. It also suggests that variations in certain constituents of the diet, such as manganese may greatly affect the vitamin B_1 requirement. With the use of large amounts of vitamin B_1 in therapy, an adequate supply of manganese must also be made available.

Our experiments further suggest that in the presence of an excess of manganese, a greater quantity of vitamin B_1 is essential. Perhaps the vitamin B_1 in the diet is rapidly exhausted under these conditions and insufficient quantities are available for normal lactation. In any case, apparently, the toxic manifestations observed with an excess of vitamin B_1 are the expression of an exhaustion of available manganese stores in the body, and the symptoms are those of insufficiency of manganese.

Summary and Conclusions. In rats fed normal adequate diets an excess of vitamin B_1 in amounts exceeding 30 or 40 times the minimal requirement results in an interference with the capacity of the mother to rear her young and with the nursing instinct. With an excess of 400 gamma this manifestation was pronounced in the parent generation but became progressively worse in the F_1 and F_2 generation. The young were neglected and eaten in over 90% of the litters in the F_2 generation.

The toxic manifestations of an excess of vitamin B_1 were found to be dependent on the ratio of manganese to vitamin B_1 in the diet. The addition of manganese to the diet in amounts of 2 mg per rat per day completely neutralized the unfavorable effects of the excess of vitamin B_1 (400 gamma daily). Practically no interference with lactation or rearing of the young was observed in animals which received both the excess of vitamin B_1 and manganese as ad-

dition to the normal diet during 3 successive generations. Apparently manganese in amounts of 1/2 mg is even more effective.

Supplements of manganese alone in amounts of 2 mg a day result in interference with lactation and cannibalism, particularly marked after one generation.

It is inferred that manganese acts as an essential catalyst in oxidative processes in which vitamin B_1 is concerned. The vitamin B_1 requirement of an animal varies with the manganese content in its diet.

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Note on the Action of X-rays on Goldfish (*Carassius auratus*).

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The following experiments were undertaken in order to study the suitability of goldfish for the problems of experimental radiotherapy. Goldfish have already been used successfully in experimental pharmacology.

A total of 408 commercial goldfish (96 of which were used as controls) were divided into series of 12 for each exposure. After irradiation each series or controls were placed in an aquarium containing 7000 cc of water at a temperature of 19° to 22°C for observation.

The radiation factors were: 200 KV, 30 MA, no filter, HVL 6 mm Al, target distance 50 cm, field 15 x 15 cm, intensity 230 r/min. The fishes were irradiated in an open Petri dish, 15 cm in diameter, suspended between 2 layers of gauze, flush with the surface of the water in a water phantom. This was 32 x 32 x 32 cm, thus permitting the maximum back scatter (Quimby and coworkers[1]). Ionization measurements with a Victoreen chamber showed an increase in back scatter from the Petri dish of 3%.

The entire dose was given in one session and varied from 500 to 10,000 r without back scatter. When a dose of 1500 r up to 10,000 r was administered, all goldfish died 10 to 18, on an average 14 days after treatment, while nearly 100% of the controls remained alive

[1] Quimby, E. H., Marinelli, L. D., and Farrow, J. H., *Am. J. Roentgenol.*, 1938, **39**, 799.