in vitamin B_1-deficiency.[11] In our rats the rise in blood pyruvic acid from the initial value of 3.74 mg of pyruvic acid per 100 cc of blood to a peak of 7.28 mg per 100 cc of blood was also observed a week earlier in animals who received additions of Mn to a vitamin B_1-deficient diet.

On a vitamin B_1-deficient synthetic diet the experimental rats stored 18% of the Mn intake, even though this was very low (60 gamma). The controls, which received the same diet but were supplied with crystalline vitamin B_1, stored 19% of the Mn intake. On a Mn intake of 100 gamma daily, contained in a normal diet fed before the start of the experiment, the balance was zero. McHargue[12] suggests that the biological potency of Mn contained in natural foodstuffs differs from that in a synthetic diet. The variation in the ability of our rats to store the Mn of different types of diet may be explained on this basis.

When 1 mg of Mn was added to the diet the vitamin B_1-deficient group stored 72% of the Mn intake while the controls retained only 25%. During the next period, when 400 γ of vitamin B_1 were administered, Mn retention dropped to only 34% of the intake, in spite of the continued high Mn content of the diet. When Mn addition was stopped, the balance became negative, since the excess of Mn which had been stored during the preceding periods was still excreted 4 weeks afterwards, when the experiment was terminated.

10908

Interdependence of Vitamin B_1 and Manganese. III. Manganese, Copper and Iron Metabolism in Normal Rats.

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The studies presented in this communication form part of an extended investigation of the metabolic interdependence of manganese and vitamin B_1.[1, 2, 3]

Sixteen male and female rats (Wistar strain) were used. Care of

11 Thompson, R. H. S., and Johnson, R. E., *Biochem. J.*, 1935, **29**, 694.

12 McHargue, J. S., *Am. J. Physiol.*, 1926, **77**, 245.

1 Perla, D., *Science*, 1939, **89**, 132.

2 Perla, D., and Sandberg, M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 522.

3 Sandberg, M., Perla, D., and Holly, O. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 368.

the animals, precautions to avoid unknown Cu intake and methods used in the chemical determinations of Mn, Cu and Fe were the same as those referred to in a previous publication.[3] Feces and food were analyzed for Mn, Cu, and Fe; urine for Mn only.[4] The animals received our stock diet, supplemented with 10% of whole milk powder and 3% of dry brewers' yeast. The Mn content of this diet was 7 γ per g. The rats were studied for a control period of 5 weeks. Then followed 4 periods of one month each, when they received (1) small additions of Mn, (2) large additions of Mn, (3) large additions of Mn plus vitamin B_1, and (4) vitamin B_1. Details about Mn and vitamin B_1 administration are given in the preceding paper.[3] *

Since changes in Fe metabolism were not significant, protocols for Mn and Cu metabolism only are presented.

During the control period when the rats were given a normal diet, supplying about 100 γ of Mn daily, they did not store any Mn. When, however, the Mn content of the food was raised about 50%, 25% of the intake was retained. The addition of 1 mg of Mn per g of food, though raising the intake to over 13 mg of Mn, did not change the percentage of retention. If, however, 400 γ of vitamin B_1 daily were given, the large amount of Mn offered in the diet was utilized to a greater extent. We have found[3] that in vitamin B_1 deficient rats administration of vitamin B_1 caused a drop in the retention of Mn to about half the amount retained during development of the avitamino-

TABLE I.
Manganese Metabolism (Daily Average per Rat).

Date	Urine, mg	Feces, mg	Total Excretion, mg	Intake, mg	Retention, mg	Retention in % of intake
Control period Mar. 8-Apr. 11	0.002	0.099	0.101	0.096	—0.005	0
+ 5 γ Mn/g of food Apr. 12-May 9	0.002	0.122	0.124	0.165	0.041	25
+ 1 mg Mn/g of food May 10-June 6	0.005	10.061	10.066	13.487	3.421	25
+ 400 γ of vitamin B_1 + 1 mg Mn/g of food June 7-13	0.005	8.049	8.054	13.640	5.586	41
+ 400 γ of vitamin B_1 + 1 mg Mn/g of food June 14-July 4	0.005	6.837	6.842	14.819	7.977	54
+ 400 γ of vitamin B_1 July 5-11	0.003	1.791	1.794	0.088	—1.706	—
+ 400 γ of vitamin B_1 July 12-25	0.002	0.113	0.115	0.102	—0.013	0

[4] Richards, M. B., *Analyst*, 1930, **55**, 554.

* We are indebted to the Medical Research Department of the Winthrop Chemical Company for a generous supply of crystalline vitamin B_1.

sis. Normal rats on a normal diet are enabled to store a greater part of an unusually high Mn intake if large amounts of vitamin B_1 are offered at the same time. Apparently this does not represent a mere retention of inert material as in vitamin B_1-deficient animals, where it is excreted again when administration of vitamin B_1 permits a normal functioning of the organism. In normal animals only a small part of the accumulated Mn was excreted during the first week when Mn addition was stopped while administration of vitamin B_1 was continued. In the following weeks when Mn intake amounted again to about 100 γ, continued vitamin B_1 administration did not influence the retention of Mn which was practically zero, just as in the control period.

Since Cu is recognized as an oxidative catalyst it was considered possible that a change in Mn metabolism might show an influence on Cu metabolism.

During the control period when the Mn balance was zero, 30% of the ingested Cu was retained. When Mn retention had been raised to 25% of the intake by addition of Mn to the ration, Cu retention dropped to as low as 15% of the intake. This suggests the possibility that if large amounts of Mn are available, less Cu may be required for oxidative processes in which one or the other of the trace metals plays a part. The administration of 400γ of vitamin B_1, while raising Mn storage to 41% of the intake, did not influence the low level of Cu retention during the first week. After that Cu retention rose to 21% of the intake. Since Mn retention amounted to 54% of the intake during this period it may be that large amounts of Mn and vitamin B_1, if offered simultaneously, may permit a better utilization of Cu as well as of Mn. When Mn addition was stopped while vitamin B_1 administration was continued, Mn retention became negative and Cu retention dropped again to 15% of the intake for one

TABLE II
Copper Metabolism (Daily Average per Rat)

Date	Feces, γ	Intake, γ	Retention, γ	Retention in % of intake
Control period Mar. 8-Apr. 11	52	74	22	30
+ 5 γ Mn/g of food Apr. 12-May 9	59	73	14	18
+ 1 mg Mn/g of food May 10-June 6	66	77	11	15
+ 400 γ of vitamin B_1 + 1 mg Mn/g of food June 7-13	64	76	12	16
+ 400 γ of vitamin B_1 + 1 mg Mn/g of food June 14-July 4	65	83	18	21
+ 400 γ of vitamin B_1 July 5-11	63	74	11	15
+ 400 γ of vitamin B_1 July 12-25	62	86	24	28

week. During the following weeks both Mn and Cu retention returned to normal proportions. Continued administration of vitamin B_1 failed to influence Cu metabolism as long as the Mn intake remained at the normal level of 100 γ. The Cu intake of about 70 to 80 γ daily remained unchanged throughout the experiment.

10909

Relation Between Latent Period and Growth Rate in Chemically Induced Tumors.

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It has been customary to rate the potency of carcinogenic action according to the shortness of the latent period or the percentage of animals developing tumors (Boyland and Warren,[1] Cook, *et al.*[2]). It is of interest to know whether malignancy of the tumors produced is correlated with the potency of carcinogenic action as measured by the latent period. Of the various criteria of malignancy, it has seemed to us most appropriate to investigate growth rate as measured directly *in vivo*, or indirectly by means of mitosis counts.

The mice in these experiments, representing various inbred strains, were injected in the subcutaneous tissues of the right axilla with carcinogenic agents dissolved in lard or in cholesterol pellets. The mice were examined biweekly and the latent period was reckoned from the time of injection to the time when tumors were first noted by palpation. Tumors were then measured biweekly in 2 dimensions by a single observer, who recorded the diameters to the nearest millimeter, using calipers with approximately constant pressure. Growth rate was estimated in terms of the increase in the mean of these diameters per week of observation. Only those tumors were included in the series in which at least 4 observations covering a period of 10 days or more were made. Most mice were killed after tumors had reached considerable size and paraffin sections were made. Mitoses were enumerated in groups of 1000 or more counted tumor cells. The findings are recorded in Table I.

1 Boyland, E., and Warren, F. L., *J. Path. Bact.*, 1937, **45**, 171.

2 Cook, J. W., Hieger, I., Kennaway, E. L., and Mayneord, W. L., *Proc. Roy. Soc. London*, 1932, **3**, 455.