the initial complex become included in the S-T segment. In the second series the deep S-waves, produced by the block of the right bundle, are still visible although they are upwardly displaced in the direction of the S-T segment; in the third series they are no longer visible and a monophasic electrocardiogram appears. In the fifth and sixth series conduction from the auricle to the ventricle becomes longer and the slower ascent of the initial deflection which becomes increasingly obvious indicate a slower activation on the ventricle. The initial and terminal deflections can no longer be separated from each other.

Stimulation of the right or left accelerans produces an acceleration of rate but the changes in the terminal deflection, typical of sympathetic stimulation, do not occur.

The high take-off after the administration of *Viscum album* is the result of a profound damage of the muscle tissue itself. No proof was obtained which suggested that a disturbance of blood supply to the heart muscle was involved. We must assume that the activation of the heart is already altered so profoundly by *Viscum album* that the appearance of a bundle-branch block is unable to produce any further changes. Through the appearance of a high take-off and through the incorporation of the terminal deflection into the initial complex, it becomes impossible to demonstrate other alterations of the initial and terminal deflections.

Summary. In cats and dogs *Viscum album* produces a characteristic high take-off. If this is distinctly developed, one can no longer recognize a disturbance of intraventricular conduction (bundle-branch block) and stimulation of the accelerans does not alter the shape of the terminal deflection.

10907

Interdependence of Vitamin B_1 and Manganese. II. Manganese, Copper, Iron Metabolism in B_1 Deficient Rats.

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Perla, and Perla and Sandberg[1, 2] in their work on the reproductive behavior of rats have demonstrated a metabolic interdependence of manganese and vitamin B_1. The present study was planned to pro-

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

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

vide information on the metabolism of manganese, copper and iron during the development of and recovery from vitamin B_1 deficiency. Since the changes in copper and iron metabolism were not sufficiently constant to suggest a definite rôle of these metals in relation to vitamin B_1, the metabolism of manganese only will be discussed.

Twenty-two male and female albino rats of about 3 months of age were used in the experiment. They were kept in metabolism cages as described previously.[3] Since only negligible amounts of Mn, Cu and Fe are excreted in the urine, feces only were collected twice a week. Feces and food were analyzed for Mn,[4] Cu,[5] and Fe.[6] Blood pyruvic acid[7] was determined weekly in control groups of rats that were kept only for this purpose.

The rats received the Sherman-Spohn vitamin B_1-deficient diet as modified by Chase. This diet contained 6 gamma of Mn per g of food. Additional Mn was given in the form of recrystallized $MnCl_2 . 4H_2O$ of highest quality dissolved in copper-free water. The controls received the same diet, but were supplied with crystalline vitamin B_1.* The rats were kept on the vitamin B_1-deficient diet for a period of 5 weeks. One mg of Mn per rat daily was then added for 3 weeks. When symptoms of vitamin B_1-deficiency were strongly developed, synthetic vitamin B_1 was injected intraperitoneally twice a week, supplying 400 gamma of vitamin B_1 daily, while the addition of Mn was continued. After 4 weeks Mn addition was stopped, but vitamin B_1 was supplied up to the end of the experiment.

The observations of the earlier workers[8, 9, 10] on the effect of Mn on growth may be interpreted to indicate that small amounts of Mn show a favorable effect, possibly because they complement the vitamin B_1 intake, while large amounts may be unfavorable because there is not sufficient vitamin B_1 available to maintain the metabolic balance. This interpretation is supported by our finding that the depletion period of rats on a vitamin B_1-deficient diet was consistently shortened from a week to 10 days if the rats received additions of 1 mg of Mn daily. It has been shown that blood pyruvic acid rises

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

5 McFarlane, W. D., *Biochem. J.*, 1932, **26**, 1022.

6 Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1926, **67**, 43.

7 Wilkins, R. W., Taylor, F. H. L., and Weiss, S., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 584.

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

8 Richet, C., Gardner, and Goodbody, *Compt. rend. Acad. d. sc.*, 1925, **181**, 1105.

9 McCarrison, R., *Indian J. Med. Research*, 1927, **14**, 641.

10 Hamamoto, E., *Orient. J. Diseases of Infants*, 1935, **18**, 21.

TABLE I.

Manganese Metabolism (Daily Average per Rat) During Development of and Recovery from Vitamin B_1 Deficiency

Date, 1938	Experimental Rats				Control Rats			
	Fecal Excret., mg	Intake, mg	Retention, mg	Retention in % of intake	Fecal Excret., mg	Intake, mg	Retention, mg	Retention in % of intake
Sept. 6-12	.068	.084	.016		.056	.069	.013	
" 13-19	.067	.084	.017		.055	.068	.013	
" 20-26	.062	.067	.004		.050	.061	.011	
" 27-Oct. 3	.038	.041	.003		.052	.066	.014	
Oct. 4-10	.027	.045	.017		.035	.043	.008	
Depletion Period Sept. 6-Oct. 10	.053	.064	.011	18	.050	.061	.011	19
Oct. 11-17	.235	1.025	.790		.837	1.048	.210	
" 18-24	.319	1.023	.704		.775	1.028	.253	
" 25-31	.288	1.009	.721		.728	1.024	.296	
+1 mg Mn daily Oct. 11-31	.281	1.019	.738	72	.780	1.033	.253	25
Nov. 1-7	.609	1.076	.467		.788	1.024	.236	
" 8-14	.783	1.103	.320		.797	1.077	.280	
" 15-21	.754	1.077	.323		.822	1.095	.274	
" 22-28	.738	1.069	.332		.803	1.070	.268	
+1 mg Mn + 400 gamma vitamin B_1 Nov. 1-28	.721	1.081	.360	34	.802	1.066	.264	25
Nov. 29-Dec. 5	.412	.073	—.340		.420	.057	—.363	
Dec. 6-12	.370	.080	—.290		.301	.059	—.242	
" 13-19	.674	.080	—.594		.560	.070	—.490	
" 20-26	.470	.091	—.379		.436	.073	—.363	
+ 400 gamma vitamin B_1 Nov. 29-Dec. 26	.481	.081	—.400		.429	.065	—.364	

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.