inhibition of lipid deposition in the cortisone-treated rabbits.

Summary. Administration of cortisone to rabbits caused hyperlipemia due chiefly to an increase of lipids other than cholesterol and phospholipid. Cholesterol-fed rabbits treated with cortisone developed severer degrees of hypercholesterolemia and hyperlipemia than rabbits on cholesterol alone. The relative proportion of cholesterol in the serum lipids of cholesterol-fed rabbits on cortisone was less than in the serum lipids of rabbits on cholesterol alone. Significantly less lipid was deposited in the arteries of the cortisone-treated, cholesterol-fed animals than in the cholesterol-fed controls. It is suggested that hyperlipemias in which the lipid fractions other than cholesterol undergo the greatest increase, are less likely to be associated with arterial lipid deposition than hyperlipemic states in which cholesterol is the major constituent.

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Copper Metabolism. XII. Influence of Manganese on Metabolism of Copper.* (21056)

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The clinical and pathological similarity between the manifestations of chronic manganese poisoning in man and the changes which occur in hepatolenticular degeneration has been noted several times(1-3). Hepatolenticular degeneration is now known to be characterized by aminoaciduria, hypocupremia, hypercupriuria and an increase in the amount of copper in the tissues(4-9). Studies on the metabolism of copper in man or in animals with manganese toxicity have not been reported, however.

It was, therefore, considered to be of interest to determine whether or not the administration of large amounts of manganese could be shown to influence the metabolism of copper in animals. In this report observations are presented on the level of copper in the plasma, urine and tissues of rats given large amounts of manganese. The influence of manganese on the red blood cells, the urinary excretion of amino acids and the content of iron in the plasma and tissues were also studied. A preliminary report of this work has been published(10).

Methods. Eighty-three male weanling rats of the Sprague-Dawley strain were divided into 4 groups (Table I), housed in individual

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TABLE I. Observations on Copper and Iron Content of Various Tissues and on Urinary Excretion of Amino Acids. Ten animals in each group, sacrificed at 86 days.

	A Control	B High Mn	C High Cu	D High Mn + Cu
Body wt, g	371 ± 9.1	171 ± 7.2	333 ± 0.9	125 ± 5.0
Plasma copper, μg %	107 ± 3.6	142 ± 3.9	126 ± 3.6	194 ± 11.7
Urine " * μg/day	1.8	1.0	3.1	1.8
Liver " μg/g	5 ± 0.2	5 ± 0.2	39 ± 14.1	670 ± 66.4
μg/rat	69 ± 3.1	29 ± 1.3	457 ± 161.4	3070 ± 276.6
Kidney " μg/g	8 ± 0.6	5 ± 0.2	23 ± 3.9	55 ± 23.4
μg/rat	19 ± 1.7	7 ± 0.2	51 ± 7.0	71 ± 29.1
Brain " μg/g	3.6± 0.21	4.4± 0.15	3.9± 0.10	5.0± 0.21
μg/rat	6.6± 0.25	7.3± 0.22	6.8± 0.11	8.0± 0.24
Plasma iron,* μg %	301	112	265	128
Liver " μg/g	160 ± 11.0	40 ± 2.2	130 ± 5.0	40 ± 2.0
μg/rat	2120 ± 198.0	200 ± 3.2	1620 ± 67.7	200 ± 11.1
Kidney " μg/g	78 ± 2.8	32 ± 2.2	75 ± 2.8	41 ± 4.1
μg/rat	194 ± 11.5	47 ± 3.6	161 ± 6.4	54 ± 4.8
Urine amino N,* mg/day	1.4	0.8	1.2	0.6

* Value obtained from pooled specimens.
Figures refer to mean ± stand. error.

cages, and fed the following basal diet *ad libitum*: purified casein, 20%; sucrose, 64%; lard, 11%; and salt mix(11), 5%. Vitamins were added to the basal diet as follows (mg/kg of diet): thiamin hydrochloride, 12.5; riboflavin, 6; nicotinic acid, 60; pyridoxine hydrochloride, 10; calcium pantothenate, 25; inositol, 5; para-aminobenzoic acid, 5; pteroylglutamic acid, 15; vit. E, 7.6; and vit. K, 7.6. Vit. A in an amount of 23000 units and vit. D in an amount of 4250 units per kilogram of diet were also added.

The general design of the experiments and the schedule of the intraperitoneal injections are recorded in Fig. 1 together with the growth curves. All animals in groups A, B, and C survived the duration of the experiment. Three animals in group D died between the 68th and 83rd days. The remaining animals in this group were thin, inactive, and failed to gain weight. Therefore, on the 86th day 10 animals in each group were anaesthetized with ether, phlebotomized by cardiac puncture and the organs removed for chemical analysis. The remaining animals continued to survive and on the 120th day of the experiment they were sacrificed by ether anaesthesia. Total body copper and iron analyses were performed on these rats. *Hemoglobin* was determined by the oxyhemoglobin method in the Evelyn photoelectric colorimeter. The other hematologic determinations were performed as outlined by Wintrobe(12). The method employed for the measurement of plasma copper has been described by Gubler, *et al.*(13); for plasma iron by Hamilton *et al.*(14); for tissue iron by Gubler *et al.*(15); and for total body copper and iron by Chase *et al.*(16). A modification(17,18) of the method of Folin was used for the determination of urinary alpha amino-nitrogen. Twenty-four-hour urine samples were collected from ten rats on the 84th day of the experiment. The samples were pooled and used for the determination of both alpha amino-nitrogen and copper(19).

Results. The administration of manganese alone was associated with a significant increase ($P < 0.01$) in the concentration of copper in the plasma and brain and a decrease in the urinary excretion of copper (Table I). The concentration of copper in the liver was not significantly influenced, and the concentration of copper in the kidneys was decreased. The administration of large amounts of copper was associated with a significant increase ($P < 0.01$) in the concentration of copper in the plasma, urine, liver, and kidneys but not in the brain. When large amounts of both copper and manganese were given (group D)

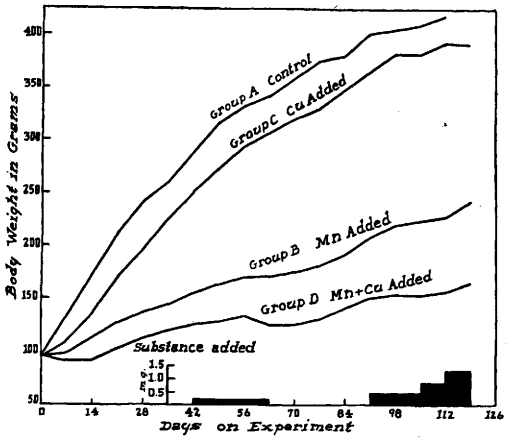

FIG. 1. Influence of manganese, copper, and copper in addition to manganese on growth of rats. All animals received the basal diet and the following supplements: Group A, none; group B, 4% manganese chloride; group C, 0.1% copper sulfate; group D, 4% manganese chloride and 0.1% copper sulfate. In addition, the animals in group B were given manganese chloride ($MnCl_2 \cdot 4H_2O$) intraperitoneally in amounts indicated; those in group C were given "Cupralene" in this way and those in group D both manganese chloride and "Cupralene." These compounds were injected 3 times weekly. Half the animals in each group were sacrificed at 86 days, the remainder at 120 days. As a result those sacrificed at 86 days received a total of 4.5 mg $MnCl_2$ intraperitoneally while the remainder received a total of 25.5 mg. The total amounts of "Cupralene" received by the animals sacrificed at these different times were also 4.5 and 25.5 mg, respectively.

the concentration of copper in the plasma, liver, kidneys, and brain was significantly increased ($P < 0.01$) as compared with the values in the animals given the same amount of supplementary copper (group C). A decreased urinary excretion of copper was associated with this retention of copper in the tissues.

The concentration of iron in the plasma, liver and kidneys was significantly lower ($P < 0.01$) in the animals given manganese, either alone (group B) or with copper (group D). Aminoaciduria was not observed in any of the 3 experimental groups.

As shown in Table II, the administration of manganese alone (group B) had little influence on total body copper; the administration of large amounts of copper (group C) caused an increase in total body copper. When large quantities of both copper and manganese (group D) were given simultaneously, the total body copper was approximately twice that found in the animals receiving the same amount of copper without added manganese. Since these animals were considerably smaller than the animals in group C, the amount of copper expressed in terms of $\mu g/100$ g of body weight was increased about 5-fold.

The administration of manganese alone (group B) was associated with a significant decrease ($P < 0.01$) in the total amount of iron in the body and a significant decrease ($P < 0.01$) in the amount of iron per 100 g of body weight. When supplementary copper was given in addition to manganese, the decrease in iron (mg/100 g of body weight) was partially overcome, but the total body iron was less than in the animals in group B. The administration of copper alone (group C compared with group A) had no significant influence on total body iron.

The hematologic observations are summarized in Table III). The animals receiving large amounts of manganese (group B) developed a mild anemia which was microcytic and slightly hypochromic and was accompanied by a slight increase in reticulocytes. The animals given large quantities of copper (group C) failed to develop anemia or morphologic alterations in the erythrocytes. When large amounts of copper were given in addition to manganese (group D) the hematologic changes observed were similar to those which occurred when large amounts of manganese alone were given.

Discussion. From the data presented it is evident that in the presence of a large amount of dietary copper, the administration of large quantities of manganese is associated with a marked increase in the retention of copper in the body, particularly in the liver and spleen but also in the plasma and brain.

The administration of large quantities of manganese, either with or without the administration of excessive amounts of copper was associated with the development of microcytic, hypochromic anemia. The concentration of iron in the body of the rats fed large amounts of manganese was significantly reduced, particularly in the liver. Therefore, it is quite possible that the anemia was the result of a mild deficiency of iron. However,

TABLE II. Total Body Copper and Iron. Ten animals in each group, sacrificed at 120 days.

	A Control	B High Mn	C High Cu	D High Mn + Cu
Body wt, g	419 ± 10.9	234 ± 12.1	392 ± 7.8	166 ± 8.7
" copper, μg/100 g	126 ± 3.5	193 ± 8.9	470 ± 31.3	2319 ±378.3
μg/rat	572 ± 16.4	445 ± 18.5	1833 ±115.0	3741 ±537.0
" iron, mg/100 g	4.72± 0.177	3.62± 0.247	4.81± 0.108	4.32± 0.193
mg/rat	19.6 ± 0.71	8.6 ± 0.85	18.8 ± 0.38	7.1 ± 0.11

Values represent mean ± stand. error.

the degree of reduction in the plasma iron level and in the total body iron would not seem to be sufficient to account for the development of anemia on this basis. It should be noted that the anemia associated with an excessive intake of manganese is also morphologically similar to the anemia which appears in swine deficient in copper(20). It is possible that manganese forms a complex with copper thus making the copper physiologically unavailable or that the manganese in some manner blocks the action of copper-containing enzymes which are essential for normal erythropoiesis. Such an interaction has been postulated by Marston(21) to account for the observation that moderate amounts of molybdenum, administered to rats given rations which supply sufficient copper to provide for maximal growth and erythropoiesis, will increase materially the copper concentration in the tissues and precipitate the symptoms of copper deficiency.

In regard to the possible role of manganese and copper in the pathogenesis of hepatolenticular degeneration, it is worthy of note that, although in our experimental animals the tissue levels of copper were increased to a degree comparable to that observed in patients with hepatolenticular degeneration(8), a syndrome simulating this disease was not produced. Neurologic abnormalities, corneal pigmentation, aminoaciduria, and histologic alterations† in the brain were not observed. Furthermore, the plasma copper level was increased rather than decreased, and the administration of manganese was associated with a decrease rather than an increase in the excretion of copper in the urine. In the animals receiving excessive manganese (group B), copper (group C), and copper and manganese together (group D) acute widespread necrosis of the liver with varying degrees of regeneration of liver parenchyma was observed.† However, increase in fibrous tissue, such as is found in the livers of patients with hepatolenticular degeneration was not present. It cannot, of course, be concluded from these relatively short term studies in rats that copper and manganese either do or do not play a role in the pathogenesis of the lesions observed in hepatolenticular degeneration.

† E. J. Eichwald, unpublished observations.

Summary. 1. Administration of large amounts of manganese to rats was associated with an increase in the concentration of copper in plasma and brain, a decrease in the urinary excretion of copper, a decrease in the concentration of copper in the kidneys, no alteration in the concentration of copper in the liver, and microcytic, hypochromic anemia. The total amount of copper in the body was not increased. 2. Administration of large amounts of copper was accompanied by an increase in total body copper and an increase in the concentration of copper in the plasma, urine, liver, and kidneys but not in the brain. Anemia did not occur. 3. Simultaneous administration of large amounts of both manganese and copper resulted in a marked increase in total body copper, an increase in the concentration of copper in the plasma, liver, kidneys and brain, and microcytic, hypochromic anemia. The total amount of copper in the body of these animals was twice as great as in rats given the same amount of supplemental copper alone. The concentration of copper (μg/100 g of body weight) was increased 5-fold. 4. It is suggested that manganese may form a complex with copper which makes the latter unavailable or that in some manner it blocks the action of copper-containing enzymes.

TABLE III. Hematologic Studies. Ten animals in each group.

	A Control	B High Mn	C High Cu	D High Mn + Cu
Red cell count $\times 10^6$/mm^3	8.91± .323	9.40± .192	8.66± .238	10.06± .334
Hemoglobin, g %	17.5 ± .19	11.7 ± .64	17.6 ± .32	12.8 ± .75
Vol packed red cells, ml/100 ml	51 ± .8	37 ±1.3	49 ± .7	40 ±1.9
Mean corpuscular vol, cμ	57 ±1.7	40 ±1.8	58 ±1.3	40 ±2.7
Mean corpuscular hemoglobin, $\mu\,\mu$g	20 ± .6	12 ± .5	21 ± .3	13 ± .9
Mean corpuscular hemoglobin conc., %	35 ± .4	32 ±1.0	36 ± .2	32 ±1.2
Reticulocytes, %	2.6 ± .23	5.7 ± .57	2.7 ± .20	6.8 ±1.19

Values represent mean ± stand. error.

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