ported for the rat. The testes of all animals injected with androgens showed spermatogenesis in progress, although the degree of tubular maintenance varied with the particular androgen. The latter was most marked in cases where androstanedione or androstanediol had been used. Even here, as is true of the rat, occasional tubules that show degenerative changes are encountered. The striking degeneration of the interstitial cells which follows hypophysectomy in the mouse is not prevented by treatment with androgens. Nor are the damaged adrenals and thyroid altered. As would be expected the male accessory organs present a normal or hypertrophied histological picture in animals which had received treatment. Figures 1 to 3 represent typical areas in the testes of normal, hypophysectomized-control, and hypophysectomized-treated animals.

Summary. The injection of crystalline androgenic hormones will prevent the marked degenerative changes in the seminiferous tubules of hypophysectomized mice for at least 23 days. The most effective substance used has been androstanedione. The degenerative changes in the interstitial cells, adrenals and thyroid were not altered.

10001 P

Toxicity of $MnCl_2.4H_2O$ when Fed to Rats.

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Findlay[1] claims that addition of manganese chloride to the "ordinary" diet of rats produces cirrhosis of the liver and death in from 6 to 10 weeks. He fed 0.3 to 0.4 g manganese chloride per rat per day but failed anywhere to indicate the composition of the diet used. McCarrison[2] fed rats 0.889 mg of MnO per day. He compared their weights with those of a similar group receiving no manganese other than that contributed by the salt mixture and the other constituents of the diet. He states, "There was no difference in the percentage weight curves of the 2 groups up to the 32nd day. Thereafter the curves diverged gradually and increasingly; that of the group receiving manganese being at the lower level." The experi-

[1] Findlay, G. M., *Brit. J. Exp. Path.*, 1924, **5**, 92.

[2] McCarrison, R., *Ind. J. Med. Res.*, 1926-27, **14**, 641.

ment lasted 135 days. To another group of rats he fed $MnCl_2$ at a level of .0327 mg per rat per day. A control group received no $MnCl_2$. He states, " . . . from the outset the rate of growth of the group receiving 0.327 mg $MnCl_2$ daily per rat surpassed that of the control group." The experiment lasted 53 days. Unfortunately, the diets used in these 2 sets of experiments were not identical either in the source of the manganese, amounts of manganese, or sources of fat or vitamins.

Orent and McCollum[3] demonstrated the need of the rat for manganese for normal reproduction. On manganese-free diets females fail to suckle their young, and males show testicular degeneration after 100 days or less. The addition of .005 to .05% manganese in the form of $MnCl_2.4H_2O$ promotes normal functioning of males and females.

As a result of these observations further experiments were conducted where manganese was added to the basal ration at the following levels of $MnCl_2.4H_2O$: 0.18%, 0.36%, 0.9%, 1.8%, 3.6%. Thus there was added to the diets .0499, .0998, .2495, .4990, and .9980 g of manganese respectively. The basal ration had the following composition:

	%
Yeast	10.0
Casein	18.0
Salts (No. 51)	6.1
Dextrin	57.9
Butterfat	8.0
Viosterol	15 drops per kilo of ration

Young animals weighing 40 to 50 g were taken from our stock diet and placed on the experimental rations.

On all the diets except where $MnCl_2.4H_2O$ was fed at 3.6% the animals grew well and produced healthy young. On the latter diet there was some depression of growth but here, as at the lower levels of manganese feeding, reproduction was excellent. The experiments on the 4 lower levels of manganese were terminated after the animals had been on the diet 240 days. Only the group receiving 3.6% $MnCl_2.4H_2O$ was kept for longer observation. At this level marked rancidity of the fat developed. When the ration was kept on ice no such changes occurred. Hence this practice was followed. As mentioned above, there were no indications of toxicity on this diet other than slight depression of growth. After 730 days on the diet 2 males still show normal testes and motile sperm as de-

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

termined by examining epididymal smears obtained by aspiration. Each of these males has subsequently been bred to 4-month-old virgin females from our stock colony. One sired 40 young when mated with 8 females, the other 25 young when mated with 5 females. Of 50 males on similar diets but without added manganese, other than that found as a contaminant of certain inorganic constituents and natural foods as shown by Orent and McCollum,[3] testicular atrophy and tubular degeneration was marked in 45 males after they had been on the rations from 117 to 833 days.

On the type of diet employed, food consumption averages from 10 to 15 g per adult rat per day. Animals on the above rations were thus consuming about .018, .036, .09, .18, and .36 g of $MnCl_2.H_2O$ per day, or .00449, .00998, .02495, .0499, and .0998 g of manganese. The rations used contained .63 g calcium and .72 g phosphorus per 100 g. Evidently the high level of phosphorus effectively prevented symptoms of toxicity by reducing the amount of absorbable manganese.

It is thus apparent that the conditions in the intestines which influence the absorbability of manganese constitute the controlling factor in determining the level at which it is toxic. From our observations on a small number of rats it also seems clear that when a high intake of this element is provided sexual function is retained in males decidedly longer than is the case in our rats on similar diets without added manganese. This observation is being checked on a larger group of male rats.

10002

Excretion of Homogentisic Acid After Oral Administration of Phenylalanine to Alcaptonuric Subjects.

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That the homogentisic acid excreted in alcaptonuria originates from the aromatic amino acids of the protein molecule, tyrosine and phenylalanine, has been accepted.[1] Further confirmation has

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1 Compare Neubauer, O., *Handbuch der normalen und pathologischen Physiologie*, 1928, **5**, 851, for an excellent review of the literature.