and the liver reaction significantly enhanced. There was reason to believe that the attendant necrosis began in the nests of tumor cells and spread outwardly to the adjoining parenchyma.

It is probable that the virus was actually carried by one or more mice of the ascites passage series before it was detected by the appearance of liver lesions. The ascites tumor may now be added to the list of agents which have been instrumental in activation of viruses of the murine hepatitis group. In addition to the tumor, the list now includes *Eperythrozoon coccoides,* Niven *et al.*(3), leukemic lymphocytes, Nelson(2), and the reagents urethane and methylformamide, Braunsteiner and Friend(4). The initial emergence of hepatitis virus in Princeton mice has never been observed in the absence of one or another of these agents.

Summary. 1) A contaminant, subsequently identified as a virus of the mouse hepatitis group, appeared in Princeton weanlings during the 132nd intraperitoneal passage of an ascites tumor. The virus was regularly demonstrable in the ascitic fluid and was readily maintained in both Princeton and Swiss mice by serial transfer of fluid. In its presence, livers showed necrotic lesions and the ascitic fluid was altered in amount, consistency, and color. An artificial mixture of the 2 agents behaved in much the same way as the natural mixture. 2) The 2 mouse strains were equally susceptible to the tumor but not to the virus. Liver lesions produced by the virus alone were much more pronounced in Princeton mice. Presence of both agents facilitated migration of tumor cells to the liver and resulted in a marked enhancement of the hepatic reaction in Swiss mice.

1. Nelson, J. B., *J. Exp. Med.,* 1956, v103, 743.
2. ——— *Bull. N. Y. Acad. Med.,* 1957, v33 2nd ser., 811.
3. Niven, J. S. F., Gledhill, A. W., Dick, G. W. A., Andrewes, C. H., *Lancet,* 1952, v2, 1061.
4. Braunsteiner, H., Friend, C., *J. Exp. Med.,* 1954, v100, 665.

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Delayed Development of Righting Reflexes in Offspring of Manganese-Deficient Rats.* (25248)

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Our previous reports(1,2) have shown that a maternal dietary deficiency of manganese in rats and guinea pigs results in the birth of offspring which exhibit ataxia with incoordination and loss of equilibrium. These symptoms appear to result from a congenital defect or defects occurring during latter part of gestation in the rat(1). Observation of defective young during early part of life span raised questions concerning development of the righting reflexes. The present communication reports results of tests designed to examine the effect of a maternal manganese deficiency in rats on development of righting reflexes in their young. Two tests were used: (1) time the animal required to right itself when placed on its back, and (2) ability of animal to right itself in air when dropped upside down.

Methods. Procedures used to obtain manganese-deficient offspring were the same as previously reported(1). Briefly, female rats of the Sprague-Dawley strain were maintained from time of weaning on fortified milk diet deficient in manganese. When they reached maturity, they were mated with normal males receiving a stock diet, and the resulting offspring were tested. First, second and third litters were used. Control animals were treated in the same way, except that the diet was supplemented with manganese. Turnover time was tested beginning at 1 day of

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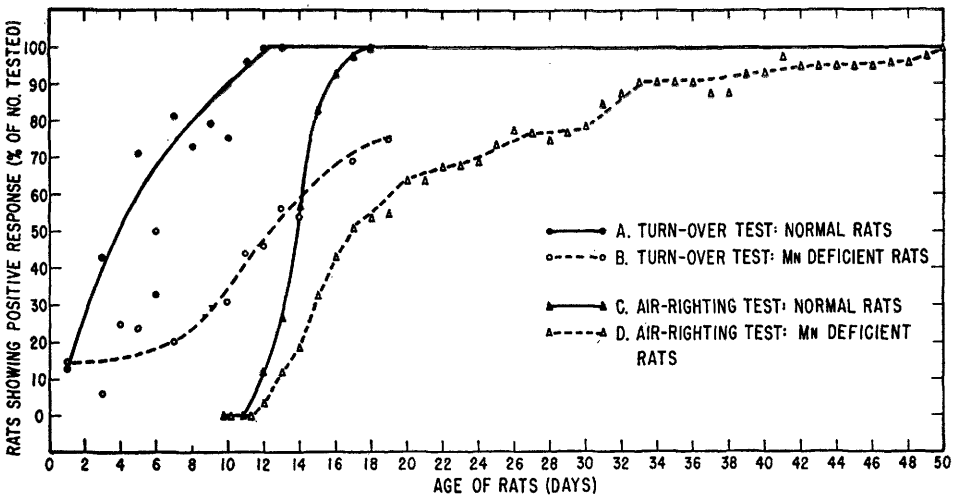

FIG. 1. Development of righting reflexes in offspring of normal and manganese-deficient rats. Curves A and B represent percentage of animals able to turn over in 2.5 sec. or less when placed on their backs. Each point represents tests on from 7 to 34 animals. Curves C and D represent percentage of animals showing a positive air-righting response. Number of normal animals tested was 162. Number of deficient animals tested ranged from 89 at 10 days of age to 42 at 50 days of age.

age. The animals were considered 1 day old 24 hours after birth. The animal was placed back downwards on a table, and the time required to turn over to its feet was measured with a stop-watch. Appearance of air-righting reflexes was tested in young beginning at 10 days of age. Each animal was held back downwards, and dropped 12 inches onto a soft cotton-filled pad. The response was recorded as positive if the rat landed on all 4 feet in 2 out of 3 trials.

Results. The results are summarized in Fig. 1. For the turn-over test (Curves A and B), the response was considered positive when the animal was able to turn from its back to its feet in 2.5 seconds or less. In both normal and deficient groups, this response developed at an earlier age than did the air-righting reaction, but a delay in appearance of the response was exhibited by the deficient animals. At 12 days of age, 100% of normal animals were capable of turning over in 2.5 seconds or less, while at the same age only 48% of deficient rats were able to do so. In addition, examination of individual data showed that until 13 days of age, many of the deficient animals required more than 60 seconds to right themselves, while after 3 days of age, not one of the normal animals needed more than 16 seconds to turn over.

The data concerning air-righting reaction (Curves C & D) also show a significant difference between response of manganese-deficient young as compared with normal young. At 18 days of age, 100% of 162 normal animals were able to right themselves in air, while at the same time, only 54% of 52 manganese-deficient animals showed a positive response. Not until 50 days of age did 100% of the 42 deficient rats tested exhibit the positive reaction. Since only 42 of the original 89 deficient animals tested at 10 days of age survived to 50 days, the data do not, of necessity, include throughout the age span those animals most severely affected by the deficiency. The data may therefore be biased in the direction of a smaller difference between the 2 groups than was actually true. It was not possible to eliminate the data obtained from animals which did not survive, because individual young in litters were not marked.

Discussion. The nature of the defect responsible for the congenital ataxia of manganese-deficient offspring has not as yet been ascertained. No histological abnormalities have been found in the nervous system(1,3). The present experiments indicate at least that the nervous system is involved in the ataxic symptoms observed in manganese-deficient offspring.

That the reflexes concerned with the turn-over test developed earlier than did those involved in the air-righting response is in accord with the work of Windle and Fish(4), who reported that body-on-body righting reflexes appeared in the fetal cat before labyrinthine reflexes could be observed.

The delayed development of the air-righting response in manganese-deficient offspring may be related to (a) impaired labyrinthine function(5,6,7) (although Hill *et al.*(8) found no differences between normal and manganese-deficient rats in histological studies of the inner ear), and (b) delayed or abnormal myelination. Windle *et al.*(9) observed some correlation between development of labyrinthine reflexes and myelination in the fetal cat. Furthermore, in the normal animals studied in the present experiments, the air-righting response appeared at the age at which myelination is being accomplished(10). The work of Eayrs and Taylor(11) indicates that the thyroid also may be involved, since thyroidectomized animals showed some delay in development of this response. These possibilities are currently under investigation.

Summary. Development of righting reflexes was studied in offspring of manganese-deficient and normal rats by 2 tests: (1) time required to turn from their backs to their feet and (2) ability to right themselves in air when dropped upside down. The reflexes concerned with the turn-over test developed at an earlier age than did those involved in the air-righting response. In both tests, however, the manganese-deficient young exhibited a marked delay in development of reflexes responsible for righting reactions.

1. Hurley, L. S., Everson, G. J., Geiger, J. F., *J. Nutrition,* 1958, v66, 309.

2. Everson, G. J., Hurley, L. S., Geiger, J. F., *ibid.*, 1959, v68, 49.

3. Cotzias, G., *Physiol, Rev.,* 1958, v38, 503.

4. Windle, W. F., Fish, M. W., *J. Comp. Neurol.,* 1932, v54, 85.

5. Fulton, J. F., *Physiology of the Nervous System,* Oxford Univ. Press, N. Y., 1949.

6. Muller, H. R., Weed, L. H., *Am. J. Physiol.,* 1916, v40, 373.

7. Magnus, R., *Lancet,* 1926, v2, 531, 585.

8. Hill, R. M., Holtkamp, D. E., Buchanan, A. R., Rutledge, E. K., *J. Nutrition,* 1950, v41, 359.

9. Windle, W. F., Fish, M. W., O'Donnell, J. E., *J. Comp. Neurol.,* 1934, v59, 139.

10. Folch-Pi., J., in *Biochemistry of the Developing Nervous System,* Waelsch, H., Ed., Academic Press, N. Y., 1955.

11. Eayrs, J. T., Taylor, S. H., *J. Anat.,* 1951, v85, 350.

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Effect of Vit. E-Deficiency on Protein Composition of Guinea Pig Skeletal Muscle.* (25249)

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Several reports concerning the status of one or more muscle protein fractions from animals maintained on a vit. E-deficient diet have appeared in the literature. Spencer *et al.*(1) observed an increase in collagen content of nutritionally dystrophic rabbit muscle. The myosin and actomyosin content of rabbit skeletal muscle fibers gradually decreases with increasing duration of the deficiency state(2). Baechtel *et al.*(3) noted that nitrogen concentration of water soluble extracts of muscles from supplemented and deficient rabbits were the same. In 21-day old vit. E-deficient rats, Rumery *et al.*(4) found a pronounced loss in actomyosin associated with a marked elevation of insoluble protein. The present investigation was initiated to examine the effect of vit. E-deficiency on the sarcoplasmic, contractile and collagen fractions of skeletal muscles from young guinea pigs. It was also of inter-

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