Prenatal Manganese Deprivation and Early Behavior of Primates

Arthur J. Riopeiie[1] and David G. Hubbard [2]

Manganese deficiency has been shown in pregnant rats to lead to a variety of behavioral disorders related to development of inner ear mechanisms in the young. Infant rhesus monkeys, whose mothers were fed a semisynthetic diet that contained less than .5 ppm of manganese, and who themselves were fed the same diet, were tested on a series of behavioral tasks during their first 60 *days of postnatal life. Their behavioral development was compared with that of animals fed a comparable diet, but containing over 40 ppm manganese. The performance of the low-Mn infants was entirely within normal limits for most tests, but their clasping response was extraordinarily vigorous and their righting performance, which required release from clasping, was inadequate.*

Little attention has been given in psychiatric literature and scarcely more in research to the importance of adequate integrated responses to gravity's constant force, or to the consequences of their inadequacy. Yet the terror created by acute inner ear disorders such as Meniere's disease or other labyrinthitides and the cautiousness and the changes in outlook which accompany deterioration of balance sensitivity of older people should remind us of their significance to the life economy. The importance of the adequate development of labyrinthine function and the consequences of incompetence have been suggested by the observations of Bender (1947), Schilder (1950), Pollack and Krieger (1958), and of Fish and Alpert (1963) in childhood schizophrenia and in aphasia by Rosen-bliit et al. (1960). The related stereotypies, rocking, whirling, somersaulting, etc., are seen in institutionalized children, mental retardates (Berkson and Davenport, 1962), and socially isolated infant primates (Davenport and Menzel, 1963). Hubbard has reported clinical evidence of vestibular dysfunction among "skyjackers" (Hubbard, 1971).

Bender, describing schizophrenic children, wrote, "Rotating and whirling motor play in all planes make up a large part of their activity. It finds expression in their dreams and in all other forms of fantasies, and is the nucleus of many of their psychological problems such as fear of or preoccupation with losing their

1 Dept. of Psychology, Louisiana State University, Baton Rouge, Louisiana 70803.

[2] Ste. 1250, DFS Tower, 8333 Douglas Ave., Dallas Tx 75225.

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limbs, inability to determine the periphery of their own body or the boundaries of their personality or 'ego boundaries,' their relationship to the reality of the outer world, to determine their own center of gravity, to relate themselves to time and space, or even be sure of their own identity" (Bender, p. 43, 1947).

A prominent feature of such stereotyped, repetitive behavior is head movement. It is as if the subject by such movement is attempting to replace the vestibular stimulation lost by either diminished input or receptor dysfunction. Nutritional deficiency, perhaps of a trace element essential to receptor function, is possibly an etiologic factor in receptor dysfunction and its behavioral sequelae.

Manganese (Mn) is the 12th most abundant element in the earth's crust. It also appears in many natural foods (National Research Council Committee, 1973). One can reasonably expect, in view of its ubiquity, that most if not all living organisms make use of it in their normal metabolic processes even if only at low concentrations. Inadequate maternal or fetal metabolism might thus contribute to the behavioral disorders. Mn's role in sensory and in cerebral function is either documented or suspected. Some cerebral enzymes demand Mn, and perhaps melanin-related semiconductor mechanisms do, too. Uneq-ivocal evidence of Mn deficiency has not been found in man "... but diets high in milk, sugar, and refined cereals might provide insufficient Mn, particularly in growing children, pregnant women, and persons with diabetes or rheumatoid arthritis, in which a slow turnover rate of Mn has been demonstrated" (Dobbing, 1968). Hurley et al. (1958) demonstrated that rats born of Mn-deficient mothers were ataxic at birth. A good portion of this ataxia was due to the anomalous development of otoconia in the inner ear. Erway et al. (1970) showed that the congenital ataxia was due to otolith defects caused by Mn deficiency in mice, since supernormal doses of Mn fed to genetically defective mice during their pregnancy prevented the defect from appearing in the offspring (Erway et al., 1966). Hurley et al. (1963) showed that rats' susceptibility to convulsions increased if they were Mn deficient.

It seems reasonable, then, that animals higher in the phyletic tree, including man, might also be susceptible to nutritional deficiency during the prenatal and early postnatal period, and failure to obtain essential nutrients during this critical period might visit permanent deficit upon the developing organism. The brain, after all, like most other organs of the body, grows first by an increase in cell number (hyperplasia) followed by and overlapping with an increase in cell size (hypertrophy) (Enesco and Leblond, 1962). Winick (1968), in particular, has argued that the time available for cell replication is genetically limited and that if the potential number of cells in an organ is not attained within the allotted time span, the animal is permanently deficient. Dobbing (1968) has put forth a related hypothesis: early growth begins slowly, accelerates to a peak, and then retards. During the period of maximal growth rate the demand by the organ for nutrients is at its greatest, and it therefore is most vulnerable to nutritional deficit during this period. The vulnerability of rats to early protein-calorie deprivation has been demonstrated amply by these and other authors, although the primate fetus is protected better than the rat fetus is, due in part to better communication between the single primate fetus and its mother (Riopelle and Hale, 1975).

These considerations lead to a concern for the role of Mn in the development of the primate fetus. The purposes of the present experiment were to determine whether or not infant rhesus monkeys born of mothers who were fed a low-Mn diet during most or all of their pregnancies would be affected in any way by the deficiency.

Methods and Procedures

Recent studies from this laboratory

have reported the effects of protein deprivation in nonpregnant (Riopelle et al., 1976) and pregnant monkeys (Riopelle et al., 1974.) Those reports describe in detail the method of handling the animals, the way in which they were housed, and the composition of the diets. Suffice it for present purposes to say that the animals were maintained in individual cages in a screened porch-like room in a semirural area which required the animals to adapt to the ambient temperature (tempered somewhat in the wintertime). The adult rhesus monkeys **(Macaca mulatta)** were mated with males for four days beginning on the 11th day after onset of menstruation. The mothers were placed on the experimental (low-Mn) diet weeks or months before they became pregnant. The experimental diet was the same adequate diet as that fed the animals before except that the Mn (previously about 40 ppm) had been removed from the salts in the diet. Assay of the diet by atomic absorption showed less than .5 ppm, the lowest sensitivity tested. Manganese content of the available water was less than .2 ppm. No industries in the area are known to expel Mn into the atmosphere.

Six pregnancies were observed, and all were carried to term. The infants upon discovery in the laboratory were removed from the mother and taken to the nursery where they were fed the same diet as their mothers except that it was served liquid rather than solid in form.

Twelve behavioral tests were administered to the infants during the first two months of their lives. Most were adopted modified in scoring detail from the tests developed by Mowbray and Caddell (1962).

1. **Rooting.** The normal newborn rhesus monkey tries to mouth an object that touches its nose or its cheek. Three grades of performance are recognized in response to such a stimulus: vigorous movement, desultory movement, and no movement, receiving scores of 2, 1, and 0, respectively. On each session three trials were run and the score for the session was the accumulated total.

2. **Startle.** In this test the infant remained on the table top. Two pieces of wood were clapped together behind its head. Three grades of response namely, no response (0), eye-blink (1), and body movement (2) were recorded.

3. **Visual orientation.** Response in this test consists in following with the head and eye a small bright object moved across the visual field. If both head and eyes moved in synchrony with the moving visual target a score of 2 was given. If only the eyes moved, a score of 1 was given, and if no response at all was evident a score of 0 was given.

4. **Extending.** This test measured the reflexive extension of the arms and legs upon loss of support. The animal was dropped from a height of 12 inches on to a soft trampolin. On falling the animal could extend **and** remain rigid for which he achieved a score of 2, extend then relax (1), or fall without extension (0).

5. **Clasping.** Placing the arms and legs around an object that touches the body's ventral surface was demanded in this task. The infant on the table was rolled on its back and a wire cylinder was placed against its abdomen, inducing a clinging response, then the cylinder was raised until the clinging animal was lifted off the table. The test was repeated with the cylinder wrapped by sandpaper. A full response was given 3 points credit, partial response 1.5 credit, and no response 0. Two trials were given during the test session.

6. **Righting.** Newborn monkeys hanging from a cylinder are lowered back down onto a table and allowed to right themselves. The score could be full release in righting (3), releasing and righting with the forelegs only (1.5), and neither (0). Two trials were given during the session.

7. **Body orienting.** This test measured negative geotropism. The animal was placed upon a large circular disc which was tilted 30° off the horizontal. It was slowly pivoted, rotating about its central point, maintaining a constant slant so that the animal's orientation shifted from head up to head down as the table was

rotated. If the animal fully corrected for the change in body orientation so as to maintain a constant head-up position it was given a score of 2, partial righting was given a score of 1, and no response earned a score of 0.

8. **Placing.** The animal, held in the tester's hands, was moved forward until its suspended arms touched the near edge of a table. The animal had to raise its forelimbs to place them on top of the table. If both limbs were raised, it was given a score of 2, if one limb was raised, it was given a score of 1, and if none was raised the response was scored 0.

9. **Platforms.** Three platforms approximately 250 mm square were constructed to be 50 mm, 100 mm, or 150 mm high. The animals were placed on the platform (three trials at each, height), and they were scored for the best response on the trial.

10. **Elevating rod walking.** A piece of dowel 2 m. long was mounted on standards 30 cm above the floor. A small platform was attached in the center of the rod. The animal, when placed on this platform, showed signs of distress; it could achieve security by crawling suspended to one of the end platforms on which was placed a wig, *a* very strong incentive. The animal could make a variety of responses which are graded in complexity. Attainment of the wig yielded a score of 2, moving part way off the platform yielded a score of 1, and no response got a 0.

11. **Rope line.** This test is similar to the previous test except that the animal, instead of being placed on the platform in the center of a rigid dowel, was suspended from a moderately taut rope line. Again, a graded series of responses was noted, with 2 points scored if the animal moved to one end of the line and achieved the security of the wig, 1 point for a partial response, and 0 for no response.

12. **Tunnel.** A transparent plastic tunnel, *22* cm wide, 30 cm high, and 1 m long was constructed. At one end of the tunnel a wig was placed and at the opposite end a starting box. The infant monkey was placed in the starting box and held there for 30 seconds and then released. If the animal stayed in the, starting box it got a score of 0, if it got its body but of the starting box but remained within the tunnel it got a score of 1, and; if it eventually reached the wig it achieved a score of 2.

The 12 behavioral tests described above measure various types of locomotive efficiency, integrity of reflexes, and awareness of external stimuli. In some tests the emotional distress occasioned merely by taking the animal from its home cage to the test room had a significant effect on the score. In general individual differences were wide and day-to-day fluctuations in performances were obvious.

Results

Gestation length

The effects of Mn deficiency can be revealed by the responses of the mother in her attempt to adapt to her new physiologic state as well as by the direct consequences to the infant. Ah important mechanism by which the mother adapts is adjustment of the gestation length. Maternal protein intake, conception weight of the mother, season of the year, and the size and sex of the infant all affect gestation length (Rio-pelle et al., 1974). Infants born to mothers fed a high-protein (13.4 percent) diet are **in utero** approximately 8.5 days less than are infants born of mothers fed diets containing one-half or .one-fourth the amount of protein. Male infants from mothers fed the high-protein diet (which is identical to the present diet except for the presence of adequate amounts of Mn) are born after about 164 days, female infants after about 156 days of gestation. Gestation lengths of the five males of the present study were 174, 195, 168, 170, and 165 days, for an average of 174 days. Even allowing for the outside possibility that the one extreme value is in error by a menstrual period *(ca 28* days) the average would be 169 days. The single female born had a gestation length

of 179 days, 21 days "overdue." Although we fully acknowledge the small numbers of animals involved, we incline toward the belief that the Mn-deficient mother, like the protein-deficient one, extends her pregnancy in accommodation to her deficiency. It is likely that a consequence of this increased length is minimized damage to her infant.

Birth weight

Birth weights of the five males averaged 466 g, which is about what we would expect for males fed the comparable diet, but with adequate manganese (467 g), (Pollack and Krieger, 1958). The animal with the longest gestation period (195 days, the longest yet recorded from our laboratory) was the heaviest of the group, weighing 555 g. The female weighed 410 g, which also is about at expectation (413 g). The finding of altered gestation lengths but average birth weights suggests that in Mn deficiency, as in protein deficiency, the mother makes adjustments, the consequence of, which is to keep her infant's physical status close to normal.

Growth

The daily records of body weight and radius length revealed that the infants of the Mn-deficient mothers grew as rapidly as the control infants did despite the fact that they were fed a Mn-deficient diet postnatally. Individual animals were usually within one standard deviation of the mean of the control infants.

Skeletal maturity scores derived from the Tanner-Whitehouse scale did not differ from control values (Riopelle et al., 1976). Growth changes that are seen in low-rMn lambs (Lassiter and Morton,. 1968) were not seen in monkeys.

Table 1 shows the percentile ranks the Mn-deprived infants would attain if placed into a matched control population fed a commercial infant formula and in which the mothers were not Mn deprived. No particular trend is in evidence.

Early behavior

The performances of the six infants of this study can be compared with those of a comparable group of infants born to mothers fed the adequate diet. Figure 1 shows the results for the 12 behavioral tests. Each panel of the figure depicts the results for a single test. The two vertical lines whose midpoints are connected denote the mean plus and minus 1 standard deviation based on the performance of 15 normal animals fed the control diet. The ticks crossing the vertical line denote the performances of individual Mn-deficient infants.

The scores of the low-Mn infants for

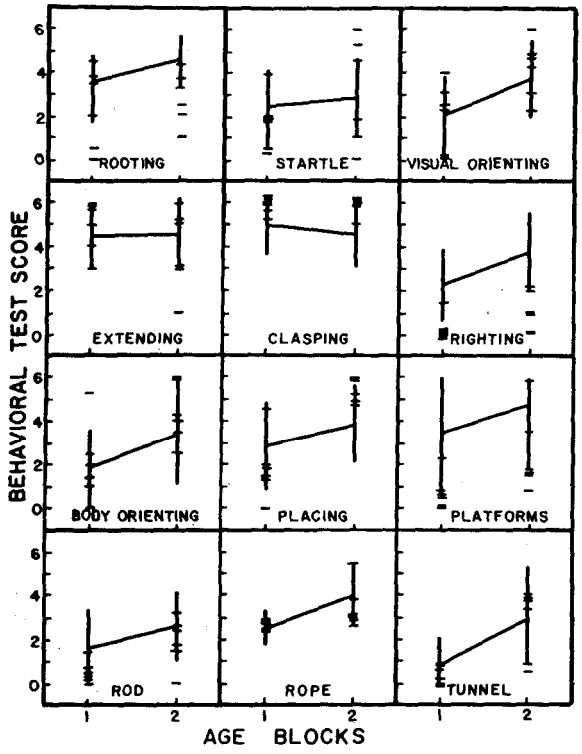
TABLE 1

Percentile ranks of individual no-Mn infants in a control population

Animal	Sex	Weight*	%ile	Radius length**	%ile	Maturity Score***	%ile
59	M	861	25	65.5 mm	55	466	15
60	M	1348	100	70	100	539	42
61	M	869	26	62	33	517	65
63	M	861	22	64	41	677	92
76	F	693	25	60.5	30	460	12
77	M	910	44	62	33	726	96
Colony Mean		965		61.8		553	
Colony S.D.		125		3.5		73	

* at 112 days
** at 180 days
*** at 217 days

FIGURE 1



Performance of six infant monkeys born of Mn-deprived mothers on 12 early behavioral tasks. Vertical lines represent mean ± 1 S. D. at two age periods. Horizontal line connects the performance means of a control group of monkeys. Individual ticks represent score for Mn-deprived infants.

Rooting, Startle, Visual Orienting, Extending, Body Orienting, Placing, Platforms, Rod Walking, Rope Walking, and the Tunnel tests are scattered at random on both sides of the means for the colony. There is thus no evidence that the low-Mn infants are deficient on any of these tests. In contrast, all of the Mn-deficient infants are above the mean at both age levels for Clasping, the test in which the animals were laid on their back and they were permitted to clasp a cylinder placed against their ventral surface. Their response to this task was usually vigorous. Performance on the Righting task, which entails release from clasping when they were returned to a table, is deficient, most of the animals clinging persistently despite the fact that they were in the abnormal, unpleasant, position of feet up.

Discussion

Manganese is a tasteless element, and its absence from food is gustatorily undetectable. Mothers fed the low-Mn diet continue to eat normal amounts, so they are not otherwise deficient. If they are not pregnant, they maintain their body weight and general physical well-being, even though they may be fed the low-Mn diet for months or years. The pregnant animal fed the same diet gains normally, but significantly extends the gestation length of her pregnancy. If this response parallels in function that seen in mothers fed a low-protein diet, it appears to be an attempt to compensate for and to minimize the effects of the protein deficiency on the growth and development of their infants.

Despite these efforts at adjustment, a few soft signs of abnormality can be detected in the performance of their infants. They have abnormally strong clasping and clinging responses, and they fail to release the response at the appropriate time when laid down on a table. Absence of evidence of deficiency on the other tests does not, of course, constitute evidence of its absence; equally, we are not in a position to assert that the deficiency is greater than is revealed by these tests.

Considerable evidence exists for rats and other species to suggest that inadequate maternal Mn metabolism results in infants that are defective in their responses to gravity and to motion. The pioneering work of Erway, Hurley, and Fraser (Hubbard, 1971) adequately demonstrated the ataxia and inability to maintain body posture in water. The present results suggest that similar or at least related effects occur in primates. The effects we are looking for are very subtle and indirect, but they can be realized even with tests that are very crude. The present data suggest that the infant's behavior may not be completely normal with respect to gravity.

Our methods of inducing Mn deprivation are equally imprecise, and we cannot say either that no Mn was ingested during pregnancy or that pre-

experimental stores did not ameliorate the deficit. Still, we are hesitant to leap to the obvious implication that the behavioral changes would have been greater if only we had been able to reduce even further the Mn available to the fetus and the growing infant. We believe it legitimate to draw the conclusion that Mn is essential for behavioral development and that its absence or diminution elicits adaptive changes in the mother during her pregnancy, in particular a lengthening of gestation, which may not be totally successful.

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