A Behavioral Review of Trace Element Deficiencies in Animals and Humans

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Trace elements in the diet have been found to be essential for the normal development of animals and humans. In animals, iron, zinc, copper, and manganese deficiencies early in life cause brain injury followed by various behavior deficits such as tremors, convulsions, reduced activity, increased emotionality, increased aggression, less tolerance for stress, retarded learning, and memory impairment. Little or no research has been done on the other trace elements. In view of the widespread malnutrition in the human population, animal research can potentially provide a better understanding of the effects of and long-term solutions to human starvation.

Key words: malnutrition, undernutrition, learning disabilities, brain development, memory deficits

INTRODUCTION

Trace element deficiencies in the human population rarely occur in isolation. The human typically suffers a multitude of nutritional deficiencies in addition to which the human is simultaneously exposed to many diseases and environmental toxins. These compounding factors make it more difficult to understand the precise effects of a specific trace element deficiency. There is also the potentially serious problem of fad diets. Many of these diets have been developed by individuals who have little or no scientific expertise in the field of nutrition. Consumption of these diets by pregnant and/or lactating women poses a serious threat to their offspring. The general population may also be at risk when consuming these inadequate diets.

The primary purpose of this paper is to provide a review of the possible effects of trace element deficiencies on brain development and subsequent aberrant behavior. The emphasis will be on animal research, but where it is relevant and available, human research will be briefly cited.

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An essential trace element is defined as an element that must be present at some minimal level in the diet in order to maintain normal metabolic, physiologic, and growth levels in animals and humans. Iron, zinc, copper, manganese, magnesium, nickel, and iodine are among the 14 essential trace elements currently listed by the American Institute of Nutrition. Other elements, such as boron and arsenic, are currently under investigation. Each element has its own daily minimum requirement. In this review, behavior is defined in its broadest sense but special emphasis will be placed on learning and memory because any permanent impairment in these areas is devastating to animals and humans.

It is hoped that sound behavioral research utilizing animals will ultimately provide useful knowledge to the clinical psychologist, nutritionist, neuropsychologist, and medical practitioner. A second purpose of this paper is to point out some of the difficulties of doing research in the area of nutrition and animal behavior.

A third purpose is to illustrate the imbalance of behavioral research for many trace elements. For instance, 73 of the 102 research papers in this review involved iron and zinc. There are 19 papers for copper and manganese and 11 papers for magnesium, iodine, and nickel. We were unable to find any behavioral research papers on the remaining trace elements. This imbalance is perpetuated when animal and human studies are considered. The use of an animal model in research is a common procedure to supplement knowledge gained in human clinical studies. Unfortunately, only two elements, iron and zinc, have been studied in both animal and human experiments on behavior. On four elements—copper, manganese, magnesium, and nickel—we have only animal data, while for iodine there are only human data. Since all of these elements are classified as essential for normal development and health by the American Institute of Nutrition, the need for behavioral research, both animal and human, on elements other than iron and zinc is obvious. We are not suggesting that research on iron and zinc be terminated or reduced but rather that more research is needed on the other elements.

IRON

As recently as 1979, it was reported [Leibel et al., 1979b] that there were no substantial data to suggest that iron deficiency impaired learning, intelligence, attention, or motivation in animals. The review further stated there was no evidence that iron deficiency damaged the brain. Recent research suggests that these conclusions need to be modified.

Bernhardt [1936] studied the effects that iron deficiency in rats had on learning. The rats were made iron deficient (ID) after weaning and then tested on a maze. The performance of ID rats was inferior when compared to the performance of the normal control rats, but the results were not statistically significant. Using nutritionally rehabilitated rats, Scarpelli [1959], Weinberg [1982], and Weinberg et al. [1979] also found no significant differences in learning between ID and normal rats. Weinberg et al. [1979] reported that rehabilitated ID rats were superior in performance in a passive avoidance task when compared to normal control rats. However, Findlay et al. [1981] also tested rats who were ID during the gestation and/or lactation periods. They found little effect on the acquisition or extinction of passive avoidance tasks for any period of deprivation. When rehabilitated ID rats were compared to weight control and normal rats in an active two-way avoidance procedure, no significant differences in
performance were found [Weinberg, 1982]. However, Massaro and Widmayer [1981] did find performance differences between ID and normal rats in an incidental learning task. The ID rats were maintained on the iron-deficient diet throughout the behavioral experiment. In this experiment, young adult rats were trained to discriminate between two visual stimuli for a food reward. ID rats could solve the simple visual discrimination task as well as control rats. After a number of trials, an auditory (redundant) stimulus was associated with the correct visual stimulus. When tested on just the auditory stimulus, it was found that the ID rats did not associate the auditory stimulus with the food reward, while the normal rats did make the association. The results suggested that ID rats were both less capable of incidental learning and deficient in the utilization of environmental cues.

There is a possibility that the poor performance of the ID rats in the Massaro and Widmayer [1981] study may have been due to a general debilitation of the animals and not due to an impaired learning capacity. However, a recent experiment [Yehuda et al., 1986] suggests that rats fed an iron-deficient diet after weaning may indeed suffer a long-lasting learning deficit. Prior to testing in a water maze, five groups of 42-day-old rats were fed an ID diet for 0,7,14,21, and 28 days. The ID diet was continued throughout testing. A significant performance deficit was observed for all ID groups and, as might be expected, the performance of the 28-day ID groups was inferior to the performance of the 21-day ID group, which in turn was inferior to the 14-day ID group, and so on. After this phase of the experiment was completed, the 28-day ID group was fed an iron-supplemented diet for 21 days and then retested in the water maze. Interestingly, the rehabilitated ID rats showed no improvement in performance. These results suggest the possibility of a permanent learning disability and are of particular interest because prior experiments, both human and animal, suggest that inducing ID after weaning does not cause irreversible cognitive impairment. Regrettably, comparing results from different experiments is complicated by the fact that Yehuda et al. [1986] used a water maze (escape) while other investigators used dry mazes (food reward), passive avoidance (electric shock), etc., to motivate and test their animals.

There have also been a series of experiments studying the effects of iron deficiencies on physical activity. When animals are ID they are less active in both a voluntary activity procedure and a forced running-to-exhaustion procedure [Edgerton et al., 1972, 1977, 1982; Finch et al., 1976; Glover and Jacobs, 1972; Ohira et al., 1981; Weinberg et al., 1980; Youdim and Green, 1977]. This reduction in activity is observed only while the rats are ID and is usually corrected by iron therapy [Edgerton et al., 1972; Glover and Jacobs, 1972; Ohira et al., 1981]. The data further suggest that the reduced activity is not due to brain injury but probably to a disruption in a peripheral mechanism [Edgerton et al., 1972; Finch et al., 1976; Glover and Jacobs, 1972; Leibl et al., 1979a; Youdim and Green, 1977].

There have been some attempts to correlate the reduced activity to stress and changes in the corticoid response, but the results have been contradictory and ambiguous [Weinberg et al., 1979, 1980, 1981; Williamson et al., 1981]. Interestingly, ID rats show a reversal of the circadian rhythm in that they are more active in daylight than in the dark [Edgerton et al., 1977; Weinberg et al., 1980]. However, a study by Dallman et al. [1984] found a normal circadian pattern in ID and control rats. Nevertheless, the overall spontaneous activity of ID rats is less than that of the normal control rats [Edgerton et al., 1977; Weinberg et al., 1980].
These behavior results from animals are similar to what has been observed in humans. Iron-deficient infants and children have been found to be less active [Lozoff et al., 1982; Pollitt et al., 1981, 1982], less attentive [Howell 1971; Pollitt et al., 1981, 1982, 1978; Webb and Oski, 1974], have reduced sensory-motor functions [Lozoff et al., 1982; Oski and Honig, 1978], have poor scholastic achievement [Soemantri et al., 1985; Webb and Oski, 1973, 1974], and have learning and memory deficits [Pollitt et al., 1978, 1981, 1982]. The inferior performance on IQ tests and scholastic achievement by ID children has generally been attributed to an inability to attend to relevant cues in the situation [Howell, 1971; Pollitt et al., 1982; Webb and Oski, 1974], but iron therapy usually corrects the problem [Howell, 1971; Oski and Honig, 1978; Pollitt et al., 1982; Soemantri et al., 1985]. Sulzer et al. [1973] also found lowered IQ scores among ID children, but the results were inconclusive due to a confounding of other uncontrolled variables, namely: motivation, attention, and activity. Although most studies found that ID children performed poorly on cognitive tests, Deinard et al., [1981] reported that 11–13-mo-old ID infants performed as well as iron-replete infants on a variety of mental and cognitive development tests. The authors attribute the discrepancy to differences in methodology in their research. Finally, ID adults were less capable of strenuous physical labor [Basta et al., 1979; Gardner et al., 1975; Pollitt and Leibel, 1976], but again iron therapy corrected the problem [Basta et al., 1979]. This necessarily short review of the human literature pertaining to behavioral deficits associated with ID does not include the related metabolic and physiologic variable of IDs in humans. For a more complete review of the human literature, the reader is referred to articles by Evans [1985] and Pollitt et al. [1986].

A few studies have observed brain injury due to perinatal ID, but the injuries were subtle and not gross [Dallman et al., 1975; Leibel, 1977; Tojyo, 1983; Youdim and Green, 1977]. Iron deficiency induced after weaning causes an irreversible reduction in iron concentration in the brain [Weinberg, 1982; Weinberg et al., 1979; Youdim and Ben-Shachar, 1985] as well as injury to the dopamine neurons in the brain [Youdim, 1984; Youdim and Ben-Shachar, 1985]. These authors have speculated that the dopamine neurons may be involved in learning and memory. These results suggest that if there are any permanent behavior deficits, the deficits may well be minimal, and therefore require sensitive behavioral testing procedures to measure them.

ZINC

An extensive review of the zinc-deficiency (ZD) literature has recently been published [Halas, 1983]. Therefore, the earlier literature will be summarized rather briefly and only the most recent developments will be reviewed in detail.

In general, the detrimental effects of ZD on animal behavior are widespread. Both severe and mild ZD can produce these behavior impairments. The time of nutritional deficiency, whether during gestation or lactation, or postweaning, or some combination of these periods, does complicate the issue. Despite these complications, a number of firm conclusions can be made with regard to the effects of ZD on animal behavior.

Although there is some controversy, there are sufficient data to suggest that perinatal ZD causes rats [Apgar, 1968; Caldwell and Oberleas, 1969; Caldwell et al.,
1970, 1976; Gordon et al., 1982; Hesse et al., 1979] and monkeys [Strobel et al., 1979] to be less active than normal animals. There are several hypotheses that attempt to explain this observation, but the data do not favor any specific explanation.

Since ZD causes anorexia, pair-fed control dams (PF) are fed the same quantity of the diet as consumed by their paired ZD mates. However, the diet consumed by the PF dams is adequate in zinc. The ad libitum control dams (AL) are fed a zinc-adequate diet ad libitum. With these dietary conditions, a variety of behavioral testing procedures found that ZD rats were more emotional [Caldwell and Oberleas, 1969; Caldwell et al., 1970, 1976; Hesse et al., 1979] and less tolerant of stress [Halas et al., 1976; Halas and Sandstead, 1975] than PF and ad libitum–fed normal control (AL) rats. Zinc-deficient rats spent significantly more time in a defensive immobility position [Hesse et al., 1979], had more and larger gastric ulcers [Halas, 1983], had longer latencies in passive avoidance [Hesse et al., 1979; Tinius et al., 1986], and were unable to learn a two-way avoidance task due to an inability to tolerate stress [Halas et al., 1976; Halas and Sandstead, 1975]. However, a recent study [Golub et al., 1983] reported that adult mice whose dams were fed a mild (9.00 ppm zinc) or severely (2.00 ppm zinc) ZD diet had shorter latencies in a passive avoidance test. Other than the possibility of species differences, there is no reasonable explanation as to why ZD rats should have abnormally long latencies and ZD mice have abnormally short latencies in the passive avoidance test. Since ZD rats are more emotional [Caldwell and Oberleas, 1969; Caldwell et al., 1970, 1976; Hesse et al., 1979], it is reasonable to predict they would have longer latencies. Unfortunately, there is no data on the level of emotionality in mice due to ZD.

The willingness to affiliate or socialize with other rats as well as the aggressive tendencies of nutritionally deprived rats were also studied. When they were given a choice, rehabilitated PF and AL rats were less willing to affiliate or associate with rehabilitated ZD rats [Peters, 1978]. Zinc-deficient rats were also less willing to affiliate with other ZD rats. They preferred to associate with PF or AL rats. Furthermore, rehabilitated ZD rats were significantly more aggressive than either the PF or AL rats [Halas et al., 1975, 1977a, 1977b; Peters, 1978]. There was a tendency for PF rats to be more aggressive than AL rats [Halas et al., 1975, 1977b; Peters, 1978].

Adult rats whose dams were fed a severely ZD diet (<1.0 ppm/zinc) during lactation were tested on a 17-arm radial maze [Halas et al., 1983]. When compared to the performance of PF and AL rats, the short-term memory (STM) and learning ability of the rehabilitated ZD rats were found to be impaired. Long-term memory (LTM) was not impaired. These results suggest that ZD animals are slow to learn, but that once they do learn, their LTM is normal. A significant learning deficit was observed in the PF rats, but it was not as severe as the impairment in the ZD rats. Neither STM nor LTM was impaired in the PF rats. Very similar results were obtained when rat dams were fed a mildly ZD diet (10.00 µg/g zinc) throughout gestation and lactation [Halas et al., 1986]. In another study [Halas et al., 1979], rat pups whose dams were fed a severely ZD diet during lactation failed to learn a light-shock association. This failure disrupted their LTM of the event. Testing occurred after the pups were nutritionally rehabilitated.

The effects of mild ZD (10.00 µg/g zinc) on behavior and hippocampal morphology have been reported [Halas and Kawamoto, 1984; Kawamoto and Halas, 1984]. Rehabilitated offspring of dams who were mildly ZD during gestation and
lactation were tested on the 17-arm radial maze. The STM and learning ability of the ZD rats were impaired when compared to PF and AL rats. The rats were killed after the completion of the behavior experiments and eight regions of their hippocampi were examined histologically. All eight regions of ZD hippocampi were significantly reduced in area, had greater neuronal density, and had fewer neurons than the PF and AL hippocampi. The PF hippocampi suffered a modest degree of injury when compared to the AL hippocampi. Severe ZD (<0.5 μg/g zinc) during the late prenatal and/or early postnatal periods has been found to significantly reduce the weights of the cerebellum and hippocampus in rats [Dreosti et al., 1981]. In this same study, the activities of two enzymes (2', 3'-cyclic nucleotide 3'-phosphohydrolase and glutamic acid dehydrogenase) critical to neural function were substantially lowered in the cerebellum and hippocampus. These results are of particular significance because an intact hippocampus is believed to be a necessary structure for STM and spatial learning [Olton et al., 1979; Olton and Samuelson, 1976].

In another study [Massaro et al., 1982], young adult rats were fed a diet mildly deficient in zinc for a period of 17 days. During the zinc-depletion period, the rats were tested for discrimination and transfer learning. The ZD rats did not perform as well as normal control rats in the transfer learning task.

A marginal zinc diet (4.00 μg/g zinc) was fed to pregnant rhesus monkeys starting on the day of conception and was continued until their offspring reached an age of 1 yr [Golub et al., 1985]. The offspring were tested on an extensive battery of behavioral tests and it was observed that ZD infants suffered from lethargy, apathy, and hypoactivity. Although the performance of the ZD infants was inferior on a discrimination reversal task, the investigators did not attribute the behavior deficit to a learning impairment but suggested that a reduced flexibility of behavior was a better explanation. This study illustrates the complexity of doing research in the area of malnutrition and behavior. It is not too difficult to observe a behavior impairment in malnourished animals; the difficulty comes in the interpretation of the data.

In summary, when ZD was imposed in animals during the perinatal period, the offspring usually suffered permanent behavioral abnormalities. When chronic ZD was implemented during adulthood, similar aberrant behavior may appear, but zinc therapy can restore the animal to normal behavior.

The human literature has been reviewed extensively and therefore the effects of ZD will be described briefly. Young adults who had subsisted on a cereal diet throughout their lives suffered from dwarfism, hypogonadism, and mental lethargy [Prasad, 1983, 1984; Sandstead, 1986]. They also exhibited irritability, emotional disorder, tremors, and cerebellar ataxia. Overall, they had the appearance and physical size of 8-10-yr-old children. An adequate diet plus zinc supplementation resulted in normal, adultsize genitalia, development of secondary sexual characteristics, and a significant growth spurt. Even though ZD tends to be more severe and prevalent in Third World countries [Prasad, 1983, 1984], it also exists in European countries [Jameson, 1976]. Jameson [1980] has postulated a correlation between maternal ZD and congenital malformation of the central nervous system; unfortunately, a definitive evaluation of the cognitive abilities of rehabilitated humans has not been done. Such an evaluation would help to determine if there were any congenital malformations of the CNS since a histological evaluation must wait until the subjects die, which could be several decades from now. There is some concern on this issue because permanent learning and memory disabilities have been observed in rehabilitated adult rats whose dams suffered ZD during gestation and/or lactation [Halas et al., 1983, 1986].
Prolonged consumption of a ZD diet in young humans produces severe physical and possibly mental abnormalities. Zinc therapy can reverse much of the physical problems but the possibility of an irreversible intellectual incapacity remains an unanswered question.

MANGANESE

Manganese deficiency in pregnant animals produces offspring that suffer from congenital ataxia, uncoordination, tremors, lack of equilibrium, and retraction of the head [Everson et al., 1959; Hurley, 1981, 1983]. Primate offspring appear to suffer similar behavioral abnormalities [Riopelle and Hubbard, 1977; Riopelle et al., 1980]. The ataxia and lack of equilibrium is caused by abnormal development of otoliths in the inner ear, which are necessary for normal vestibular function [Erway et al., 1966, 1970]. When a combined manganese- and zinc-deficient diet was fed to pregnant monkeys, the offspring demonstrated timidity and labile emotionality [Riopelle et al., 1980]. Dietary rehabilitation does not reverse these abnormalities. The offspring of guinea pigs who were deprived of manganese throughout gestation suffered permanent neurological damage [Chandra, 1983], but the learning and memory capabilities of these animals have not been studied.

COPPER

Copper deficiency during the perinatal period in animals produces a variety of behavioral and brain abnormalities in the offspring. The offspring suffered from reduced activity, hyperirritability, convulsive seizures, and loss of balance. All of these behavioral abnormalities were associated with a wide variety of brain injuries. Reduced concentrations of noradrenaline [Hunt, 1980] and norepinephrine [Prohaska and Smith, 1982] as well as hypomyelination [Dipaolo et al., 1974; Zimmerman et al., 1976] were observed in the brains of copper-deficient animals. Prohaska and Smith [1982] suggested that reduced norepinephrine concentration may be responsible for the widely observed phenomenon of hypomyelination. Furthermore, the overall brain weight, including the cerebral cortex, corpus striatum, and cerebellum, was significantly reduced [Carlton and Kelly, 1969; Everson et al., 1967]. Dietary rehabilitation does not reverse these abnormalities. Although the copper-deficient offspring have not been tested for learning and memory disabilities, a recent study reported that normal animals who made a large number of errors on a radial maze were later found to have unusually low concentrations of copper in the hippocampus [Chafetz and Bernard, 1984]. In another study [Thorne et al., 1983], weanling rats who were fed a low (0.7 ppm Cu) or a marginal (3.9 ppm Cu) diet for 10 wk were not significantly different in muricide, open-field activity, pain sensitivity, or learning and memory when compared to weanling rats fed a normal copper diet (9.1 ppm Cu). This study suggests that if a trace element deficiency occurs after weaning, little or no behavioral deficit may be observed.

MAGNESIUM

The major behavioral deficit in adult animals due to severe magnesium (Mg) deficiency seems to be convulsive seizures [Harriman, 1974, 1978, 1980]. Startle response to novel stimuli and tremors have also been observed [Dalley et al., 1975;
Harriman, 1974]. The seizures become progressively worse as the Mg deficiency is prolonged, resulting ultimately in death if Mg therapy is not initiated [Harriman, 1978]. Convulsions during open-field tests among Mg-deficient gerbils occurred with greater frequency than among gerbils deprived of five essential trace elements and six minerals [Harriman, 1980]. Young kittens fed a Mg-deficient diet (50 mg/kg Mg) for a period of 8 wk exhibited muscular weakness, hyperirritability, and convulsions [Chausow et al., 1986]. In the early stages, the Mg-deficient kittens were readily startled by quick movements or loud noises. Convulsions induced by loud noises were observed in the latter stages of Mg deficiency. In an attempt to measure memory deficits, normal adult rats were initially trained on a bar-pressing avoidance response [Dalley et al., 1975]. After they had learned to avoid the shock, the experimental rats were fed a Mg-deficient diet for 10 days. The performance of the Mg-deficient rats gradually deteriorated over the 10 days when compared to the performance of normal control rats. Although the performance of the Mg-deficient rats was impaired, the data do not suggest that memory was impaired. Since Mg deficiency affects both the nervous system and the muscle system, the poor performance of Mg-deficient rats was probably due to the physiological debilitation of the animals, and therefore the behavior data probably have little psychological significance. Another study reports that young adult rats fed an Mg-deficient diet were inferior in learning a water maze, were less active, and may have exhibited altered motivation when performing behavioral tasks [Oberleas et al., 1978]. Magnesium therapy can reverse some of these deficits.

**CHROMIUM, COBALT, IODINE, MOLYBDENUM, SELENIUM, AND THE ULTRATRACE ELEMENTS**

To date there has not been any research in animals on the effects of the remaining trace elements on behavior. Despite this lack of animal behavioral experimentation, clinical studies suggest there is a potential need for research in this area. Although cretinism, accompanied by severe mental retardation, is a common result of iodine deficiency in humans, less severe development deficits have also been observed [Connolly et al., 1979; Hetzel and Potter, 1983]. Presumably normal children of iodine-deficient mothers exhibited subclinical deficits when they were tested on motor tasks requiring both speed and accuracy. It was felt this subtle motor deficit could have led to a long-term developmental disadvantage if appropriate therapy had not been implemented. In animals, retardation in brain development has been reported in the offspring of rats, guinea pigs, and sheep who were iodine deficient during pregnancy [Hetzel and Potter, 1983; McIntosh et al., 1981]. For instance, iodine deficiency in rats was associated with reduced fetal brain weight, as well as delayed maturation of the cerebellum, hippocampus, and motor cortex. Reduced myelination throughout the brain was also observed. Unfortunately, no behavioral research was done on these animals. A number of ultratrace elements (arsenic, silicon, nickel, and vanadium) have recently been discovered to be essential for life [Nielsen, 1982]. Behavioral research on the ultratrace elements has been nonexistent with the exception of one study on nickel [Nielsen et al., 1974]. After three generations of nickel deficiency, it was observed that nickel-deficient rats were less active than normal control rats. No other behavioral animal research has apparently been done in this field. Deficiencies in humans have not been reported for any of the ultratrace elements [Nielsen, 1982].
DISCUSSION

All of the animal data suggest that any trace element deficiency that occurs during the perinatal period usually results in offspring that display some form of aberrant behavior that is not reversible by future nutritional therapy. If the deficiency is induced after weaning, abnormal behavior symptoms may appear while the animal is nutritionally deprived, but appropriate nutritional therapy can usually rehabilitate the animal. Iron, zinc, and iodine are the only trace elements the deficiencies of which have been studied in humans. Iodine deficiency in pregnant women definitely results in mentally retarded offspring; iron deficiency may cause cognitive impairments; the long-term consequences of zinc deficiency are unclear at this time.

Behavioral research on trace elements in animals can be divided into two broad categories which roughly approximate the two types of malnutrition found in humans:

1. A deficient diet can be fed to pregnant and/or lactating dams. An adequate diet is then fed to the offspring after they have been weaned. The rehabilitation offspring can then be tested on various behavioral tasks.

Research by Dobbing [1968, 1974], Smart and Dobbing [1971], and Smart et al. [1973] suggests that there is a period of vulnerability when the brain is most susceptible to injury due to nutritional deficiency. This period occurs during gestation and lactation when the brain is in the process of rapid development, but once the brain matures it is highly resistant to nutritional insult. Therefore, nutritional deficiencies should be induced during the gestation and/or lactation periods in order to investigate possible permanent brain injury and subsequent aberrant behavior.

2. Studying the acutely malnourished animal is equally important because large segments of the human population suffer acute malnutrition. Acute malnutrition occurs in the elderly, young adults, and children, but unfortunately the short-term and long-term consequences of acute malnutrition are not understood for many of the trace elements. Testing an animal while it is acutely malnourished provides information on whether the animal can perform the specific task, but because of the debilitated state of the animal, the underlying cause of the poor performance cannot always be determined. For instance, failure to perform well in a learning and/or memory task may be due to impaired cognitive functions, but it may also be due to lack of motivation, inability to attend to the task, sensory-motor dysfunctions, altered emotional state, and other factors. After the animal has been tested while malnourished, the animal should be rehabilitated and then retested on the same task [e.g., Yehuda et al., 1986]. If the performance continues to be poor after rehabilitation, the investigator can have greater confidence in his/her interpretation of the data. If, however, the performance returns to a normal level after rehabilitation, then the behavioral deficit is obviously transient and the mental abilities of the animal have not been permanently impaired.

The same problem exists when testing humans. Iron-deficient children frequently do poorly in learning and memory tasks [Pollitt et al., 1982, 1981, 1978; Webb and Oski, 1973, 1974; Oski and Honig, 1978]. After iron therapy, the children perform at a normal level on these tasks [Pollitt et al., 1982, 1978; Oski and Honig, 1978]. These children apparently did not suffer any permanent brain injury or learning impairment while they were iron deficient. Their poor performance can more readily be attributed to other factors such as poor motivation, lack of attention, perceptual
disturbances, physical debilitation, or other dietary deficiencies [Leibel et al., 1979b; Oski and Honig, 1978; Pollitt and Leibel, 1976; Pollitt et al., 1982; Webb and Oski, 1974].

Appropriate behavioral testing procedures should be selected when studying the effects of nutrition on behavior. All too often behavioral testing procedures are selected by scientists because they are easy to use and not because they have proven to be valid measures of some specific behavior. Two prime examples are the passive avoidance test and the open-field test. The passive avoidance test is frequently used to "test" malnourished animals for learning or memory deficit. The use of a learning or memory interpretation for this type of behavioral testing procedure is inappropriate. For malnourished animals, the observed differences in passive avoidance behavior can be more readily attributed to differences in motivation or emotion. The open field test is also widely used, but an excellent review of the literature has shown that the interpretation of the data is very difficult [Walsh and Cummins, 1976].

In addition, some authors [Golub et al., 1983] have criticized the use of food reward in learning experiments because animals who have experienced a dietary deficiency early in life exhibit a different motivation for food as compared to normal animals. The criticism is valid [Halas et al., 1980; Smart and Dobbing, 1977], and therefore other motivators such as a mild electric shock, or water deprivation, or escape from water have been suggested as a vehicle for testing learning and memory deficits in rehabilitated malnourished animals. Unfortunately, early nutritional deprivation alters many behavioral mechanisms such as food and water motivation, attention, curiosity, exploration, emotion, pain sensitivity, social affiliation, and physical activity. All of these mechanisms have an impact on the performance of the animal in a behavior test. For this reason, it seems highly unlikely that there will ever be an experiment in which all of these variables can be rigidly equated for both normal and malnourished animals. The scientist must be aware of these problems and design the experiments accordingly.

Food reward can be used in studies where animals are exposed to a dietary deficiency early in life if the experimenter is aware of the effects of the deficiency on food motivation. Many studies involve three groups of animals: the normal control, the dietary-deficient group, and pair-fed or weight control group. The food motivation of each group can be accurately measured by using a progressive ratio reinforcement schedule in an operant conditioning box [Halas et al., 1980]. This information can then be used to evaluate data obtained in other studies such as maze learning [Halas et al., 1983; Halas and Kawamoto, 1984; Kawamoto and Halas, 1984].

Another guideline to be followed in conducting research on nutrition-behavior interactions concerns replication. The results of a study should be replicated within the original laboratory before they are submitted for publication, and this is especially true if the study involves an interdisciplinary problem studying a number of variables such as nutrition, behavior, biochemistry, and neuroanatomy. These interdisciplinary problems are potentially the most interesting and challenging, but their inherent complexity increases the probability of not having all of the experimental variables adequately controlled. The data from such a poorly controlled experiment may very well be unreliable or random; therefore replicating the study will help to determine if the data are reliable or stable.

Genetic differences constitute another variable which can cause problems in the interpretation of experimental data. Although no behavioral data were reported, it
was observed that perinatal deficiencies of manganese, copper, and zinc caused different morphological abnormalities in different strains of mice [Hurley, 1976; Hurley and Bell, 1974]. These data suggest that some strains of mice are apparently more sensitive to a specific trace element deficiency while other strains are more resistant to the same deficiency. Therefore, the behavioral testing of different strains of animals who were fed the same deficient diet may produce quite different data.

Studying the interaction of two or more trace element deficiencies or the interaction of an essential trace element and a toxic element also increases the complexity of an experiment. A perinatal deficiency in zinc [Halas and Kawamoto, 1984; Kawamoto and Halas, 1984], copper [Carlton and Kelly, 1969; Everson et al., 1967; Hunt, 1980], iron [Dallman et al., 1975; Leibel, 1977; Tojo, 1983; Youdim and Green, 1977], or manganese [Chandra, 1983] can injure the brain. What the consequences might be if an animal or human were to suffer multiple deficiencies is presently unknown, but the interactive effects may be multiplied. In order to determine the effects, each trace element would have to be studied independently and then in combination. Unfortunately, very little behavioral research has been done on the individual trace elements and only one study [Riopelle et al., 1980] has examined the combined effects of two trace elements. The interactive effects of an essential trace element and a toxic element on brain development and subsequent behavior is another area of interest. For example, zinc deficiency in animals increases the retention of lead in the brain [Bushnell and Levin, 1983], with the subsequent result that the animals are seriously impaired in learning a maze; yet the area of interactive effects suffers from a paucity of research. In view of the fact that environmental pollution and malnutrition may coexist, particularly in large cities, these interactive effects are a cause for social concern and need to be researched.

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