humans the severe hypoxia that we used to alter pain threshold in animals.

In comparison of the 2 species, ground squirrels maintained pain sensitivity with more severe hypoxia than could the rat, but the rat's righting ability was much more susceptible. The response to pain as we measured it may be a spinal reflex(1) while the righting response utilizes higher centers. This indicates that the ground squirrel supported some functions, perhaps at the expense of others, during oxygen deficiency. The greater hypoxic survival of the ground squirrel could be due to partial functional depression which conserves energy for maintenance of more vital functions. Which functions are maintained and how they are maintained, and which are depressed are avenues of future research.

Summary. The response to thermal pain in the rat was decreased by breathing 7.5% oxygen and abolished with 5% oxygen. The 13 lined ground squirrel had no alteration of response with 5% oxygen but did with 2.5%. However, the righting reflex of the ground squirrel was much more susceptible to hypoxia than that of the rat. Loss of righting ability occurred at a barometric pressure of 183 mm Hg for the ground squirrel and 154 mm Hg for the rat in simulated altitude ascent in a chamber.

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Threshold and Pattern of Electroshock Seizures in Ataxic Manganese-Deficient Rats.* (26332)

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Offspring of manganese-deficient females exhibit a congenital ataxia characterized by incoordination, loss of equilibrium, retraction of the head, and tremor. This abnormal condition has been shown to result from an irreversible defect or defects occurring between the 14th and 18th days of gestation in rats (1). Recent studies, both physiological and morphological, have revealed abnormalities of the inner ear in manganese-deficient young; these studies indicate that impaired vestibular function may be involved in the etiology of the ataxic syndrome(2,3,4). However, certain manifestations of the ataxia, such as tremor, could not be attributed to loss of vestibular function. It was therefore of interest to examine another aspect of nervous system function.

The present communication describes experiments investigating brain excitability, as measured by response to electroshock, in offspring of normal and manganese-deficient rats.

Materials and methods. Animals and diets. Ataxic, manganese-deficient offspring were produced by procedures similar to those previously reported(3). Briefly, female rats of the Sprague-Dawley strain were maintained from time of weaning on a fortified milk diet deficient in manganese. Control groups were treated in the same way, except that manganese was added to the diet.‡ At maturity the animals were mated with normal males.

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† U.S.P.H.S. Pre-doctoral Fellow.

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Deficient females continued to receive the manganese-deficient milk ration during pregnancy and lactation, except for 24 hours on the 16th day of gestation,§ when they were given the manganese-supplemented ration used for control animals.‖ After weaning at 28 days of age, the young were maintained on diets received by their mothers (either manganese-deficient or manganese-supplemented). All of the manganese-deficient rats used in this study showed marked ataxia.

Electroshock technics. The apparatus and methods for producing electroshock seizures in rats have been described in detail by Woodbury and Davenport(5), and are at present used routinely in several laboratories. These methods are: 1. *minimal electroshock seizure threshold (EST) test,* as a measure of brain excitability; 2. *maximal electroshock seizure (MES) pattern test,* as a measure of spread of excitation; and 3. *return of the righting reflex (RRR) test,* as a measure of brain recovery processes.

The EST is defined as the minimal amount of alternating-current in milliamperes (ma), delivered for 0.2 seconds through corneal electrodes, which will just elicit barely detectable clonic convulsions of ears, jaws, and forepaws without loss of equilibrium and lasting only a few seconds. The MES is produced by delivering 150 ma for 0.2 seconds through corneal electrodes. With this supramaximal stimulus, normal rats show typical maximal (tonic-clonic) convulsions. Total duration of the seizure, duration of the tonic phase with its flexor and extensor components and duration of the clonus are determined for each animal. The RRR test measures duration of the depression period elapsing between the end of MES and the spontaneous rise of the animal.

In the present experiments, EST was determined 2 or 3 times a week for 2 weeks or until threshold stabilization occurred. At least one day of rest was allowed between tests. Three days after the last EST test, the MES pattern and the RRR were determined. Body weight was measured at beginning and end of the electroshock testing period.

Results. The effects of manganese deficiency on EST in rats are summarized in Table I. The EST was lower in the deficient rats than in the corresponding normal controls of the same age; the difference in EST was statistically significant.

Body weight of the manganese-deficient rats was smaller than that of the controls. This is in agreement with previous experiments(6) in which a slower weight gain was observed in deficient rats as compared with their controls. On the other hand, the electroshock testing did not interfere with the growth of either control or deficient rats as judged by the increase in body weight with time during the experimental period.

The effects of manganese deficiency on MES and RRR in rats are also presented in Table I. A statistically significant decrease was observed in duration of the flexor tonic phase in the manganese-deficient rats as compared with the manganese-supplemented controls. The other parameters of the maximal convulsions were similar in the two groups of animals. The RRR was longer in the deficient rats but the difference with the controls was not statistically significant.

Discussion. The data presented indicate that ataxic offspring of manganese-deficient rats, maintained on a deficient diet, show a marked increase in brain excitability as measured by an EST significantly lower than in normal rats. It may be noted that the manganese-deficient rats were smaller in size than the corresponding controls of the same age. In view of the positive correlation between body weight and EST described elsewhere (7) it might be postulated that the lower EST in the manganese-deficient animals is related to the smaller body weight. However, both phenomena, greater excitability and smaller body size, may be concomitant and yet independent expressions of the same metabolic factor, in this case, manganese deficiency.

§ The day of finding sperm in the vaginal smear is considered day zero.

‖ Supplementation for one day during gestation was used as a means of increasing survival of the young. Data on the effects of this procedure on survival will be presented later.

TABLE I. Minimal Electroshock Seizure Threshold (EST), Maximal Electroshock Seizure (MES) Pattern and Postictal Depression in Normal and Manganese-Deficient Rats.

Description	No. of rats	Age, days	Body wt (g) Initial	Body wt (g) Final	EST,* mAmp	MES† pattern, duration of each phase (sec.) Tonic flexion	Tonic extension	Total tonus	Clonus	Total seizure	Postictal depression, min.‡
Normal controls	17	80	181.2§ ±9.4	184.5 ±9.2	21.09 ±.08	3.83 ±.35	11.21 ±.52	15.05 ±.41	17.56 ±1.42	32.61 ±.76	2.06 ±.14
Manganese-deficient	10	80	137.4 ±7.1	143.8 ±7.1	18.60 ±.36	1.67 ±.31	12.90 ±1.15	14.98 ±.47	18.06 ±3.18	33.06 ±3.23	3.11 ±1.42
P‖ (control *vs* manganese deficient rats)					<.01	<.01	<.001	<.01			

* EST is defined as minimal amount of alternating current delivered for 0.2 sec. through corneal electrodes which will elicit barely detectable clonic convulsions of ears, jaws and forepaws without loss of equilibrium and lasting only a few seconds.

† MES were produced by delivering 150 milliamperes of a.c. for 0.2 sec. through corneal electrodes.

‡ Postictal depression after a maximal seizure was the time required to recover righting reflex.

§ Mean ± stand. error.

‖ P values based on t-tests for significance of difference between means.

The increased brain excitability in the manganese-deficient rats is also evidenced by changes in the MES pattern. Toman *et al.* (8) showed that the response to a supramaximal stimulus, such as the one used in producing maximal seizures, is independent of current density and of the passive influence of body mass. In the present study, a shorter duration of the tonic flexion was observed during the MES in the manganese-deficient rats. Duration of the tonic flexion is considered to be proportional to intensity of the inhibitory state which must be overcome before the maximal spread of excitation occurs. Maximal spread of excitation is indicated by appearance of the tonic extension. Thus, the shorter the flexion, the shorter the period of inhibition and the greater the excitability. The fact that a higher degree of convulsability could be demonstrated in the manganese-deficient animals with both tests used, whether correlated (EST) or independent (MES) from body mass, adds further support to the conclusion that brain excitability is increased by manganese deficiency.

These results are consistent with the gross behavior of the deficient animals: they were hyperactive and appeared to be hypersensitive to stimuli.

Previous researches seeking to determine the origin of the congenital ataxia of manganese-deficient animals have failed to reveal any histological lesions in the central nervous system(9,10,11,1). The present study may indicate that the brain is involved in the ataxic syndrome of manganese-deficient offspring. This work does not show, however, whether the increased brain excitability observed in this study is the result of an irreversible congenital defect, as is known to be the case in regard to ataxia(1), or whether it results from a deficiency of circulating manganese as such.

Summary. Electroshock convulsions were produced in ataxic manganese-deficient rats. The threshold for minimal seizures was significantly lower in the manganese-deficient than in the control animals. During a maximal seizure, duration of the tonic flexor phase was markedly shorter in the deficient than in control rats; duration of the other phases and of postictal depression were similar in both groups. The data are interpreted to mean that brain excitability and convulsability are increased in ataxic manganese-deficient rats; these data may indicate that the brain is involved in the ataxic syndrome characteristic of offspring of manganese-deficient animals.

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Facilitation of Pituitary-Induced Frog Ovulation by Progesterone in Early Fall.* (26333)

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Since the original investigations of Wolf (1) on *Rana pipiens* and Houssay *et al.*(2) on *Bufo arenarum,* induction of ovulation in anurans by homoplastic pituitary injections or implantations has become a common laboratory procedure. Freshly-ovulated eggs for fertilization studies and embryological material may be obtained at any time during the academic year, but minimal effective dosages of pituitary glands vary widely with the season(3). Nearly complete ovulation in March or April may be induced in the average frog 24 hours following injection of 1 or 2 frog anterior pituitary lobes, but 3 to 4 lobes are usually required in January and February. From mid-September often into December, significant ovulation in a single female necessitates slaughter of 6 to 8 other females, only for their pituitary glands; and 48-56 hours must ordinarily elapse following injection before any appreciable response is obtained. Recent success with steroids in inducing ovulation *in vitro* in *Xenopus laevis* (4) and in *Rana pipiens*(5,6) has quite naturally led into a study of possible use of steroid hormones as substitutes for part or all of the large pituitary dosages required for ovulation *in vivo* in the fall.

Methods. Frogs for these studies were obtained from both Vermont and Wisconsin. Suppliers were instructed not to ship animals which had been kept in a warm room. Frogs were stored upon arrvial in large aquaria in a refrigerator (5°C) with a little water, changed frequently. Pituitary glands were dissected from freshly-killed animals, ground in a small mortar, and injected (#27 needle) in a small volume of Ringer's solution into the dorsal lymph sac. Progesterone or other steroid, as aqueous suspension or dissolved in oil, was administered similarly. Following injection, each animal was kept at room temperature in a large finger bowl containing about a half inch of water and covered to prevent escape but loosely enough to admit air. Evidence of ovulation was often indicated by ability to force ova from the ovisacs, but percentages of ovulation were determined by laparotomy.

Results. Very satisfactory responses (Table I) were obtained when frogs were injected with a single pituitary gland in combination with from 0.5 to 5.0 mg progesterone. Ova could be stripped in profusion from recipients within 12-16 hours following injection. The fact that these ova were readily fertilizable and underwent normal development into swimming larvae indicates that ovulation so obtained is entirely normal. At the time of laparotomy (24 hours following injection), the coeloms of these animals were still crowded with freshly-ovulated eggs, signifying that abundant ovulation was still in process.

Small amounts of ovulation were also obtainable by injecting progesterone alone

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