The rat has been observed to exhibit a behavior pattern characterized by violent convulsive seizures when exposed to intense auditory stimulation. Such seizures were first observed by Maier (22) when rats were forced to respond to a difficult problem. "Conflict" imposed by forced reaction in a situation involving an insolvable problem was at first thought to be a necessary determinant of the abnormal behavior (23). Subsequent studies (1, 28, 31), however, demonstrated clearly that auditory stimulation alone was an effective means of eliciting such convulsions and, in addition (29), that the existence of a problem was not of basic importance for the appearance of the seizures.

In previous studies in our laboratories, Patton, Karn, and King (32–34) obtained evidence that nutritional factors play an important rôle in the susceptibility of the rat to convulsive seizures induced by exposure to sound. Furthermore, inanition, vitamin B1, and vitamin B complex deficiencies were found to be contributing factors to increased sensitivity. A significant finding of one of these studies (34) was the fact that such convulsions, associated with low food intake and persisting despite high vitamin B1 supplements, could be quickly reduced by the addition of a group of vitamin and mineral supplements. Reduction in sensitivity occurred even with a low caloric intake. Thus, there was the indication that protection was afforded either by the supplements per se, or that they permitted a more efficient utilization of food substances already present in the reduced food allowance.

A continuation of the work has entailed the investigation of the specific or combined functions of individual components of the vitamin B complex. In the present experiments the effects of pyridoxine deficiency have been studied.

* This investigation is part of a research program supported by a grant from the Buhl Foundation, Pittsburgh.

Part of the data given in this paper was presented at the meeting of the American Chemical Society at Pittsburgh, September, 1943.
A number of investigators have reported the occurrence of a convulsive state in experimental animals subjected to pyridoxine deficiencies. Chick, El Sadr, and Worden (2) found that fits of an epileptiform nature appeared in rats maintained for long periods on a diet deficient in vitamin B6. The seizures were characterized by hyperexcitability and circular running, tonic clonic convulsions, and a comatose recovery period. Daniel, Kline, and Tolle (6) found that similar seizures appeared in young rats while being nursed by mothers maintained on pyridoxine-deficient diets. Although the diets were satisfactory from the standpoint of growth and reproduction, convulsions and failure of the young appeared suddenly toward the end of the lactation period. Such symptoms could be cured or prevented by pyridoxine supplements. Hyperirritability and convulsions accompanying pyridoxine deficiencies have been observed by other investigators in the pig (3, 39, 40), the chick (19), and in the dog (11). In all of these studies auditory stimuli have been found capable of precipitating attacks.

The present experiments were designed primarily to investigate possible relationships existing between the sound-induced seizures studied previously by Patton, Karn, and King and the convulsive fits referred to above which accompany pyridoxine deficiency in the rat. As a technique to impose varying degrees of pyridoxine deficiency upon young animals, the mothers at parturition were placed on pyridoxine-free synthetic diets or on diets having this vitamin present in graded amounts. Seizures which ranged from fatal spontaneous convulsions through a less acute convulsive state capable of being repeatedly elicited by exposure to sound have been observed. A decreasing sensitivity to sound-induced seizures has been correlated with increased dietary levels of pyridoxine.

EXPERIMENTAL

The rats used in these experiments were select male and female breeders1 with relatively uniform genetic and dietary backgrounds. The females were approximately 100 days old when procured and averaged 220 to 250 gm. in weight. When pregnant, the females were transferred to individual cages with raised, screen bottoms to prevent reflection. Purina dog chow constituted the diet through pregnancy until shortly before parturition, when synthetic diets (see below) were used. Litters were reduced to six on the 2nd day after birth and were regularly weaned at 21 days of age.

Two basal experimental diets, essentially free of pyridoxine, were used throughout this work. The major components of these diets (in gm. per 100 gm.) were sucrose 71, casein (S. M. A. vitamin test) 18, fat (Crisco) 5, salt mixture (6) 4, and cod liver oil (Mead’s) 2. To each 20 gm. of this

1 Sprague-Dawley, Inc., Madison, Wisconsin.
mixture were added choline and two levels of thiamine, riboflavin, and pantothenic acid \(^\text{2,3}\) to complete basal Diet I and basal Diet II, as given in the accompanying tabulation. The thiamine, riboflavin, and pantothenic acid content of Diet I was comparable to that employed by Daniel, Kline, and Tolle (6) who found that complete protection against spontaneous seizures was afforded by 40 \(\gamma\) of pyridoxine per day. The amounts of pyridoxine provided in our experiments are shown in Table I.

### Table I

<table>
<thead>
<tr>
<th>Diet No.</th>
<th>Pyridoxine (mg)</th>
<th>Diet No.</th>
<th>Pyridoxine (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>0</td>
<td>IIa</td>
<td>0</td>
</tr>
<tr>
<td>Ib</td>
<td>25</td>
<td>IIb</td>
<td>25</td>
</tr>
<tr>
<td>Ic</td>
<td>50</td>
<td>IIc</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IID</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IId</td>
<td>150</td>
</tr>
</tbody>
</table>

**Auditory Tests**—All auditory tests were given in a sound-proofed box containing a resonated buzzer. (This apparatus has been previously described (30).) A standard exposure of 2 minutes was used for all tests.\(^4\) Five consecutive daily tests were given the females prior to breeding. No trace of sensitivity appeared in any animal during these tests. Three similar tests were again made on all females when the young were weaned. Unless otherwise specified all young animals were given standard tests every other day beginning at the 17th day.

**Pyridoxine-Deficient Diets**—Spontaneous convulsions occurred in young rats suckled by mothers maintained from parturition on the pyridoxine-free diets (Nos. Ia and IIa). 63 young rats from eleven different litters were

\(^2\) The vitamins used in these experiments were generously provided by Merck and Company, Inc., Rahway, New Jersey.

\(^3\) Diets were made up in 2 kilo lots. Vitamins in the amounts required were first mixed with the casein for 2 hours in a ball mill, after which the sucrose and salts were added and the total mixed for 3 additional hours.

\(^4\) For detailed investigations of the essential characteristics of auditory stimuli capable of precipitating seizures, see Morgan and Galambos (27) and Galambos and Morgan (12).
studied (Table II). Spontaneous seizures were observed in forty-nine cases but undoubtedly occurred in many other cases at times when no one was in the laboratory.

There was some variation in the age at which spontaneous convulsions were first observed, but the majority occurred between the 17th and 19th days. Initial symptoms included tremors, chewing movements, face washing, and retraction of the head with extension of the fore legs. Some hours later these animals showed bursts of circular running or crawling,

<table>
<thead>
<tr>
<th>Diet No.</th>
<th>Litter No.</th>
<th>No. in litter</th>
<th>Age at which convulsions were observed</th>
<th>Supplemented with pyridoxine*</th>
<th>Not supplemented with pyridoxine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>days</td>
<td>No. of animals</td>
<td>Amount of pyridoxine</td>
</tr>
<tr>
<td>Ia</td>
<td>1</td>
<td>6</td>
<td>19-23</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>&quot;</td>
<td>2</td>
<td>6</td>
<td>18-20</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>&quot;</td>
<td>3</td>
<td>6</td>
<td>17-19</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>IIa</td>
<td>4</td>
<td>5</td>
<td>15-20</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>&quot;</td>
<td>5</td>
<td>6</td>
<td>14-18</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>&quot;</td>
<td>6</td>
<td>6</td>
<td>12-15</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>&quot;</td>
<td>7</td>
<td>6</td>
<td>13-15</td>
<td>3</td>
<td>10</td>
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<td>&quot;</td>
<td>8</td>
<td>6</td>
<td>18-21</td>
<td>2</td>
<td>50</td>
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<tr>
<td>&quot;</td>
<td>9</td>
<td>6</td>
<td>18-20</td>
<td>1</td>
<td>50</td>
</tr>
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<td>5</td>
<td>11-13</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>&quot;</td>
<td>11</td>
<td>5</td>
<td>12-15</td>
<td>3</td>
<td>50</td>
</tr>
</tbody>
</table>

Total........... 63                   21                               15

* The supplement (pyridoxine hydrochloride in 0.1 ml. of distilled water) was administered daily by pipette immediately after convulsive symptoms were first observed.

gave loud cries, and had convulsions. Seizures recurred at shorter and shorter intervals, with death generally ensuing on the following day. Stimuli of various types, such as sounds, a puff of air, or handling, were all effective in inducing spasms in these animals after the initial symptoms were noticed.

Litters 4, 5, and 6 (cf. Table II) were subjected to the standard auditory tests immediately after the initial spontaneous symptoms of sensitivity were observed. Out of sixteen animals in which spontaneous seizures were later observed, nine convulsions were elicited immediately by the buzzer. Of
the seven remaining animals, four showed hyperactivity in response to the buzzer and three of these developed severe convulsions approximately 5 minutes after being returned to their cages. In Litter 7, auditory tests were given from the 10th day after birth. These tests were ineffective in producing convulsions until after the initial symptoms (tremors, etc.) had appeared spontaneously.

No convulsive seizures were observed in any of the mothers during this time, nor was there any sign of sensitivity in the mothers during three additional sound tests given 21 days after parturition.

It is interesting to note that fewer spontaneous seizures were observed in litters from mothers which had been maintained on Diet Ia (less thiamine, riboflavin, and pantothenic acid content than in Diet IIa). These litters also appeared less well nourished than those on Diet IIa. Records of the food intakes of the lactating mothers receiving Diets Ia and IIa showed great individual variations, but in general there was a decreased consumption of food during the lactation period, from an average of 21.5 gm. at parturition to approximately 16 gm. when the young were weaned.

Effect of Pyridoxine Supplements on Sound-Induced Convulsions—As shown in Table II, pyridoxine supplements in the amounts of 10 and 50 \( \gamma \) of pyridoxine hydrochloride daily were effective in alleviating convulsions in eleven out of fourteen young on Diet IIa, and four out of seven on Diet Ia. The convulsions stopped in most of the supplemented animals as suddenly as they had appeared.

To determine the continued effect of pyridoxine on sensitivity to sound, these animals were maintained on their respective diets and supplements until they were 60 days of age. From weaning at 21 days, auditory tests were given every other day, resulting in a total of twenty tests for each animal.

The total incidence of seizures throughout the 40 day observation period is shown in Table III. It is evident that, although spontaneous seizures were alleviated by pyridoxine, complete protection did not seem to be afforded against exposure to sound. Most of these animals showed a high and continued level of sound-induced seizures with the convulsive type predominating. Two animals on Diet IIa showed an abrupt cessation of seizures and no further signs of sensitivity during these tests.

Incidence of Sound-Induced Seizures in Rats Maintained on Different Levels of Pyridoxine—In the next experiment, 147 young rats from twenty-five litters were observed during periods from just before weaning to 60 to 90 days of age. The litters raised on Diets Ib, Ic, and IIb were subnormal in weight and appearance at weaning. Also, weight gains of all six groups were subnormal during the experimental periods. Regular auditory tests, given every other day, were begun on 17 day-old suckling rats and discon-
continued when it seemed that a stable level of sensitivity had been reached or, as in those groups receiving Diets Ib and Ic, an increasing number of deaths made further group comparisons difficult. No spontaneous convulsions were observed in any of these young animals, but sound-induced seizures were observed in all six groups (Table IV). The incidence of sensitivity for the groups raised on Diets IIb, IIc, IIId, and IIe is shown in Fig. 1.

Significant aspects of the seizure curves of these animals include the levels of sensitivity reached, the elapsed time before maximum sensitivity was attained, and the severity of attack (convulsion or hyperactivity) characteristically shown by a group.

Three additional daily tests were given to all the mothers beginning 21 days after parturition. These tests revealed signs of sensitivity (running) in only one animal (on Diet Ib). The average daily food intake in the mothers at the beginning of the lactation period was 20.5 gm. With a number of individual variations, the average daily food intake decreased to 16 gm. for groups on Diets Ib and Ic, 16.5 gm. for groups on Diets IIb and IIc, and approximately 19 gm. for those on Diets IIId and IIe.

TABLE III
Effect of Pyridoxine Supplements on Sound-Induced Convulsions

<table>
<thead>
<tr>
<th>Diet No.</th>
<th>No. of animals</th>
<th>Pyridoxine supplement</th>
<th>Total No. of tests</th>
<th>Convulsive seizures</th>
<th>Running attacks</th>
<th>No effect</th>
<th>Proportion of total observations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Convulsive seizures</td>
</tr>
<tr>
<td>Ia</td>
<td>4</td>
<td>10</td>
<td>80</td>
<td>34</td>
<td>27</td>
<td>19</td>
<td>42</td>
</tr>
<tr>
<td>IIa</td>
<td>7</td>
<td>50</td>
<td>140</td>
<td>73</td>
<td>49</td>
<td>18</td>
<td>52</td>
</tr>
</tbody>
</table>

TABLE IV
Incidence and Type of Sound-Induced Seizures in Groups of Young Rats Maintained on Diets Containing Different Levels of Pyridoxine

<table>
<thead>
<tr>
<th>Diet No.</th>
<th>Pyridoxine per 20 gm. diet</th>
<th>No. of litters</th>
<th>No. of animals</th>
<th>Average weight at 21 days (gm.)</th>
<th>Total No. of seizures</th>
<th>Type of sound-induced seizure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Convulsive</td>
</tr>
<tr>
<td>Ib</td>
<td>25</td>
<td>4</td>
<td>24</td>
<td>26</td>
<td>333</td>
<td>31</td>
</tr>
<tr>
<td>Ic</td>
<td>50</td>
<td>3</td>
<td>18</td>
<td>28</td>
<td>193</td>
<td>35</td>
</tr>
<tr>
<td>IIb</td>
<td>25</td>
<td>4</td>
<td>24</td>
<td>27</td>
<td>230</td>
<td>48</td>
</tr>
<tr>
<td>IIc</td>
<td>50</td>
<td>4</td>
<td>24</td>
<td>31</td>
<td>169</td>
<td>41</td>
</tr>
<tr>
<td>IIId</td>
<td>100</td>
<td>5</td>
<td>28</td>
<td>30</td>
<td>99</td>
<td>6</td>
</tr>
<tr>
<td>IIe</td>
<td>150</td>
<td>5</td>
<td>29</td>
<td>30</td>
<td>130</td>
<td>9</td>
</tr>
</tbody>
</table>
A high level of sensitivity developed rapidly in animals receiving Diets IIb and IIc. The development of sensitivity in these animals followed a similar sequence with seizures at first consisting only of short bursts of circular running. A steadily increasing number of convulsions emphasized the increasing severity of the attacks. This trend was well illustrated by the results of the last three tests given to those animals on Diet IIb. All the seizures observed during these trials were severe convulsive attacks.

The course of sensitivity observed in the above groups contrasts with that for animals receiving Diets Ib and Ic. It will be recalled that the latter diets included lower levels of thiamine, riboflavin, and pantothenic acid but the same levels of pyridoxine supplied by Diets IIb and IIc. Along with a less satisfactory gain in weight and general appearance, seizures in the animals receiving Diets Ib and Ic tended to be less severe, with running attacks predominating. When the highest level of sensitivity for a single day on Diet Ib was 66 per cent of the total number of animals, a higher percentage (79 per cent) of the twenty-four animals in the group showed seizures at some time during the experimental period. An increasing number of deaths from undetermined causes occurred in these two groups during the latter part of the testing period.

In groups receiving Diets IIa and IIc (higher levels of pyridoxine) sensitivity was delayed for significantly longer periods of time. Very few convulsions were noted (Table IV) and the attacks consisted only of short bursts of running. In two of the litters maintained on Diet IIc, there were three animals which showed scattered signs of sensitivity. The litters were from females which had produced convulsive young at an earlier breeding (Litters 4 and 5) when maintained on pyridoxine-free Diet IIa.
DISCUSSION

The spontaneous seizures observed during the course of these experiments seemed identical with the convulsive symptoms previously found by Daniel, Kline, and Tolle (6) to be associated with pyridoxine deficiency. In addition, it appears that the sound-induced convulsive seizures found associated with varying dietary levels of pyridoxine are identical with those described in previous studies by Patton, Karn, and King (32–34). A continuum of sensitivity ranging from spontaneous and eventually fatal seizures through a subacute convulsive state easily precipitated by sound has thus been found to be correlated with increasing levels of pyridoxine.

The present experimental findings seem to indicate that such an observed correlation does not necessarily imply a direct cause and effect relationship. In our first experiments, it was found that, when Diets Ia and IIa were supplemented with pyridoxine, the acute symptoms (spontaneous convulsions) rapidly disappeared and most of the young animals survived. In the second experiment, however, it appeared that such supplements given after the acute symptoms had begun were not effective in providing continued protection against exposure to sound. Later, the results again indicated a high degree of sensitivity to be associated with low levels of pyridoxine. A steadily increasing severity of attack was noted in animals receiving Diets Ib and IIb. With higher levels of pyridoxine a greater degree of protection was afforded. However, even in animals receiving Diets IId and IIe, sound-induced seizures did appear, although they were delayed and consistently less severe.

There is thus some indication that dietary factors other than a specific deficiency of pyridoxine alone may be of importance in the etiology of the seizures. Other necessary food constituents may not have been present in optimum amounts in the synthetic diets used or their functions may have been impaired by the lower levels of pyridoxine intake.

Strikingly similar convulsive seizures have been found to be associated with magnesium deficiency (15, 16, 20, 37). The extreme vasodilatation of the exposed body surfaces which was found to be coincident with hyperirritability and convulsions in magnesium-deficient rats has not been observed in any of our animals. It is of interest for the present problem, however,

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5 There is the possibility that certain irreparable damage had resulted from the low level of pyridoxine intake or from the seizures themselves. These young animals in convulsion were frequently observed to be cyanotic. The arrest of breathing and symptoms of oxygen lack are well known in older susceptible animals (9, 30). There is evidence in the literature (13, 36, 38) that irreversible and summated damage to the central nervous system results in experimental animals repeatedly subjected to short periods of anoxia. Additional experimental work on the relation of oxygen lack to the seizures seems indicated.
that there is evidence (14) of a synergistic relation between a lack of magnesium and a lack of certain members of the vitamin B complex.

Recent studies of sound-induced seizures have emphasized the physiological aspects of the problem. Evidence has been presented concerning autonomic discharge (1, 9, 17, 18, 21, 24, 25, 30), blood changes following seizures (5), and the action of drugs in alleviating seizures (4, 17). Farris and Yeakel (7, 8) and Finger (10) have observed that age seems to be correlated with sensitivity.6 Lindsley, Finger, and Henry (21) found phases of the attack to be associated with abnormalities of the electroencephalogram similar to those observed during the epileptic seizure.

Apparantly, a finely balanced mechanism is represented by the seizure threshold which may be influenced by a number of factors. It is to be expected that differences in the testing environment such as variations in the type of auditory stimulation or conflict-producing situations would be capable of modifying the susceptibility of an experimental animal. The results of the present experiments indicate that before these effects may be properly interpreted, the basic physiological factors underlying the seizures must be more fully investigated. There is also the indication that the results of these experiments may be applicable to the early detection and evaluation of marginal deficiencies when physiological injury has occurred without external evidence of malnutrition. Such studies offer a new approach to an understanding of the mechanism of convulsive seizures.

SUMMARY

Spontaneous convulsive seizures have been observed in young rats suckling from mothers maintained since parturition on synthetic diets deficient in pyridoxine but supplemented with thiamine, riboflavin, pantothenic acid, and choline. Such symptoms which appeared towards the end of lactation could be quickly alleviated by the administration of pyridoxine in amounts as low as 10 \( \gamma \) per day. However, neither 10 nor 50 \( \gamma \) of pyridoxine per day gave continued protection against similar convulsive seiz-

6 Recent studies (26, 35) appear to have demonstrated that the intestinal flora of the rat is capable of synthesizing in varying degrees many vitamins in the B complex. It is of interest that the cecum of young rats (where such bacterial synthesis is thought to occur) does not reach its maximum capacity until after the rat has passed 100 gm. in weight. At weaning, the cecum is only one-fourth as large relative to the digestive tract as it is in the adult animal. In the opinion of the above investigators this may account for the well recognized fact that rats at this stage are less resistant to some vitamin deficiencies than they are later. Coupled with the added requirements during a period of active growth, these facts may have a bearing on the above findings of Farris and Yeakel that there is a high incidence of seizures in young animals and that such susceptibility decreases with advancing age.
ures which regularly appeared when these animals were given standard auditory tests over a 40 day period.

When pyridoxine was included in the mother's diet at levels varying from 25 to 150 \( \gamma \) per day, no spontaneous seizures were observed in the young. However, a high incidence of sound-induced convulsive seizures was found when these young animals were given regular auditory tests from weaning at 21 days until they were from 45 to 90 days of age. No level of pyridoxine, from 25 \( \gamma \) to approximately 150 \( \gamma \) per day, was sufficient to afford continued protection from sound-induced seizures, but with higher levels of pyridoxine, the seizures were both delayed and less severe.

These experiments appear to have demonstrated a correlation between the degree of pyridoxine deficiency and susceptibility to seizures. However, in view of the relatively large amounts of pyridoxine required to obtain a reduced sensitivity, it is possible that another dietary factor or factors in addition to pyridoxine may be of importance in the etiology of the seizures.

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R. A. Patton, H. W. Karn and Herbert E. Longenecker


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