Effect of Pyridoxine and Pantothenic Acid Deficiency on Conditional Reflexes

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Thiamine and niacin in the diet have been recognized for many years as being necessary for life and normal mental function; their absence results in disease—e.g., beriberi, pellagra. However, the literature contains little direct evidence of such an effect of pantothenic acid or pyridoxine.

Our curiosity was aroused in regard to the possible effect on mental function of the deficiency of pantothenic acid and pyridoxine in 1940 when we became acquainted with the work of Wintrobe, who was then at the Johns Hopkins Hospital. Depriving young pigs under one year of age of these two vitamins, he noted various neurologic abnormalities—ataxia, degeneration in the spinal motor cells, as well as in the sensory neurons.[9–13] Anemia and blood alterations also occurred. These nervous symptoms and blood changes did not appear, however, until the deprivation had lasted for several months.

Would there be a corresponding impairment of higher nervous activity (at the basis of mental function)? How soon would it follow complete deprivation? Would it be reversible? The conditional reflex measures, being quantitative, seemed well suited to give answers to these questions.

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PROCEDURES

For this purpose we used two types of animals, rats and dogs; two kinds of inborn activities (unconditional reflexes), related to pain and to food; and we tested for both formation and retention of learned behavior (conditional reflexes). In the dogs we tested also for reversibility.

Four adult dogs were used, including one old one. These animals had been used in previous experiments for periods ranging from several months to three to four years, for the formation of stable conditional reflexes, both excitatory and inhibitory. Two tones were used, T256+ and T512−, the first given for ten seconds and immediately followed by faradic shock to the foot of sufficient intensity to cause withdrawal of the foot, and the second tone, given in random order and never reinforced. This procedure had been repeated so many times that the conditional reflexes could be considered as stable and well established. Moreover, the difference between two tones an octave apart is an easy one for the animal.

The dogs were switched to the stock diet containing all synthetic B group vitamins as control. Then the same diet was used with the omission of either pantothenic acid or pyridoxine. The vitamins were added to the diets in the form of capsules. The dogs were maintained on the deficient diet for several months, and then returned to the diet containing the vitamins formerly omitted. Conditional reflex records were taken for the two tones, according to the routine of repeating the two tones eight to ten times daily, five times weekly. This work was done in the early 1940s.

In the rats a different procedure was employed. Beginning this work in 1957, we

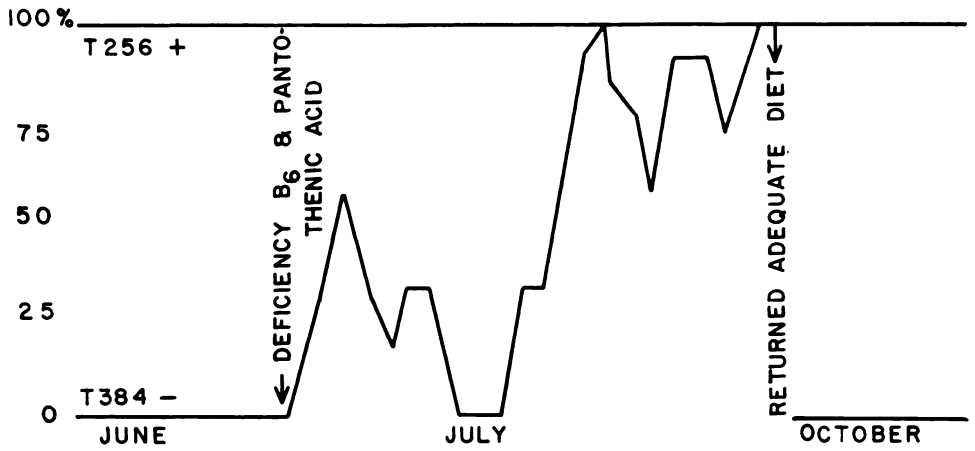

Fig. 1. Impairment differentiation caused by vitamin deficient diet. Impairment begins three days after deficiency.

divided six-week-old rats into three groups of five to ten each. Group I was placed on the adequate stock diet, containing the essential foodstuffs in natural form—milk, liver, yeast, corn oil, and salt. Group II had vitamin-free casein 29 per cent, sugar 60 per cent, corn oil 5 per cent, and salt 4 per cent, to which were added all known synthetic vitamins. The diet of group III was exactly the same as that of group II except that pyridoxine was omitted from the added vitamins.

Conditional reflex training in the rats, in contrast to the procedure used in the dogs, was not started until after the rats were placed on the diets. Furthermore the reinforcement was food instead of pain, and the conditional reflex used was running a maze to obtain food. Thus *formation* of conditional reflex was tested in the rats, and *retention* of conditional reflex in the dogs. The rats, starved 24 hours before experimentation, were run on a fairly simply elevated maze, without variation; i.e., the maze was not changed from day to day. Three grams of the same diet used for the respective group was the unconditional stimulus, placed at the end of the run. Each rat was given five runs daily. At the end of the run he was returned by hand to the starting point of the maze. His daily feeding was given immediately after his five runs.

The records taken were (1) time to make the run measured from the instant when the rat was placed on the maze and (2) the number of errors, i.e., the false turns taken in negotiating the maze. The rats were allowed water *ad lib.**

RESULTS

In the experiments with the dogs, impairment in differentiation began after 4 to 15 days' vitamin deprivation in animals which had shown no loss of differentiation between the two tones. This impairment was sometimes in the direction of failure on the excitatory side, sometimes on the inhibitory. Whether excitation or inhibition suffered was not due to the vitamins omitted but to the type of animal. (Type or "temperament," according to Pavlov, is a determining factor in how an individual will act under stress, i.e., whether by a predominance of excitation or inhibition.) The loss of differentiation was never complete; it became progressively worse for several weeks, and then leveled off for the three to four months' period during which it was continued (Figs. 1 and 2).

When the dogs were then returned to the adequate diet, conditional reflex differentiation was restored to normal. In two dogs a second repetition of deficiency and return to normal showed essentially the same result, i.e., the process was reversible.

In dogs, in contrast to rats, there were no

* This procedure differs somewhat from that of Dr. O. D. Murphree, to whom I am indebted for instruction in maze running.

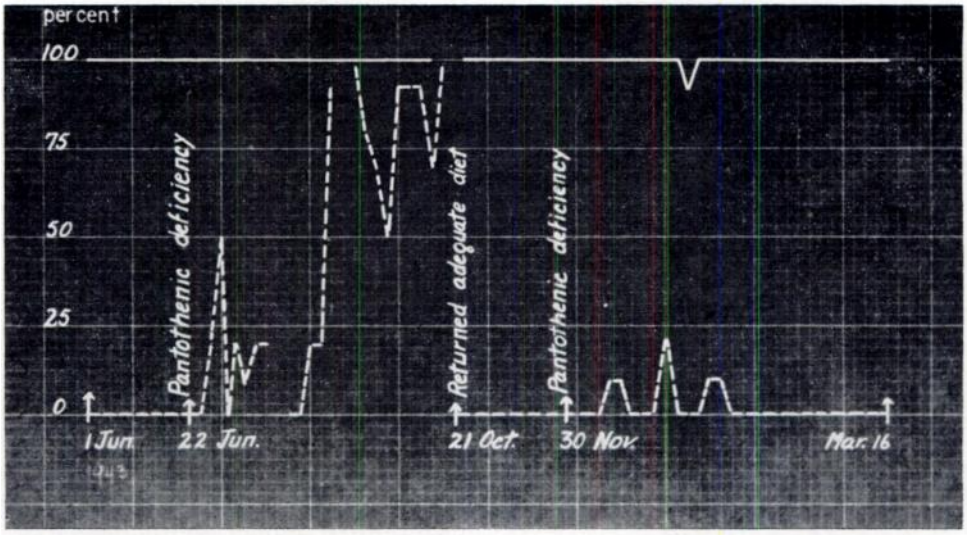

Fig. 2. Effect of vitamin deficiency on conditional reflexes. Note impairment of differentiation and return of differentiation with adequate diet.

other changes in behavior, and there was no alteration of reflexes—knee jerk, hopping, placing—or of artificial sexual reflexes.[4,5]

The dietary deprivation had a greater effect on the rats than on the dogs. There were gross behavioral alterations, marked physical changes, and in group III there was loss of weight and death.

The description of the behavior in the rats follows: group I, they were active, hungry, undisturbed by noises or people; group II, they were restless, nervous when handled, easily startled, increased thirst; group III, they were indifferent to food, squealing, attempting to escape, trembling, chattering teeth, stiff and motionless when handled, snuffling, startled by loud noises.

The details of the behavior in the rats were as follows: group I, these rats showed no fear of noises or people; group II, these rats trembled sometimes when picked up and some of the members did not seem to be as active as those in group I. The notes made on the rats in group III at the time of experimentation follow:

"These rats now range from complete apathy to biting and from motionless behavior on maze to dashing about the cage when replaced, and chattering, attempting to escape, squealing at times and batting heads against the top of the cage wire to push it up. When handled some will bite, then remain still and tremble, then excrete in experimenter's hand, something that the rats in the other two groups have not yet done. Their performance on the maze entails long periods of simply sitting on end of maze where originally placed and not moving for periods of five minutes and sometimes longer. When they do move it is only a small distance, then they wait. If they run rapidly they make many errors. Generally their program is to wait a long time, for the first five to ten minutes, with errors at that time being fairly common, but when speed is picked up later in the running, they make many errors and run wildly up and down the maze, sometimes without any interest evinced in the food after they reach it, but they will run and go back and forth on the maze. They neglect food at times when fed in the cage but drink water. At times they curl up in a ball refusing to be handled or placed on the maze and will not run at all. They show much ruffling of their coats. There is a dingy hue to the fur, and the feet and nose seem quite red and irritated at times."

When the rats were demonstrated to visitors, there was sometimes difficulty in seeing a gross behavior or physical deficiency between the rats in group I and those in group II, but even untrained observers had no hesitancy in deciding that the rats in group III were abnormal and different from those in groups I and II.

The weights of animals in groups I and II were not markedly different, but those in group III showed a definite loss of weight. The figures on the average for August to March were in groups I and II, 675 g; in group III, 450 g. The running times (520 trials) were

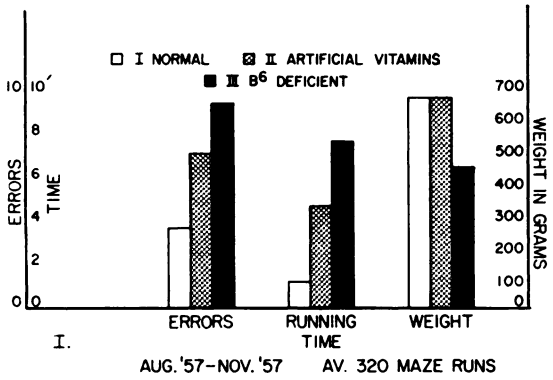

Fig. 3. Effect of vitamin deficiency on conditional reflexes: maze running rats (I) natural diet; (II) synthetic vitamin diet; (III) pyridoxine deficient synthetic diet.

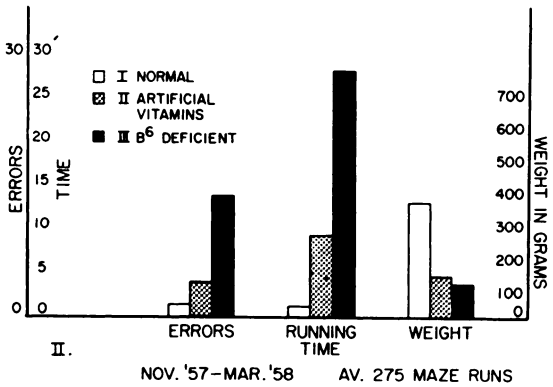

Fig. 4. Effects of vitamin deficiency on conditional reflexes: maze running in rats. (I) natural diet; (II) synthetic vitamin diet; (III) pyridoxine deficiency synthetic diet.

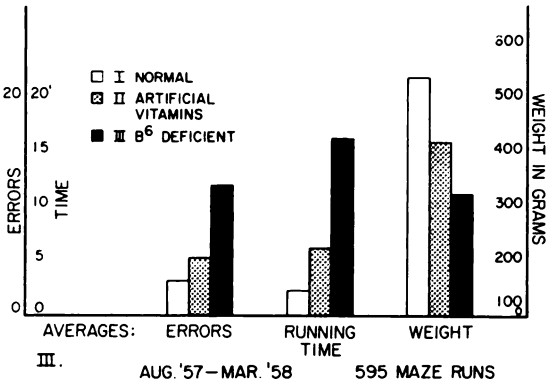

Fig. 5. Effect of vitamin deficiency on conditional reflexes: maze running in rats. (I) natural diet; (II) synthetic vitamin diet; (III) pyridoxine deficient synthetic diet.

group I, 1 minute 38 seconds with 3.8 errors; group II, 4 minutes 22 seconds with 6.9 errors; group III, 7 minutes 33 seconds with 9.8 errors (Fig. 3, 4, and 5).

DISCUSSION

Of the two species of mammal (dog and rat) tested for the effect of pantothenic acid or pyridoxine deficiency, the rats showed much greater disturbance in both general behavior and degree of conditional reflex disturbance. The rats, however, were young when the deficiency was started, while the dogs were adult. The dogs, moreover, did not show neurologic changes, while the rats did.

In the dogs, owing to the more accurate quantitative studies which had been carried out by one of us (W. H. G.), it is possible to equate the impairment of the conditional reflex with that of other agents, such as alcohol, anoxia, morphine. The vitamin deficiency produced as great a loss of the conditional reflex function as did either 2 cc alcohol kg,[1] 18,000 feet altitude for four hours, 3½ mg/kg morphine.[2,3,6–8] However, there were important differences: The alcohol in this dose produced marked ataxia, with return of function after the acute stage; the impairment from the anoxia continued for a year and was only slowly reversible; and morphine often causes ataxia. It is interesting that in the human subject suffering from chronic alcoholism in whom there is perhaps a vitamin deficiency, e.g., in Korsakov's psychosis, the conditional reflex loss is irreversible.[6]

We do not have an explanation of why the rats suffered more than the dogs, unless it be the age factor. In the rats there was no opportunity to eat their own feces, while in the dogs this was not controlled.

However, both these experiments indicate that whether pain or food is used as the unconditional stimulus, whether the subject tested is a dog or rat, whether the function tested is the ability to retain well-established conditional reflexes (dogs) or to form new conditional reflexes (rats), the impairment is marked.

The difference between the best natural diet and the best synthetic diet containing all

known added vitamins is clear in the rat experiments. There is thus some superiority of the natural diet over the synthetic diet, but the tremendous difference is between the best synthetic diet (group II) and the same diet with omission of pyridoxine.

The loss of conditional reflex function corresponds to features equivalent to or related to mental function in man.

CONCLUSIONS

Pantothenic acid or pyridoxine deficiency in dogs causes a loss of conditional reflex performance comparable to loss of mental function in man, e.g., in Korsakov's psychosis. This loss appears within four to ten days before any other neurologic symptom or blood alteration, and before or without observable changes in behavior. The effect is reversible; i.e., the conditional reflex function becomes normal when the animal is returned to the adequate diet.

In rats placed on (I) a natural adequate diet, (II) the best synthetic vitamin diet containing all *known* vitamins, and (III) the same diet as II with the omission of pyridoxine, the animals in group I did slightly better than those in group II, but there was a significant superiority of the animals in group II over those in group III.

Both *retention* of conditional reflexes (dogs) and the ability to *form* conditional reflexes (rats) suffered during pyridoxine deficiency. There was a loss of conditional reflex function with pyridoxine deficiency whether the conditional reflex was based on food or on pain.

The loss of conditional reflex function in the dogs by pyridoxine deficiency was equated to the loss resulting from a moderately large dose of alcohol or of morphine or of anoxia. Pyridoxine apparently is necessary in the diet not only for physical health, but for normal conditional reflex function, which is the basis of mental performance.

REFERENCES

1. Gantt, W. H.: Acute effect of alcohol on autonomic (sexual, secretory, cardiac and somatic) responses; in *Alcoholism*. Am. Assn. Advance. Science, 1957, pp. 73–89.
2. Gantt, W. H.: Effect of alcohol on cortical and subcortical activity measured by the conditioned reflex method. *Bull. Johns Hopkins Hosp.* 56: 61, 1935.
3. Gantt, W. H.: Effect of alcohol on sexual reflexes of normal and neurotic male dogs. *Psychosom. Med.* 16: 174, 1952.
4. Gantt, W. H.: Effect of B-Complex vitamins on conditional reflexes in dogs. Am. J. Clin Nutrition 5: 121, 1957.
5. Gantt, W. H.: *Experimental Basis for Neurotic Behavior*. Harper, New York, 1944.
6. Gantt, W. H. and Muncie, W.: Analysis of the mental defect in chronic Korsakov's psychosis by means of the conditional reflex method. *Bull. Johns Hopkins Hosp.* 70: 467, 1942.
7. Gantt, W. H., Thorn, G., and Dorrance, C.: Anoxia on conditional reflexes in dogs. *Fed. Proc.* 8: 53, 1949.
8. Stephens, J. H. and Gantt, W. H.: The differential effect of morphine on cardiac and motor conditional reflexes—schizokinesis. *Bull. Johns Hopkins Hosp.* 98: 245, 1956.
9. Wintrobe, M.: Attempts to produce pernicious anemia experimentally. *Bull. New England M. Center* 3: 13, 1941.
10. Wintrobe, M.: Thiamine deficiency in swine. *Bull. Johns Hopkins Hosp.* 71: 141, 1942.
11. Wintrobe, M.: Sensory neuron degeneration in pigs; protection afforded by calcium pantothenate and pyridoxine. *J. Nutrition* 24: 345, 1942.
12. Wintrobe, M.: Thiamine deficiency in swine. *Bull. Johns Hopkins Hosp.* 71: 141, 1942.
13. Wintrobe, M., Miller, J. L., Jr., and Lisco, H.: Relation of diet to occurrence of ataxia and degeneration in the nervous system of pigs. *Bull. Johns Hopkins Hosp.* 67: 377, 1940.