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Serotonin Deficiency in Infancy as One Cause of a Mental Defect in Phenylketonuria

Abstract. *Serotonin deficiency induced in newborn mice and maintained to adulthood resulted in reduced ability to learn a maze. The serotonin deficiency was produced by overloading with phenylalanine plus tyrosine (to cause phenylketonuria), by feeding of reserpine, and by feeding of chlorpromazine from birth to maturity.*

The accumulation of evidence to show that mental aberrations can be produced in adults by changes in the serotonin contents of their brains (*1*) has led us to inquire whether some of the inherited idiocies also might arise from a defect in the serotonin metabolism and, that this defect, because it is imposed early in infancy, might be peculiarly harmful to the development of the mind. With this in view, our attention has been drawn particularly to the disease, phenylketonuria. This is an inherited metabolic defect in which the enzyme for hydroxylation of phenylalanine to tyrosine is lacking because of a single-gene mutation. As a result, the phenylalanine which is ingested in the food accumulates in the tissues and is converted to metabolic products such as phenylpyruvic acid and phenyllactic acid, which also accumulate. Infants suffering from this defect develop normally physically, but grow into morons or idiots. If the phenylalanine intake is severely restricted, the mental failure seems not to develop. The mental defect is thus attributable to the excess of phenylalanine and its metabolic products. Why should these substances cause idiocy?

It is now well established [a few of the studies are in (*1–3*)] that phenylketonuric human beings and laboratory animals suffer a deficiency of serotonin and catechol amines. These deficiencies seem to arise (at least in part) from the fact that the enzyme which synthesizes these hormones from their amino acid precursors is inhibited by phenylpyruvic acid and phenyllactic acid (*4*, *5*). Normal animals can be made phenylketonuric by continuous ingestion of very large amounts of phenylalanine or of phenylalanine plus tyrosine (*6*, *7*).

Woolley (*1*) has suggested that the mental defect might arise from the serotonin deficiency. In particular, it was suggested that the deficiency imposed early in infancy with consequent permanent damage to the developing intellect, might be the cause of the mental failure. Others, too, had implied (*2*, *5*), but without direct evidence, that the mental defect might be related to the abnormalities of the metabolism of serotonin. However, they had not stressed the importance of the deficiency in early infancy. The reasons for thinking that the deficiency must be established early in infancy are two. It is well known clinically that such deficiencies imposed in adult life do not cause permanent damage to the mind. It is also well known that to succeed in the control of the idiocy of phenylketonuria, one must start the phenylalanine-low diet early in infancy. The agreement with the idea just mentioned has by no means been general, and many other hypotheses to explain why excess phenylalanine damages the mind have been put forward (*1*).

The purpose of the present work was to produce phenylketonuria in an experimental animal, and to show that it caused a mental failure. If this could be accomplished, the way would be open to show whether, by correction of the serotonin deficiency, the mental failure could be prevented, and thereby, the proof of the idea could be made. It is impossible to do such an experiment in human beings.

Several earlier attempts have been made to show that experimental phenylketonuria induced in rats or mice will cause a mental change. In all of these cases the phenylketonuria was established at weaning time and not in early infancy. The attempts have met with varying success and in only one instance has any evidence been offered to show that the intellectual change was related to the deficiency of serotonin. Yuwiler and Louttit (*7*) for example, concluded that a slight defect in maze-learning ability which they found in phenylketonuric rats was not the result of serotonin deficiency. Polidora *et al.* (*8*) reported a slight decrease in the rate at which phenylketonuric rats swam through a water maze, but made no effort to relate this behavioral change to serotonin. Woolley and van der Hoeven (*9*) and Woolley (*10*) showed that phenylketonuric adult mice exhibited an increase in learning ability in a maze, and related this increase specifically to the deficiency of serotonin.

To produce the mental defect of phenylketonuria in mice, it was necessary to begin with newborn animals, and to maintain in them an excessive amount of phenylalanine. Excess tyrosine was also given because of the claim of Auerbach *et al.* (*6*) that, in rats, this amino acid was needed to suppress the biosynthesis of the hydroxylase enzyme and thus to intensify the disease. DL-Phenylalanine, rather than L-, was used because these same authors had found it to be effective in causing excretion of phenylpyruvic acid in rats, possibly because of the longer persistence of the D-isomer.

Within 24 hours of birth, infant mice were given by stomach tube, daily, nine times each day, 0.01 ml of a fine suspension of DL-phenylalanine (20 mg/ml) and L-tyrosine (10 mg/ml). A curved, 24-gauge needle with a ball tip was used for the intubation. As the mice grew and the capacities of their stomachs increased, the hourly dose was increased until, when they were 2 weeks old, they were receiving about 0.1 ml per hour. During all this time they were allowed to nurse their mothers, which were fed stock ration (Purina chow). At 2 weeks of age, DL-phenylalanine and L-tyrosine (35 g each per kilogram of food) were added to the food and the intubation was stopped. After weaning, the young were continued on this diet until they were 7 to 8 weeks old. They were then fed normal ration for at least 3 days before they were tested for learning ability. This was done in order to clear the tissues of phenylalanine and thus to allow repletion of the serotonin. We had shown earlier (*9*) that, unless this was done, the serotonin deficiency in the mature mice would be reflected by an increase in learning ability. Two kinds of controls were run. In one, the mice were dosed hourly, merely with water, until weaned, and then fed normal ration. In the other, untreated, normal animals were used.

The learning ability of the adult animals was measured in a T-maze as described earlier (*9*). A score of 10 in this test meant that the animals had learned their lesson perfectly, and a score of 5 meant that they had learned nothing at all. It has already been dem-

Table 1. Learning ability of mice raised from birth on diets which caused serotonin deficiency.

Treatment	No. of mice	Av. score
None	90	7.5
Water, hourly	15	7.5
DL-Phenylalanine + L-tyrosine	103	6.3
Reserpine	99	6.6
Chlorpromazine	32	6.5

onstrated that the results of this maze test were reproducible within a standard deviation of ± 0.2 in the score.

Excess phenylalanine (by way of phenylpyruvic acid) causes deficiency of serotonin and of catechol amines, probably by the inhibition of the decarboxylase which forms these hormones, and possibly also by other related inhibitions (*1*). Two other independent means of bringing about the deficiencies were also studied. One was to feed reserpine (10 mg/kg of ration) to the mothers, starting 2 days before parturition, and then to the young (5 mg/kg) until they were mature. This is known to cause a lack of the hormones by combination of the drug with some of the hormonal receptors (particularly those in the storage vesicles), with consequent displacement of the hormones from the tissues. The other was to feed chlorpromazine (500 mg/kg of diet) in the same way. Although chlorpromazine does not displace serotonin from tissues, it does block the receptors for serotonin and for catechol amines in such a way that the tissues will no longer respond to these hormones (*1*). The end result is a *functional* lack of the hormones.

The data in the table show that mice that had been reared from birth under these various conditions which caused deficiencies of these hormones had a subnormal learning ability as measured in the maze test. This was true regardless of the way in which the deficiency was induced (*11*). The fact that the animals fed phenylalanine and tyrosine actually were excreting phenylpyruvic acid was established by chromatography and the ferric chloride test of the urine. The fact that they were deficient in serotonin has been demonstrated earlier (*9*) by analysis of the tissues of animals similarly treated.

The importance of beginning the treatments at birth was demonstrated. When any one of the treatments was not started until weaning, and the mice were then tested when they were mature, no deficit in learning ability was found. Thus, mice fed phenylalanine plus tyrosine from weaning until maturity gave an average score of 7.3. The need for establishment of the disease early in infancy in mice thus corresponded with the findings in the human disease.

The phenylketonuric mice were physically quite normal when they were mature just as is the case with phenylketonuric human beings. They tended to be somewhat less active and perhaps more quarrelsome than normal mice, but it is difficult to be sure of such differences. Sometimes the infant mortality was high but with adequate technique in the dosing procedure, this could be eliminated. The animals raised on reserpine showed a variable and sometimes high infant mortality and a slight retardation of development as indicated by lateness in the opening of the eyes. When higher doses of reserpine were used, the learning deficit was considerably greater, but the infant mortality was so high and so variable from litter to litter that it was not feasible to use these higher doses.

Proof was found that the mental defect was the result of serotonin deficiency and not the result of other changes. Hourly administration of serotonin derivatives (melatonin or hydroxytryptophan) to phenylketonuric mice did not correct the excretion of phenylpyruvic acid, but did produce animals with normal scores for learning ability.

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Markovian Model of Time Patterns of Speech

Abstract. *The time pattern of speech is describable as a first-order Markov process when presence or absence is sampled at a rate of 200 times per minute. Two types of monolog were generated under different conditions of environmental constraint. Although both fit the model, estimates of their mean range of statistical dependency differed significantly.*

The temporal patterns of speech may be described in terms of the frequency distributions of (i) the durations of sound bursts, and (ii) the intervening durations of silence. Both of these distributions have been found to be approximately exponential, with frequency inversely related to duration (*1*). Mosteller (see *1*) has proposed a stochastic model of such vocal time patterns based upon alternate random drawings from the sound and silence distributions respectively. Using this model he was able to hand-simulate a reasonable approximation to actually observed data.

We are exploring such probabilistic hypotheses by sampling the presence or absence of the speech signal. The sampling time unit, which is initiated at a rate of 200 times per minute, is 30 msec in duration. Each successive unit is either a state of sound or silence, depending upon the presence or absence of a signal above threshold at some time during the sampling interval. Our initial attempts to generate observed frequency distributions were

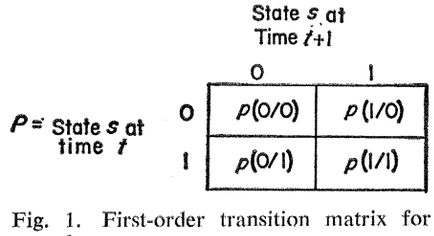

Fig. 1. First-order transition matrix for monolog.