Cadmium ion probably functions by trapping hydrogen sulphide and preventing it from reacting further with unreacted starting material[9,10], or other products of the reaction. However, the possibility that it is involved in a direct electrophilic attack on the disulphide bond[10] leading to products capable of quantitatively releasing hydrogen sulphide cannot be ruled out.

F. M. Robbins*
Joseph A. Fioriti

Research Center,
General Foods Corporation,
Tarrytown,
New York.

* Present address: U.S. Army Quartermaster, Natick, Mass.

[1] Kuratomi, K., Ohno, K., and Akabori, S. J., *J. Biochem.* (Tokyo), **44**, 183 (1957).
[2] Stapleton, I. W., and Swan, J. M., *Austral. J. Chem.*, **13**, 416 (1960).
[3] Segall, S., and Proctor, B. E., *Food Tech.*, **13**, 679 (1959).
[4] Marbach, E. P., and Doty, D. M., *J. Agric. Chem.*, **4**, 881 (1956).
[5] Clarke, H. T., and Inouye, J. M., *J. Biol. Chem.*, **89**, 399 (1930).
[6] Nicolet, B. H., *J. Amer. Chem. Soc.*, **53**, 3066 (1931).
[7] Rosenthal, N. A., and Oster, G., *J. Soc. Cosmetic Chem.*, **5**, 286 (1954).
[8] Schoberl, A., *Ann.*, **507**, 111 (1933).
[9] Rao, G. S., and Gorin, G., *J. Org. Chem.*, **24**, 749 (1959).
[10] Demarco, C., *et al.*, *Arch. Biochem. Biophys.*, **100**, 51 (1963).
[11] Hirs, C. H. W., *J. Biol. Chem.*, **235**, 633 (1960).
[12] Katyal, J. M., and Gorin, G., *Arch. Biochem. Biophys.*, **82**, 319 (1959).
[13] Carter, J. R., *J. Biol. Chem.*, **234**, 1705 (1959).

Mechanisms for the Decrease of Brain Serotonin

There is general agreement that 5-hydroxytryptamine (5HT) is formed from 5-hydroxytryptophan (5HTP) in the presence of 5-hydroxytryptophan decarboxylase (5HTPD). It is then oxidized to 5-hydroxy indolylacetic acid (5HIAH) with monoamine oxidase (MAO). A decrease of brain 5HT has been reported in new-born animals[1,2] and those made phenylketonuric by means of a diet high in phenylalanine content[3,4]. In this communication investigations of the differing mechanisms responsible for this decrease are described.

For this work, guinea pigs were killed by decapitation and the brains quickly removed for analysis. The brain 5HT was determined by the method of Bogdanski *et al.*[5,6]. The 5HTPD and MAO were assayed by the procedure of Kuntzman *et al.*[7] using iproniazid as the MAO inhibitor. Three groups of guinea pigs were examined: (1) weanlings placed on a regular diet for two to three weeks, which served as controls; (2) weanlings made phenylketonuric by adding 4 per cent L- and 4 per cent DL-phenylalanine to the diet[4]; and (3) new-born animals, which were either less than 48 h of age, or were delivered by Caesarean section one to two days prior to the expected date of delivery.

The animals fed the high phenylalanine diet showed serum phenylalanine-levels of $20{\cdot}4 \pm 18{\cdot}5$ mg per cent as compared with controls which showed levels of $1{\cdot}9 \pm 0{\cdot}5$ mg per cent. In the phenylketonuric animals, there was a decrease of 5HT, but the levels of 5HTPD and MAO remained unaltered. Similar results were observed when the data were expressed in terms of protein or RNA units and when the animals were pair fed. When 20 μg pyridoxal phosphate was added to the reaction mixture, there was an increase of 5HTPD and MAO in both groups, but the values remained comparable. On the other hand, a 23 per cent decrease of 5HTPD was noted when $3{\cdot}3 \times 10^{-2}$ M phenylpyruvic acid was added to the reaction. These results agree with the previous findings in both kidney[8] and brain[4] that the decrease of 5HT does not result from the inhibition of 5HTPD by phenylalanine metabolites in the intact animal.

In contrast, the decrease of brain 5HT in the new-born animal is associated with a decrease of 5HTPD, suggesting a functional immaturity of 5HTPD[9,10].

Table 1. Levels of brain 5-hydroxytryptamine (5HT), 5HTP decarboxylase (5HTPD) and monoamine oxidase (MAO) in adult, new-born and phenylketonuric guinea pigs. The values are expressed as means and standard deviations with the number of experiments done given in parentheses

	Brain 5HT*	Brain 5HTPD†	Brain MAO‡
Controls	0·56 ± 0·23 (23)	19·8 ± 6·8 (18)	1,371 ± 535 (18)
Phenylketonurics	0·31 ± 0·16 (23)§	19·7 ± 6·0 (18)	1,216 ± 535 (18)
Newborns	0·28 ± 0·14 (15)§	13·4 ± 3·7 (17)§	338 ± 200(15)§

* μg/g wet wt.
† μg 5HT formed/g wet wt./h.
‡ μg 5HT destroyed/g wet wt./h.
§ $P < 0{\cdot}001$.

The cause of the mental defect in phenylketonuria remains obscure. Clinical investigations have shown that a diet low in phenylalanine content is effective in preventing the mental defect if it is started early in infancy, but becomes less effective as the child gets older[11]. Recently, Crome *et al.*[12] have demonstrated a decrease of the cerebroside content of white matter, suggesting that the toxic substances in the body fluids interfere with the development and function of neurones. A number of theories have been suggested to explain these abnormalities. First, the excessive phenylalanine might inhibit the hydroxylation of tryptophan and thus reduce the amount of 5HTP available[13]. Second, this excess might inhibit the active transport of 5HTP across the blood-brain barrier or brain cell membrane[14,15]. Third, the excess of phenylalanine and its metabolites might inhibit the decarboxylation of 5HTP[16]. Fourth, the defect may be accentuated by the functional immaturity of 5HTPD or MAO, or both[9,10].

Of these concepts, it appears unlikely that inhibition of 5HTPD by phenylalanine and its metabolites plays a major part. Instead, it seems more likely that excessive phenylalanine interferes either with the hydroxylation of tryptophan or the active transport of 5HTP into brain. When this is superimposed on functionally immature enzyme systems such as 5HTPD or MAO, this could result in an imbalance of the neurohumoral compounds in the brain and cause the mental defect seen in phenylketonuria.

This work was supported by U.S. Public Health Service grant *M*-2630. One of us (K. N.) is a trainee under U.S. Public Health Service grant 2*A*-5186.

David Yi-Yung Hsia
Kazuhiko Nishimura
Yvonne Brenchley

Genetic Clinic,
Children's Memorial Hospital,
and
Department of Pediatrics,
Northwestern University Medical School,
Chicago.

[1] Kato, R., *J. Neurochem.*, **5**, 202 (1960).
[2] Nachmias, V., *J. Neurochem.*, **6**, 99 (1960).
[3] Yulwiler, A., and Louttit, R. T., *Science*, **134**, 831 (1961).
[4] Wang, H. L., Harwaklar, V. H., and Waisman, H. A., *Arch. Biochem. and Biophys.*, **98**, 181 (1962).
[5] Bogdanski, D. F., Pletscher, A., Brodie, B. B., and Udenfriend, S., *J. Pharm. Exp. Therap.*, **117**, 82 (1956).
[6] Bogdanski, D. F., Weissbach, H., and Udenfriend, S., *J. Neurochem.*, **1**, 272 (1957).
[7] Kuntzman, R., Shore, P. A., Bogdanski, D., and Brodie, B. B., *J. Neurochem.*, **6**, 226 (1961).
[8] Huang, I., and Hsia, D. Y. Y., *Proc. Soc. Exp. Biol. and Med.*, **112**, 81 (1963).
[9] Huang, I., Tannenbaum, S., and Hsia, D. Y.-Y., *Nature*, **186**, 717 (1960).
[10] Karki, N., Kuntzman, R., and Brodie, B. B., *J. Neurochem.*, **9**, 53 (1962).
[11] Knox, W. E., *Pediatrics*, **26**, 1 (1960).
[12] Crome, L., Tymms, V., and Woolf, L. I., *J. Neurol., Neurosurg. and Psych.*, **25**, 143 (1962).
[13] Freedland, R. A., Wadzinski, I. M., and Waisman, H. A., *Biochem. Biophys. Res. Comm.*, **6**, 227 (1961).
[14] McKean, C. M., Schanberg, S. M., and Giarman, N. J., *Science*, **137**, 604 (1962).
[15] Guroff, G., and Udenfriend, S., *J. Biol. Chem.*, **237**, 803 (1962).
[16] Davison, A. N., and Sandler, M., *Nature*, **181**, 186 (1958).