3. E. A. CARLINI and J. P. GREEN, *Biochem. Pharmac.* **12**, 1448 (1963).
4. R. J. LEVINE, T. L. SATO and A. SJOERDSMA, *Biochem. Pharmac.* **14**, 139 (1965).
5. L. T. KREMZNER and C. C. PFEIFFER, *Biochem. Pharmac.* **15**, 197 (1966).
6. L. T. KREMZNER and I. B. WILSON, *J. biol. Chem.* **238**, 1714 (1963).
7. H. M. ADAM, in *Regional Neurochemistry* (Eds. S. S. KETY and J. ELKES), p. 293. Pergamon Press, New York (1961).

Biochemical Pharmacology, 1966, Vol. 15, pp. 1629–1632. Pergamon Press Ltd., Printed in Great Britain.

Brain amines and brain weights in growing chicks: some normal values and effects of feeding excess dietary L-phenylalanine*

(*Received* 4 *March* 1966; *accepted* 23 *May* 1966)

THE CONCENTRATIONS of serotonin and norepinephrine in the brains of adult chickens have been determined.[1–3] However, the changes in concentration with age are not known for an avian species, although this information is available for several mammalian genera such as rabbit;[4] rabbit, rat, and guinea pig;[5] and cat.[6] This communication provides information on increases in brain amine levels in growing chicks from hatching to 8-week age. In addition, the effect of feeding excess dietary L-phenylalanine on brain amines was studied in chicks, since this amino acid has been shown to depress the brain serotonin level when fed to rats.[7] In the chick, incorporation of L-phenylalanine into the diet causes growth retardation and a variety of physical deficits.[8, 9]

MATERIALS AND METHODS

Three strains of chickens (*Gallus domesticus*) were employed. One strain designated broiler stock (males) was obtained from the Munroe Hatchery, Inc., Joliet, Ill. The other two, white leghorn (females) and a crossbred strain of Rhode Island reds and Plymouth barred rock (males), were from a local source. The normal values were obtained primarily with the broiler stock, and this strain was also fed a diet containing 5% L-phenylalanine. The other two strains were fed 2, 4, or 8% phenylalanine. All animals were maintained under standard poultry-raising conditions with a basal diet consisting of a 19 per cent protein commercial corn-soy ration (Wayne-Allied Mills). Appropriate amounts of L-phenylalanine (Mann) were intimately mixed with the basal diet to obtain the required percentages of this amino acid.

Normal chicks from the broiler stock were killed at 1, 7, 14, 28, or 56 days of age. Those receiving 5% L-phenylalanine were killed at 28 or 56 days. In addition, three chicks which had been on the normal diet for 28 days were placed on 5% L-phenylalanine, and three chicks receiving 5% L-phenylalanine were transferred to the basal ration for 28 days. The white leghorns and crossbred chicks were killed after receiving the L-phenylalanine diets for 28 days. After decapitation the brains were rapidly removed, weighed, frozen on solid carbon dioxide, and subsequently analyzed for their content of serotonin and norepinephrine by the method of Shore and Olin[10] as modified by Mead and Finger.[11] In a few experiments dopamine was also determined by the oxidizing conditions described by McGeer and McGeer.[12] Statistical comparison of results was based on Student's *t* test. Further methodological details are reported in previous publications.[2, 8, 9]

RESULTS AND DISCUSSION

The brain amine values in normal growing chicks of the broiler strain are summarized in Table 1 along with body and brain weights. After the first week of age the brain concentrations of both norepinephrine and serotonin show no further increases. Mammals, which, with the exception of the guinea pig, are born relatively undeveloped compared to the maturational level of the chick at the time of hatching, require several weeks or months to attain constant amine concentrations in the brain. There was no difference in the brain serotonin content among the three strains when compared at 4 weeks of age (Table 2). However, the norepinephrine content of the broiler strain was appreciably higher than those of the other two breeds (Table 2).

* This work was supported in part by United States Public Health Service Grant MH 12344-01.

TABLE 1. BRAIN AMINES, BRAIN WEIGHT, AND BODY WEIGHT IN NORMAL CHICKENS AT VARIOUS AGES

Measure	1 day	1 week	2 weeks	4 weeks	8 weeks
Body weight (g)	37·7 ± 4·5* (9)	78·1 ± 7·9 (15)	215 ± 17 (15)	575 ± 36 (10)	1433 ± 75 (3)
Brain weight (g)	0·93 ± 0·05 (9)	1·18 ± 0·04 (6)	1·59 ± 0·06 (7)	2·19 ± 0·08 (7)	2·84 ± 0·14 (5)
Brain serotonin (μg/g)	0·68 ± 0·06 (9)	0·82 ± 0·06 (6)	0·84 ± 0·07 (7)	0·88 ± 0·11 (7)	0·84 ± 0·13 (3)
Brain norepinephrine (μg/g)	0·63 ± 0·08 (5)	0·94 ± 0·13 (5)	0·76 ± 0·12 (7)	0·87 ± 0·10 (7)	0·88 ± 0·13 (3)

* Average ± S.D. Numbers in parentheses refer to number of animals per group.

TABLE 2. BRAIN AMINES IN NORMAL FOUR-WEEK-OLD CHICKS FED A DIETARY EXCESS OF L-PHENYLALANINE FROM TIME OF HATCHING

Breed	Sex	No. of animals	Levels of phenylalanine				
			0%	2%	4%	5%	8%
				Serotonin (μg/g)			
White leghorn	F	3	0·80 ± 0·09	1·01 ± 0·07*	0·82 ± 0·08		0·86 ± 0·07
Crossbred	M	3	0·79 ± 0·05	0·97 ± 0·08*	0·98 ± 0·08*		0·84 ± 0·04
Broiler	M	7	0·88 ± 0·11			0·55 ± 0·16*	
				Norepinephrine (μg/g)			
White leghorn	F	3	0·55 ± 0·04	0·63 ± 0·09	0·57 ± 0·05		0·57 ± 0·05
Crossbred	M	3	0·58 ± 0·06	0·58 ± 0·10	0·50 ± 0·10		0·48 ± 0·03
Broiler	M	7	0·87 ± 0·10			0·88 ± 0·16	
				Dopamine (μg/g)			
White leghorn	F	3	0·35 ± 0·05	0·39 ± 0·15	0·28 ± 0·09		0·39 ± 0·10
Crossbred	M	3	0·37 ± 0·06	0·35 ± 0·06	0·41 ± 0·12		0·44 ± 0·09

*Difference from control (0%) at $P < 0·05$ level. Other values not significantly different from control.

Feeding L-phenylalanine had no effect on either norepinephrine or dopamine levels in the brain (Table 2), although there was a difference in the normal level of norepinephrine among the three strains of chickens employed. In the broiler strain, feeding 5% L-phenylalanine caused a decrease in the serotonin content of the brain. In the other two strains, slight increases in brain serotonin levels were found after feeding 2 and 4% L-phenylalanine but not at the 8% level. In rats fed excess phenylalanine, only depression of serotonin levels has been reported.

The body weights, brain weights, and brain serotonin levels of the broiler-strain chicks which had their diets switched at 4 weeks of age are given in Table 3. There was no effect on brain serotonin

TABLE 3. EFFECT OF DIETARY PHENYLALANINE ON BRAIN SEROTONIN BRAIN WEIGHT AND BODY WEIGHT IN BROILER-STRAIN CHICKS

Group*	Body weight		Brain weight at 8 weeks (g)	Serotonin at 8 weeks (μg/g)
	4 weeks (g)	8 weeks (g)		
C8	600	1433 ± 75†	2·84 ± 0·14	0·84 ± 0·13
C4–P4	602	437 ± 110	2·43 ± 0·36	0·89 ± 0·23
P4–C4	288	1262 ± 44	2·68 ± 0·09	1·02 ± 0·14

* C8, chicks fed basal diet for 8 weeks; C4–P4, chicks fed basal diet for 4 weeks then changed to diet containing 5% L-phenylalanine; P4–C4, chicks fed 5% L-phenylalanine in diet for 4 weeks then changed to basal diet for 4 weeks.

† Three chicks per group; figures are averages ± S.D.

levels when feeding of 5% L-phenylalanine was instituted at 4 weeks of age, even though these animals lost about one-third of their initial weight at age 4 weeks. In contrast, the chicks previously on a 5% L-phenylalanine diet gained weight rapidly and had normal or higher brain amine levels.

Further work is needed to clarify the action of excess dietary L-phenylalanine on the brain serotonin level with respect to strain and sex differences.

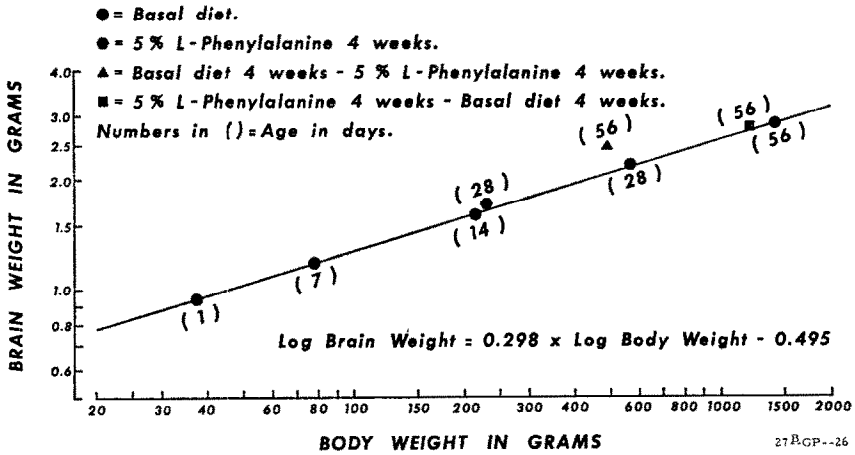

FIG. 1. Brain and body weight relationships in growing broiler-stock chicks.

It is instructive to compare the brain and body weights of the broiler strain on a logarithmic basis (Fig. 1). The average brain and body weights in each age group of the chicks fed the basal diet fit closely to the relationship:

$$\log \text{brain weight} = 0{\cdot}298 \times \log \text{body weight} - 0{\cdot}495$$

from age 1 day to 56 days. There is no indication of any inflection points in the curve or interphase periods, as is seen in mammals (rat,[13] rabbit,[14] dog,[15] mouse[16]). Kobayashi[16] has suggested that the interphase period may be an indicator of the maturation level of the brain in mammals, because several parameters, particularly the electrical activity of the brain, achieve mature development conjointly with the appearance of the interphase period. Although cortical activity in the chicken is well developed at hatching,[17] appreciable development takes place thereafter in terms of complexity of rhythms and increases in amplitude, which may indicate the divergence of this species from a normal mammalian maturation pattern.

Feeding 5% L-phenylalanine for 4 weeks caused a marked retardation in the growth of the chicks, yet the brain weight was proportional to the body weight in these animals (Fig. 1). Similarly, when the chicks receiving the phenylalanine were returned to a basal diet and had recovered most of their growth potential by 8 weeks, their brain weight was still proportional to their body weight. The only animals not fitting the log–log relationship were those in which feeding of the diet containing the amino acid was instituted at 4 weeks of age. They lost appreciable amounts of body weight, yet their brain continued to grow slightly.

Thudichum Psychiatric Research Laboratory, G. R. PSCHEIDT
Galesburg State Research Hospital, H. S. TAMIMIE
Galesburg, Ill., U.S.A.

REFERENCES

1. G. R. PSCHEIDT and H. E. HIMWICH, *Life Sci.* **2**, 524 (1963).
2. G. R. PSCHEIDT and B. HABER, *J. Neurochem.* **12**, 613 (1965).
3. G. R. PSCHEIDT and H. E. HIMWICH, *Progress in Brain Research* (Eds., W. A. HIMWICH and J. P. SCHADÉ), vol. 16, pp. 245–49. Elsevier, Amsterdam.
4. G. R. PSCHEIDT, A. SCHWEIGERDT and H. E. HIMWICH, *Life Sci.* **4**, 1333 (1965).
5. N. KARKI, R. KUNTZMAN and B. B. BRODIE, *J. Neurochem.* **9**, 53 (1962).
6. H. E. HIMWICH, G. R. PSCHEIDT and A. K. SCHWEIGERDT, paper presented at the Symposium on the Nutritional Maturation of the Nervous System in Early Life, Paris (1963).
7. A. YUWILER and R. M. LOUTTIT, *Science, N.Y.* **134**, 831 (1961).
8. H. TAMIMIE and G. R. PSCHEIDT, *Comp. Biochem. Physiol.* **17**, 609 (1966).
9. H. TAMIMIE and G. R. PSCHEIDT, *Comp. Biochem. Physiol.* **17**, 1159 (1966).
10. P. A. SHORE and J. S. OLIN, *J. Pharmac. exp. Ther.* **122**, 295 (1958).
11. J. A. R. MEAD and K. F. FINGER, *Biochem. Pharmac.* **6**, 52 (1961).
12. E. G. MCGEER and P. L. MCGEER, *Canad. J. Biochem.* **40**, 1141 (1962).
13. H. H. DONALDSON and S. HATI, *J. comp. Neurol.* **53**, 263 (1931).
14. J. P. SCHADÉ, *J. Neurophysiol.* **22**, 245 (1959).
15. M. W. FOX, *Experientia* **22**, 111 (1966).
16. T. KOBAYASHI, *Am. J. Physiol.* **204**, 343 (1963).
17. B. S. KEY and E. MARLEY, *Electroenceph. clin. Neurophysiol.* **14**, 90 (1962).

Biochemical Pharmacology, 1966, Vol. 15, pp. 1632–1637. Pergamon Press Ltd., Printed in Great Britain.

Erythrocyte membrane stabilization by steroids and alcohols; a possible model for anesthesia

(*Received* 24 *February* 1966; *accepted* 18 *May* 1966)

It is known that an extremely wide variety of drugs will stabilize erythrocytes against hypotonic hemolysis.[1–3] This stabilization of the erythrocyte membrane may be in some respects similar to the stabilization of the nerve cell membrane that occurs in anesthesia. In order to see whether erythrocyte stabilization would serve as a useful model for the nerve membrane stabilization of anesthesia it was decided to obtain and compare the values for erythrocyte stabilization by alcohols and steroids with the anesthetic values available in the literature. It is known that some steroids will reduce the degree of hemolysis induced by mechanical stress, by sulfhydryl inhibitors, by prolonged