problems, hardly germane to the present considerations, are raised by the demonstration of a condition whereby small slowly conducting fibers on one side of the central nervous system can balance the conduction of impulses by larger rapidly conducting contralateral fibers well enough to maintain symmetrical activity of the organism as a whole. The described condition is an important one, however, in relation to future analyses of the function of parts of the developing central nervous system. Any such analyses must take into account the degree to which, and the mechanisms by which, the embryonic brain can successfully compensate for the addition of supernumerary units.

Summary. Locomotor reactions have been observed in *Fundulus heteroclitus* embryos abnormal in number and arrangement of Mauthner's neurones. Five out of 11 embryos lacking Mauthner's axones on one or both sides of the cord were abnormally passive or immobile; the remaining six exhibited vigorous swimming movements. Out of 7 embryos in which the 2 Mauthner's fibers coursed posteriorly both on the same side of the cord, 2 were abnormally passive and 5 exhibited vigorous movements. Axones from supernumerary Mauthner's cells were present in the cord in 22 embryos; one of these embryos was abnormally passive, but the others exhibited no variations of behavior attributable to the altered number of Mauthner's fibers. The apparently negative results of the experiments in which supernumerary axones were added, and the fact that asymmetry in the arrangement of fibers was not reflected in asymmetry of reaction, demonstrate a high degree of regulability of function in the developing brain of Fundulus.

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Activity of Pyridoxine Derivatives in Chick Nutrition.*

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The discovery[1] and confirmation[2] of the existence of pseudo-pyridoxine, which has very high bacterial potency, raises a question regarding its activity in animal nutrition. Carpenter *et al.*[3] reported preparations of pseudopyridoxine to be less active for the rat than the original pyridoxine. We wish to report in this paper similar studies on the activity of pseudopyridoxine in chick nutrition.

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‡ Called pyracin-5 in this paper to distinguish it from the lactone of 2-methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine, which has also been reported to be active.7. Pyracin-5 has also been termed α-pyracin and pyridoxic acid.

1 Snell, E. E., Guirard, B. M., and Williams, R. J., *J. Biol. Chem.*, 1942, **143**, 519.

2 Carpenter, L. E., and Strong, F. M., *Arch. Biochem.*, 1944, **3**, 375.

3 Carpenter, L. E., Elvehjem, C. A., and Strong, F. M., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 123.

TABLE I.
Composition of Rations.

Constituent	494	494-B_6	494K
Dextrin* (g)	61	61	61
Casein† (g)	18	18	18
Gelatin (g)	10	10	10
Salts V (12) (g)	6	6	6
Soybean oil (g)	5	5	5
1(-) Cystine (g)	0.3	0.3	0.3
Thiamine HCl (mg)	0.3	0.3	0.3
Riboflavin (mg)	0.6	0.6	0.6
Calcium pantothenate (mg)	2	2	2
Choline Cl (mg)	150	150	150
Nicotinic acid (mg)	5	5	5
Pyridoxine HCl (mg)	0.4		0.4
Biotin (mg)	0.02	0.02	0.02
i-Inositol (mg)	100	100	100
B_{10} and B_{11} concentrate (mg)‡	100	100	
Vitamin D_3 (mg)	0.004	0.004	0.004
α-Tocopherol (mg)	0.03	0.03	0.03
2-Methyl-1, 4-naphthoquinone (mg)	0.05	0.05	0.05
Vitamin A (U.S.P. units)	1700	1700	1700

* Made by adding corn starch to water until the consistency of putty is obtained, autoclaving for 3 hr drying and grinding.
†SMACO vitamin test casein.
‡ Made as described for the superfiltrol eluate.[12]

When the two microbiologically potent derivatives of pyridoxine, pyridoxal (2-methyl-3-hydroxy-4-formyl- 5 -hydroxymethylpyridine) and pyridoxamine (2-methyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine), became available in crystalline form[4,5] it seemed desirable to test them for vitamin B_{10} and B_{11} activity as well as for vitamin B_6 activity in the chick. Snell[6] has recently reported these compounds to be slightly more active than pyridoxine in rat nutrition. Pyracin-5,‡ the lactone of 2-methyl-3-hydroxy-4-hydroxymethyl-5-carboxypyridine, was included in these tests because Scott *et al.*[8] found it to be active in chick nutrition. Bound forms of pyridoxine which have been found to be active for both the rat and bacteria[9] are not considered here.

Symptoms of the vitamin B_6 deficiency syndrome in the chick, not previously described, are also reported.

Experimental. The methods used were similar to those previously described.[10-12] Day-old White Leghorn chicks, obtained from the University Poultry Department or commercial hatcheries, were placed in heated cages with raised screen bottoms and fed basal ration for three days. They were then divided uniformly into groups of 6 and fed *(ad libitum)* the basal ration with the proper supplement added.

The composition of the different rations used is given in Table I. The vitamin B_6 activity of the different preparations was tested by using ration 494-B_6. Likewise, the ability of the compound to replace vitamins B_{10} and B_{11} was determined on ration 494-K. The compounds were also added to a ration containing the vitamin B_{10} and B_{11} concentrate and the response noted.

Blood samples for prothrombin estimation were drawn by heart puncture from a few of the chicks at 2, 3 and 4 weeks. Recovery from this procedure appeared rapid since the blood picture of these chicks on the following week was very similar to that of other chicks within the same group. Whole blood clotting time was determined by the capillary method.[13]

Results. The data in Table II show that the addition of pyridoxal, pyridoxamine, or pyracin-5 to the vitamin B_{10} and B_{11} deficient diet has no effect on growth or feathering in the chick. Pyridoxamine is also inactive when fed in a mixture with xanthopterin,§ thymine and guanine. Groups 9-12 inclusive indicate that

4 Snell, E. E., *J. Biol. Chem.*, 1944, **154**, 313.

5 Harris, S. A., Heyl, D., and Folkers, K., *J. Biol. Chem.*, 1944, **154**, 315.

6 Snell, E. E., *Abstracts Am. Chem. Soc. Meeting,* New York, 1944, p. 7B.

7 Scott, M. L., Norris, L. C., Heuser, G. F., and Bruce, W. F., *J. Am. Chem. Soc.*, 1945, **67**, 157.

8 Scott, M. L., Norris, L. C., Heuser, G. F., Bruce, W. F., Coover, H. W., Jr., Bellamy, W. D., and Gunsalus, I. C., *J. Biol. Chem.*, 1944, **154**, 713.

9 Hochberg, M., Melnick, D., and Oser, B. L., *J. Biol. Chem.*, 1944, **155**, 119.

10 Briggs, G. M., Jr., Mills, R. C., Hegsted, D. M., Elvehjem, C. A., and Hart, E. B., *Poultry Sci.*, 1942, **21**, 379.

11 Briggs, G. M., Jr., Luckey, T. D., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1945, **158**, 303.

12 Briggs, G. M., Jr., Luckey, T. D., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1944, **153**, 423.

13 McGowan, J. P., *Brit. Med. J.*, 1907, **2**, 1580.

§ Prepared by Dr. R. C. Mills, now in the service of the armed forces.

TABLE II.
Results on the Vitamin B_{10} and B_{11} Deficient Ration.

No.	Supplements/100 g of Ration 494K	Dead/chicks	B_{10}* %	B_{11}† %	4 wks avg wt g
1	None	1/18	30	0	125
2	500γ pyridoxine	2/12	30	14	139
3	50γ pyridoxal	0/6	20	—17	108
4	100γ "	1/5	45	—35	90
5	50γ pyridoxamine	0/6	30	— 7	118
6	500γ "	2/12	30	14	139
7	50γ " + 50γ xanthopterin + 100γ thymine + 100γ guanine	1/6	25	3	128
8	500γ pyracin-5	0/11	35	5	130
9	100 mg vit B_{10}, vit B_{11} concentrate (control diet)	0/18	100	100	225
10	100 mg vit B_{10}, vit B_{11} concentrate + 200γ pyridoxine	0/6	100	103	228
11	100 mg vit B_{10}, vit B_{11} concentrate + 200γ pyridoxamine	0/6	100	112	237
12	100 mg vit B_{10}, vit B_{11} concentrate + 200γ pyracin-5	0/5	100	85	210

* Feathering scale: 0 = very poor; 25 = poor; 50 = fair; 75 = good; 100 = very good.
† Growth results expressed as % or $\frac{\text{g gain over basal}}{\text{control weight—basal weight}} \times 100$

pyridoxine and pyracin-5 give no significant response when fed with the complete ration. These compounds produced no consistent effect on hemoglobin formation.

The growth and survival data from 3 vitamin B_6 experiments, Table III, indicate that pyridoxal is slightly more than one-half and pyridoxamine is about four-fifths as active as pyridoxine, while pyracin-5 is completely inactive. In one experiment pyridoxal and pyridoxamine were fed at a very low level to eliminate the possibility that they might be toxic at the higher levels fed previously. The results of groups 7 and 9 indicate that these compounds have no pseudopyridoxine-like activity in the chick.

B_6 Deficiency Syndrome. Most of the symptoms previously reported,[14-16] for vitamin B_6 deficient chicks were observed. These include reduced food consumption and growth, asthenia, poor feather development (resembling that obtained in an arginine-glycine deficiency), occasional fits and convulsions, and death. Hyperexcitability, reported by Lepkovsky[16] as stage I, was not observed although strong stimuli were applied, *e. g.*, various loud noises, sudden movements, and short air blasts.

The onset of a fit (whether mild or ending in convulsions) is usually accompanied with short, high-pitched chirps. Hemoglobin values (Table IV) indicate that vitamin B_6 deficiency in chicks is accompanied by a definite anemia. The anemia is quite pronounced in some of the deficient chicks while in other chicks a dehydration seemed to have occurred which manifested itself as a hemoglobinemia.

While making hemoglobin determinations, difficulty in preventing clotting was unexpectedly encountered in some of the vitamin B_6 deficient chicks. Upon further investigation it was found that the deficient chicks (only 3 were alive at this time) had a whole blood clotting time of 2.5 (1.8-4.0) min, while the value for one of the controls was 4.5 min and that of 2 others was greater than 10 min. The results of the next experiment indicated that 2-week-old chicks have similar whole blood clotting times, while 3- and 4-week-old chicks, deficient in vitamin B_6, have a decreased clotting time (when compared with the control chicks). At 4 weeks it was found (Table V) that 25γ of pyridoxine supplement per 100 g of ration 494-B_6 gave no response,

14 Hegsted, D. M., Oleson, J. J., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1939, **130**, 423.

15 Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 180.

16 Lepkovsky, S., and Kratzer, F. H., *J. Nutrition*, 1942, **24**, 515.

TABLE III.
Growth Results Using Vitamin B_6 Deficient Ration.

No.	Supplement per 100 g 494-B_6	Weight g at 4 wks	Approximate activity†	Dead/ chicks
1	None	69		14/24
2	5γ pyridoxine HCl	3* wks	1	6/6
3	25γ ”	74	1	0/6
4	50γ ”	108	1	1/11
5	100γ ”	197	1	0/12
6	400γ ”	222	1	0/24
7	5γ pyridoxal HCl	2* wks	low	6/6
8	50γ ”	95	.6	3/6
9	5γ pyridoxamine HCl	2* wks	low	6/6
10	50γ ”	127	.9	3/6
11	100γ ”	167	.8	0/6
12	100γ pyracin-5	50	0	5/6
13	200γ ”	3* wks	0	6/6
14	400γ ”	4* wks	0	6/6
15	As 14 + 100γ pyridoxine HCl	138	no effect	0/6

* Indicates time of death of the last chick in the group.
† Activity values are based on survival data as well as growth data in groups No. 8 and No. 10.

50γ gave an intermediate value and chicks receiving 100γ had clotting times comparable to the controls. Chicks which received 100γ of pyridoxine per 100 g of ration for the first week and the vitamin B_6 deficient ration for the next 3 weeks (group 5) had a short whole blood clotting time (2 min) at 4 weeks. Control chicks receiving 5% alfalfa or 1 mg of 2-methyl-1, 4-naphthoquinone per 100 g of ration 494 (groups 7 and 8 respectively) had comparatively long whole blood clotting times.

Determinations of the prothrombin level (or activity) (Table V) made by Dr. John B. Field indicate that the decrease in whole blood clotting time is accompanied by a detectable hyperprothrombinemia in the B_6 deficient chicks.

No gross pathology of the internal organs was observed at autopsy although the spleens of the vitamin B_6 deficient chicks appeared abnormally small. Further investigation indicated that the spleens of the vitamin B_6 chicks were proportionately smaller than those of control chicks (the spleens were respectively 0.09 and 0.20% of the body weights).

Discussion. Since the activities found for pyridoxal and pyridoxamine using the chick assay are not in accord with the high bacterial potencies, pyridoxine remains the most important of the B_6 vitamins in chick nutrition. Assays of natural materials for nutritionally active vitamin B_6 should thus be made using either biological methods, or corrections for the

TABLE IV.
Hemoglobin Values of 4-Weeks-Old Chicks.

Diet	No. chicks	Hb g/100 cc	Range
B_6 deficient	10	6.5	(3.1- 9.7)
Complete	12	8.7	(6.4-10.4)
Difference		2.2	
Standard error = 0.68*			

* The statistical validity of these data was confirmed by Dr. S. Ames by use of "Students" *t*-test for unique samples.

differences found between the biological and microbiological activities of compounds such as pyridoxine, pyridoxal and pyridoxamine.

Pyracin-5 appears inactive under all of the conditions used, in contradiction to the first report of the Cornell workers.[8] A later report[18] indicates that a concentrate in their ration was contaminated with some *L. casei* factor. Recently Scott *et al.*[7] have reported that the lactone of both the 4- and the 5-carboxypyridine derivatives are active for the chick in the presence of a *Lactobacillus casei* factor. This finding, presented without data, is difficult to evaluate, but it should be pointed out that the third *Lactobacillus casei* factor gives a significant response without feeding any lactone.[11,18] This response, as well as the

17 Campbell, H. A., Smith, W. K., Roberts, W. L., and Link, K. P., *J. Biol. Chem.*, 1941, **38**, 1.

18 Norris, L. C., *Flour and Feed*, 1944, **45**, 44.

19 Hutchings, B. L., Stokstad, E. L. R., Bohonos, N., and Slobodkin, N. H., *Science*, 1944, **99**, 371.

TABLE V.
Whole Blood and Prothrombin Clotting Times of 4-Weeks-Old Chicks.

No.	Supplement/100 g 494-B_6	No. chicks	Whole blood clotting time (min)	Prothrombin time (sec)*
1	None	3	4 (3.3- 5.0)	31 (28.0-32.2)
2	25γ pyridoxine	4	4 (3.0- 6.0)	
3	50γ ,,	4	8 (1.2-13.5)	
4	100γ ,,	4	17 (8.8-29.3)	
5	100γ ,, (1st week only)	2	2 (2.0- 2.5)	
6	400γ ,,	4	16 (14.5-19.0)	39 (33.9-46.3)
7	400γ ,, + 5 g alfalfa	4	21 (9.3-34.0)	
8	400γ ,, + 1 mg 2-methyl-1, 4-naphthoquinone	4	11 (6.3-13.8)	
9	100% Practical broiler ration	4	20 (10.5-29.0)	

* Determinations by Dr. John B. Field using the 12.5% plasma method[17] with thromboplastin prepared from chick brain.

response to other compounds such as vitamin C[20] and p-aminobenzoic acid,[21] is not obtained when the bacterial flora is altered by feeding sulfa drugs or corn meal.[11] While we have been unable to observe any effect due to the feeding of pyracin-5 it is entirely possible that certain responses may be obtained when it is fed in combination with other factors.

The decreased whole blood clotting time, as well as the decreased prothrombin clotting time, observed in the vitamin B_6 deficient chicks is obtained with a ration containing adequate amounts of vitamin K.[22,23] Whether the anemia and small spleens are correlated in some way with the blood clotting mechanism is not known.

Summary. Pyridoxal, pyridoxamine and pyracin-5 were found to be inactive singly in replacing vitamin B_{10} or vitamin B_{11} in chick nutrition. The biological vitamin B_6 activity of these three compounds is approximately $>1/2$, $4/5$, and 0 respectively. Vitamin B_6 deficiency symptoms in the chick were found to include a decreased clotting time, hyperprothrombinemia, small spleens and anemia.

20 Briggs, G. M., Jr., Luckey, T. D., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 130.

21 Briggs, G. M., Jr., Luckey, T. D., Mills, R. C., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 7.

22 Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 194.

23 National Research Council, *Recommended Nutrient Allowances for Domestic Animals*, 1944, Bull. No. 1, p. 3.

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Effect of Growth Influencing Factors on the *In Vitro* Deamination of dl-Alanine.

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There is evidence concerning specific effects upon intermediary protein metabolism of hormonal factors capable of influencing growth (for review, see Long[1]). Thus the growth factor of the pituitary has been shown to cause a fall in blood NPN, chiefly amino and urea N;[2] a decrease in total NPN in the body and especially in the liver, all nitrogenous fractions

* Aided by a grant from the Charlton Research Fund.

1 Long, C. N. H., *Cold Spring Harbor Symposia on Quant. Biol.*, 1942, **10**, 91.

2 Teel, H. M., and Watkins, O., *Am. J. Physiol.*, 1929, **89**, 662.

3 Shaffer, N. K., and Lee, M. O., *J. Biol. Chem.*, 1935, **108**, 355.