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 $>\frac{1}{2}$, $\frac{1}{5}$, 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.

## 14948

## 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

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

[2] Teel, H. M., and Walkins, O., *Am. J. Physiol.*, 1929, **89**, 662.

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

TABLE I.
Deamination of dl-Alanine by Rat Tissue Slices.

| | Amino N* disappearance γ/mg dry wt/hr | Ammonia N* production γ/mg dry wt/hr | Urea N* production γ/mg dry wt/hr | $QO_2$* |
|---|---|---|---|---|
| | | Liver-Adult | | |
| Normal—No injections | —1.6±0.20 (15) | 0.19±0.03 (25) | 0.30±0.05 (25) | 0.07±0.11 (11) |
| Pituitary growth complex | —1.1±0.29 (5) | 0.17±0.04 (15) | 0.30±0.04 (14) | 1.1 ±0.25 (5) |
| Adrenal cortical extract | | 0.03±0.08 (15) | 0.43±0.11 (15) | |
| Thyroxin | —1.6±0.43 (13) | 0.13±0.04 (15) | 0.44±0.02 (14) | 1.2 (3) |
| " + growth compl. | —1.2±0.11 (12) | 0.13±0.02 (16) | 0.49±0.04 (16) | |
| Thyrotropic hormone | —1.0±0.04 (4) | 0.11±0.07 (23) | 0.43±0.14 (16) | |
| " " + growth complex | —0.3±0.21 (5) | 0.17±0.05 (16) | 0.44±0.16 (16) | |
| | | Kidney-Adult | | |
| Normal—No inj. | —4.6±0.27 (22) | 2.65±0.15 (31) | —0.12±0.12 (23) | 7.9 ±0.64 (22) |
| Pituitary growth compl | —4.9±0.31 (20) | 2.59±0.09 (38) | 0.00±0.06 (37) | 8.6 ±0.40 (22) |
| Adrenal cortical extr | —4.7±0.73 (3) | 2.04±0.36 (14) | 0.10±0.31 (14) | 8.5 (3) |
| Thyroxin | —4.8±0.37 (6) | 2.42±0.13 (9) | —0.19±0.04 (9) | |
| " + growth compl | | 2.15±0.13 (7) | —0.06±0.06 (7) | |
| Thyrotropic hormone | | 2.39±0.21 (7) | 0.04±0.02 (7) | |
| " " + growth compl | | 2.31±0.15 (8) | 0.05±0.06 (8) | |
| | | Kidney-Young Animal | | |
| Normal | —4.1±0.27 (16) | 2.32±0.12 (27) | 0.21±0.07 (20) | 6.8 ±0.41 (14) |
| " + growth compl | —4.6±0.33 (16) | 2.38±0.15 (27) | 0.01±0.16 (27) | 7.0 ±0.43 (9) |
| Hypophysectomized | —3.7±0.70 (5) | 1.44±0.29 (5) | 0.38±0.18 (4) | 6.5 ±0.41 (5) |
| " + growth compl | —4.4±0.94 (4) | 1.66±0.58 (4) | 0.02±0.07 (4) | 6.5 ±0.92 (4) |
| Adrenalectomized | —5.2±0.25 (16) | 2.17±0.11 (16) | 0.05±0.05 (15) | 6.2 ±0.16 (12) |
| " + growth complex | —4.9±0.24 (18) | 1.92±0.15 (19) | —0.21±0.18 (18) | 5.5 ±0.43 (17) |

* Difference between tissues in medium without substrate and with 0.01 M alanine. Figures in parentheses indicate the number of experiments.
± = Standard mean deviation.

participating except $NH_3$;[3] and a decreased urinary N, chiefly urea.[4] These findings have received later confirmation, but the nature and locus of the effects is still not clear. In the case of other hormones the results are even more obscure. The thyroid, at least at certain age levels, is concerned with growth, but evidence associating this with protein metabolism is scanty. Sternheimer[5] observed increase in protein liver N following a single thyroxin injection, and Zitovskaya[6] noted an increased synthesis by the liver of alanine from pyruvic acid and $NH_3$, following thyroxin injection. As for the functions of the adrenal cortex in this connection, Russell and Wihelmi[7] described a lower than normal rate of deamination by the kidney of dl-alanine and 1 (+) glutamic acid, in adrenalectomized rats, with restoration of function on administering adrenal cortical extract; while Jiminez-Diaz[8] reported a rather remarkable decrease in kidney $Q_{NH_3}$ as a result of adrenalectomy in the rat. On the other hand, in the functionally nephrectomized animal Evans[9] found that the blood amino and urea N were unaffected by adrenalectomy.

In the following we report the fact that we have been unable, under the conditions of our experiments, to obtain any unequivocal evidence of an effect on the *in vitro* deamination of dl-alanine by various growth influencing factors.

Kidney and liver slices of adult and young (30-40-day-old) rats were used, the medium

[4] Gaebler, O. H., *J. Expt. Med.*, 1933, **57**, 349.
[5] Sternheimer, R., *Endocrinology*, 1939, **25**, 899.
[6] Zitovskaya, F., *Bull. biol. med. exptl. U.S.S.R.*, 1939, **7**, 114.
[7] Russell, J. A., and Wilhelmi, A. E., *J. Biol. Chem.*, 1941, **137**, 713.
[8] Jiminez-Diaz, C., *Lancet*, 1936, **2**, 1135.
[9] Evans, G., *Endocrinology*, 1941, **29**, 737.
[10] Canzanelli, A., Rogers, G., and Rapport, D., *Am. J. Physiol.*, 1942, **135**, 309.

being a previously described modified phosphate-buffered Ringer's solution.[10] The hormones were injected as follows: (1) Pituitary Growth Hormone: 0.05 cc Ayerst, McKenna and Harrison Growth Complex† (5 Collip units) per 100 g wt intraperitoneally, once 2 days before the expt., twice (A.M. and P.M.) one day before, and once about 1 hr before (on the basis of the period of maximum N retention—M. Lee, personal communication); (2) Thyroxin: 1 mg (1 cc ampoule La Roche) subcutaneously 2 days before experiment (on the basis of the maximum effect on the metabolic rate of the intact animal); (3) Thyrotropic Hormone: Either our own preparation, made according to the method of Rowland and Parkes, 10 mg intraperitoneally on 5 successive days before experiment, or Armour Thyrotropic Factor, 0.2 cc intraperitoneally daily for 5 days (on the basis of the maximal metabolic effect); (4) Adrenal Cortical Hormone: 1 cc Upjohn Extract (2.5 rat units) every hr for 5 hr before the experiment; (5) Combinations of hormones were given according to the above dosages.

In the medium from which the tissue had been removed, amino N was determined by the method of Van Slyke and Neill and ammonia and urea by the method of Conway and Byrne.[11] The tissue was incubated in the medium with and without alanine at 37°C for 110 minutes. In most cases each recorded experiment represents the determination of the metabolism in the pooled media of 5 Warburg flasks, each of which contained 3 cc of medium and 30 to 40 mg dry weight of tissue. In the remaining experiments, about 150-200 mg dry weight of tissue were incubated with 15 cc of medium in a single Erlenmeyer flask.

It will be seen in Table I that the $NH_3$ and urea formation by adult liver in the presence of alanine were entirely unaffected by any previous injection. The amino N disappearance was always greater than could be accounted for by the $NH_3$ and urea, except in the experiments where thyrotropic and growth hormones were given together, but even here there was no apparent change in deamination.

The kidney, as Krebs[12] first showed, deaminated alanine much more rapidly than did liver; there was no evidence of urea formation; and the $NH_3$ did not account for the disappearance of amino N. In both adult and young kidney, as in liver, there was no unequivocal evidence in any of the experiments that either the injection of hormonal preparations, or adrenalectomy, had the slightest effect on the catabolism of the amino acid. In the case of the adrenalectomized animals our results are in conflict with those of Russell and Wilhelmi, quoted above. Even in the case of the hypophysectomized animal where a possible statistical case might be made for a lessened ability to deaminate alanine, we do not believe the evidence warrants such a conclusion.

Apart from the fact that decreased deamination may have occurred, but have been of an order of magnitude too small for measurement, the negative results may be due to (1) the fact that the catabolism of "non-essential" amino acids like alanine are unaffected by growth factors; (2) that protein synthesis may be independent of deamination, following rather than forestalling it, in which case one would have to assume that the synthesis is occurring elsewhere than in the tissue studied, else it would be observable by our technic. The foregoing factors require further study.

*Summary.* Experiments on the effect of hormonal factors capable of influencing growth have failed to yield evidence of a decreased deamination of dl-alanine by rat liver and kidney.

† We are indebted to Ayerst, McKenna and Harrison for a generous supply of their Growth Complex.

[11] Conway, E. J., and Byrne, A., *Biochem. J.*, 1933, **27**, 419, 430.

[12] Krebs, H. A., *Zeitsch. f. Physiol. Chemie*, 1933, **217**, 191.