was a decrease in the tributyrinase of the serum of hypertensive and arteriosclerotic males as compared with the normal, while the serum of the females shows no difference from the normal.

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## Growth-Promoting Activity of L-Lyxoflavin.*† (19131)

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Pallares and Garza(1) isolated L-lyxoflavin from the heart myocardium. These workers did not suggest any biological significance of this finding, but recently Emerson and Folkers(2) found that lyxoflavin stimulated the growth of rats on a hyperthyroid soybean ration, equal to the response given by an insoluble liver fraction.

A chick assay for unidentified factors has been described by Bruins *et al.*(3). Briefly, the diet is an isolated soybean protein-sucrose diet, properly supplemented with salts, known vitamins and aureomycin. Thiamine is supplied by injection. It seemed desirable to test the activity of L-lyxoflavin under these conditions; accordingly studies were initiated in April, 1951.

*Results and Discussion.* A summary of the results of 5 experiments with chicks is given in Table I. These results show that lyxoflavin consistently elicits a growth response, alone and in combination with dried whey, wheat bran and liver residue. The magnitude of this response is not large, being of the order of a 10% increase. Certainly, it does not represent all of the unidentified factor activity involved.

In fact, these results do not clearly show what crude supplements might owe their activity to their content of lyxoflavin. As a working hypothesis, it was earlier believed that whey, being a good source of riboflavin, might fall into this class, but that has not been demonstrated in these trials since lyxoflavin complements a level of whey which is believed to be sufficient to supply the "whey factor"(4).

The mode of action of this compound is not elucidated by these trials. It will be noted in Experiment 5 that lyxoflavin does not have riboflaviin activity for the chick, even though these compounds differ only in the configuration about $C_4$ of the side chain. Emerson and Folkers reported similar results for the rat.

Snell *et al.*(5) showed that lyxoflavin similarly is without riboflavin activity for microorganisms, although its addition in appropriate quantities does stimulate growth of *Lactobacillus casei* when riboflavin is limiting. At high concentrations, lyxoflavin inhibits growth of this microorganism, acting as an antimetabolite of riboflavin.

The possibility exists that the growth-promoting action of lyxoflavin for chicks might be due to a similar "antibiotic" activity. However, no action of this sort is indicated since the basal ration contains 50 mg/kg of aureomycin, a level more than adequate to promote maximum antibiotic response. That combinations of antibiotics might stimulate growth

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‡ Now with the University of Texas, Austin, Texas.

1. Pallares, E. S., and Garza, H. M., *Arch. Biochem.*, 1949, v22, 63.

2. Emerson, C. A., and Folkers, K., *J. Am. Chem. Soc.*, 1951, v73, 2398.

3. Bruins, H. W., Sunde, M. L., Cravens, W. W., and Snell, E. E., in press.

4. Hill, F. W., *Poultry Sci.*, 1948, v27, 536.

5. Snell, E. E., to be published.

TABLE I. Tests with L-lyxoflavin.

| Exp. No. | Chicks per group* | LSD, g | Group No. | Additions to basal ration† | Two wk wt, g‡ |
|---|---|---|---|---|---|
| 1 | 10 | — | 1 | 0 | 90 |
| | | | 2 | 3 mg/kg L-lyxoflavin | 114 |
| | | | 3 | 5% wheat bran | 115 |
| | | | 4 | 5% wheat bran + 3 mg/kg L-lyxoflavin | 132 |
| | | | 5 | 10% dried whey + 10% liver residue | 168 |
| 2 | 14 | 13.7 | 1 | 0 | 85 |
| | | | 2 | 3 mg/kg L-lyxoflavin | 84 |
| | | | 3 | 15 mg/kg L-lyxoflavin | 109 |
| | | | 4 | 5% wheat bran | 110 |
| | | | 5 | 5% wheat bran + 3 mg/kg L-lyxoflavin | 128 |
| | | | 6 | 5% wheat bran + 15 mg/kg L-lyxoflavin | 129 |
| 3 | 13 | 9.8 | 1 | 0 | 87 |
| | | | 2 | 5 mg/kg L-lyxoflavin | 93 |
| | | | 3 | 10 mg/kg L-lyxoflavin | 97 |
| | | | 4 | 15 mg/kg L-lyxoflavin | 96 |
| | | | 5 | 30 mg/kg L-lyxoflavin | 99 |
| | | | 6 | 4% dried whey + 4% liver residue | 153 |
| 4 | 13 | 14.8 | 1 | 0 | 83 |
| | | | 2 | 2% liver residue | 99 |
| | | | 3 | 2% liver residue + 15 mg/kg L-lyxoflavin | 108 |
| | | | 4 | 2% liver residue + 4% dried whey | 119 |
| | | | 5 | 4% liver residue + 4% dried whey | 128 |
| 5 | 12 | 14.8 | 1 | 0 | 94 |
| | | | 2 | 15 mg/kg L-lyxoflavin | 111 |
| | | | 3 | 4% liver residue | 132 |
| | | | 4 | 4% liver residue + 15 mg/kg L-lyxoflavin | 145 |
| | | | 5 | 4% dried whey | 115 |
| | | | 6 | 4% dried whey + 15 mg/kg L-lyxoflavin | 125 |
| | | | 7 | 4% dried whey + 4% liver residue | 151 |
| | | | 8 | Less riboflavin | 51 |
| | | | 9 | Less riboflavin + 15 mg/kg L-lyxoflavin | 54 |

* Chicks weighed wing banded and randomized among groups and placed on experimental rations at day old.

† Supplements were made at the expense of sucrose.

‡ Mortality was negligible in all groups.

beyond what would be observed from a single antibiotic has been suggested, but thus far no evidence in support of this suggestion has been published.

In preliminary experiments, the supplementation of simplified practical-type rations with lyxoflavin has resulted in slight growth stimulation. However, further experiments are under way to evaluate the practical significance of this result.

*Summary.* L-lyxoflavin stimulates growth of chicks under these conditions. Its mode of action remains obscure. Since it has no riboflavin activity, and its growth-promoting action occurs on a ration rich in riboflavin, it is quite possible that it acts as a true vitamin-like entity. The possibility that it acts in the same manner as the known antibiotics is unlikely, since the ration contained aureomycin.

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