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A Critique of Biological Activity of L-Lyxoflavin.* (19776)

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Pallares *et al.*(1) claimed that lyxose was isolated from human myocardium treated with cobra venom. This group later reported(2) the isolation of lyxoflavin from human myocardium and postulated the release of lyxose by the enzymes of cobra venom. Gardner *et al.*(3,4) and Heyl *et al.*(5) subsequently described syntheses of L-lyxoflavin. Gardner *et al.*(3,4) failed to obtain lyxose from synthetic L-lyxoflavin treated with either cobra venom or Russell's viper venom. It was then reported that L-lyxoflavin has vitamin activity for the rat fed a diet containing 0.5% dessicated thyroid(6,7), and growth promoting activity for the chick(8).

Snell and Strong(9) have shown that other stereoisomers of riboflavin, namely the d- and l-arabityl analogs, enhanced the growth response of *Lactobacillus casei* to limiting amounts of riboflavin although these are devoid of activity when assayed in the absence of riboflavin. Other workers(10,11) have demonstrated that the d-xylityl and l-arabityl analogs of riboflavin have limited effectiveness in replacing riboflavin in rats receiving a riboflavin deficient ration. We wish to report our observations on the similar behavior of L-lyxoflavin in the nutrition of *Lactobacillus casei*, the rat and the chick.

Microbiological experiments. L-Lyxoflavin was tested for riboflavin activity using the assay of Snell and Strong(12). The results are shown in Fig. 1. It is apparent that lyxoflavin shows little activity in the absence of added riboflavin. The failure of the higher levels of lyxoflavin to give increased growth indicates that the slight activity of lyxoflavin is not due to contamination with riboflavin. However, in the presence of 0.10 γ of riboflavin, lyxoflavin approximates the activity of riboflavin at levels of 0.02 to 0.10 γ. At levels of 150 γ or more, lyxoflavin inhibits the response of the microorganisms to 0.10 γ of riboflavin. When these results were completed, Bruins *et al.*(8) stated without detailing the data that lyxoflavin stimulated the growth of *Lactobacillus casei* in the presence of limiting amounts of riboflavin and acts as an antimetabolite at higher concentrations.

Rat experiments. Emerson and Folkers(7) found that lyxoflavin was without activity in a prophylactic rat assay for riboflavin. In our experiments, L-lyxoflavin was tested for riboflavin activity in both prophylactic and curative assays. The ration of Street(13) was modified so that the daily vitamin supplement contained 20 γ B_1, 20 γ B_6, 200 γ calcium pantothenate, 250 γ p-aminobenzoic acid, 500 γ i-inositol, 2 γ biotin, 2 γ folic acid, 1 mg niacin and 4 mg choline chloride. Male, weanling Sprague-Dawley rats were used in these experiments. In the prophylactic assay 3 levels of L-lyxoflavin (7, 35 and 70 γ per day) and 2 levels of riboflavin (3.5 and 7 γ per day) were employed in addition to a negative control. Ten rats were used in each group. The results are plotted in Fig. 2. The greatest growth-promoting effect of lyxoflavin was manifested within the first 2 weeks of the assay, after which time the growth rate declined in contrast to the sustained weight gain of the rats receiving riboflavin. It is also

* Roche Publication No. 317.

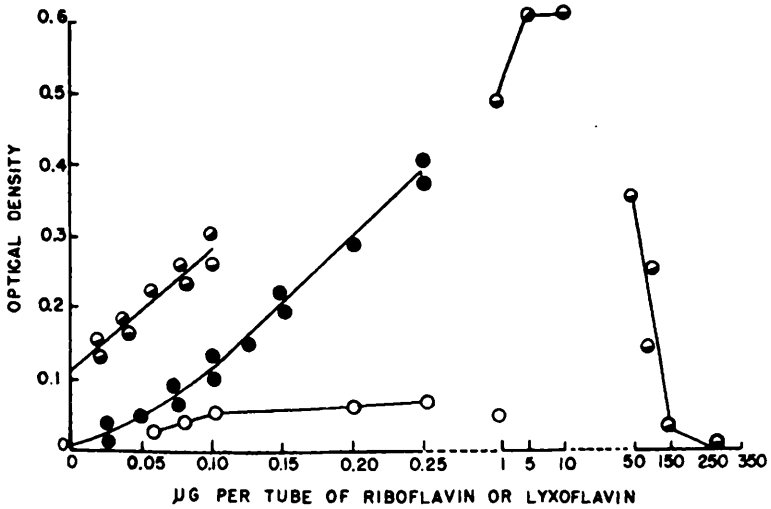

FIG. 1. Growth of *L. casei* with riboflavin and/or L-lyxoflavin in a riboflavin-deficient medium. ●——● riboflavin, ○——○ L-lyxoflavin, (half closed circle)——(half closed circle) riboflavin 0.1 γ + L-lyxoflavin.

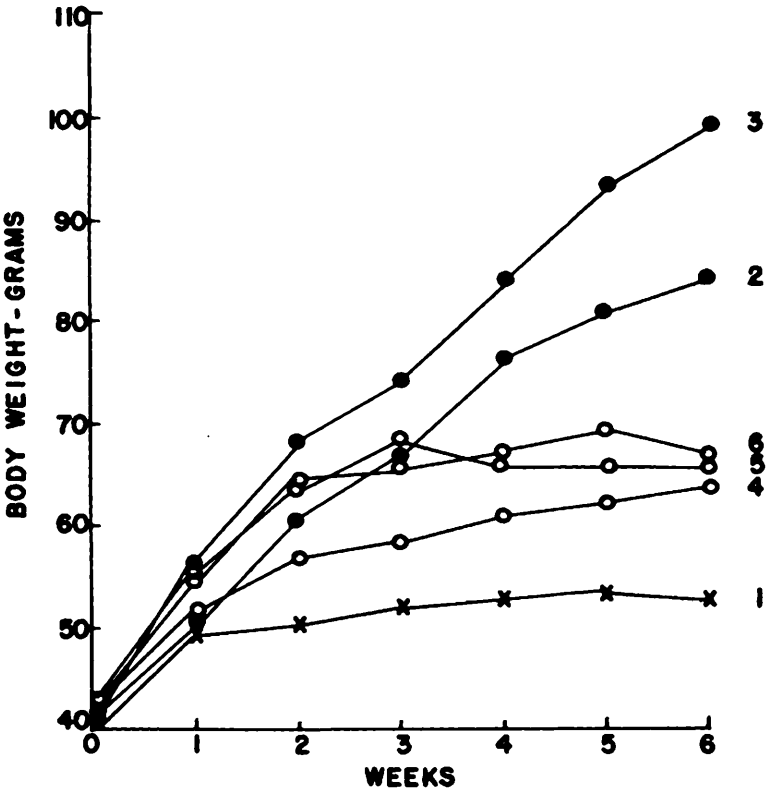

FIG. 2. Growth of riboflavin-deficient rats after dosage with riboflavin and L-lyxoflavin: prophylactic assay.

Group	Dosage in γ per day
×——× 1	none
●——● 2, 3	riboflavin, 3.5 and 7, resp.
○——○ 4, 5, 6	L-lyxoflavin, 7, 35 and 70, resp.

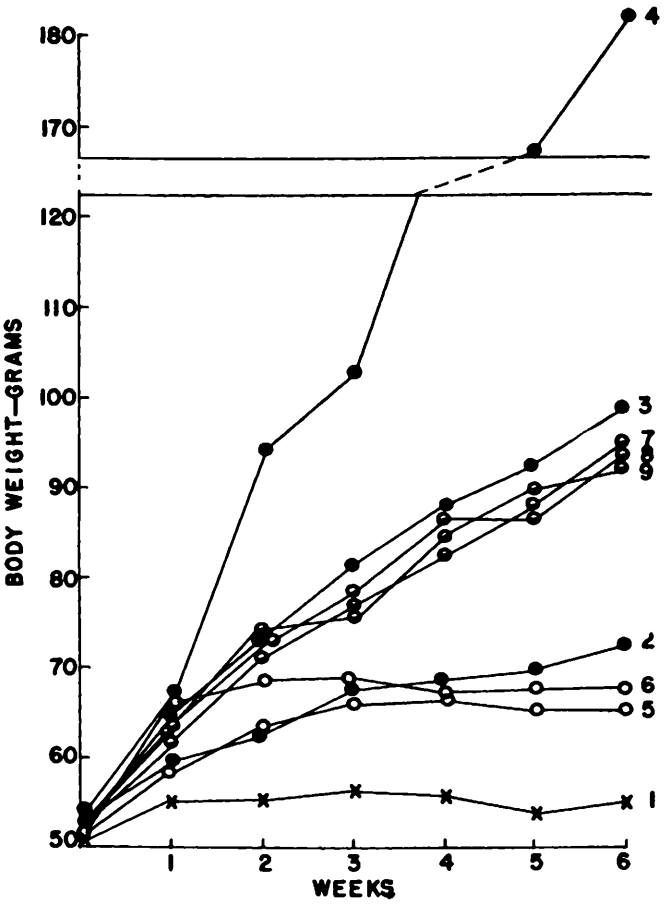

FIG. 3. Growth of riboflavin-deficient rats after dosage with riboflavin and/or L-lyxoflavin curative assay.

	Group	Dosage in γ per day
×——×	1	none
●——●	2, 3, 4	riboflavin, 3.5, 7, and 20, resp.
○——○	5, 6	L-lyxoflavin, 20, and 70, resp.
(Half closed circle) —— (Half closed circle)	7, 8, 9	riboflavin, 3.5 + L-lyxoflavin 3.5, 7, and 70, resp.

of interest to note that there was little difference in the weight gain after 6 weeks among the groups receiving the three levels of lyxoflavin.

In the *curative assay,* the rats were given the depletion diet for 3 weeks at the end of which time they were divided into groups of 10 each according to weight. In these experiments, lyxoflavin was fed a) alone (20 and 70 γ per day) and b) at 3 levels (3.5, 7.0 and 70 γ per day), each combined with 3.5 γ of riboflavin. In addition, 3 levels of riboflavin were included (3.5, 7.0 and 20γ per day). The results are shown in Fig. 3. Again, as in the prophylactic assay, the greatest slope in the growth curve was observed within the first two weeks of the assay for the groups receiving lyxoflavin alone, and little difference in growth rate was noted between the 2 levels of lyxoflavin. Of particular interest is the fact that those groups of rats receiving 3.5 γ of riboflavin plus 3.5 γ of lyxoflavin grew as well throughout the assay period as those receiving 7 γ of riboflavin. However, the groups receiving higher levels of lyxoflavin combined with 3.5 γ of riboflavin did not attain higher weights.

In another experiment it was found that a

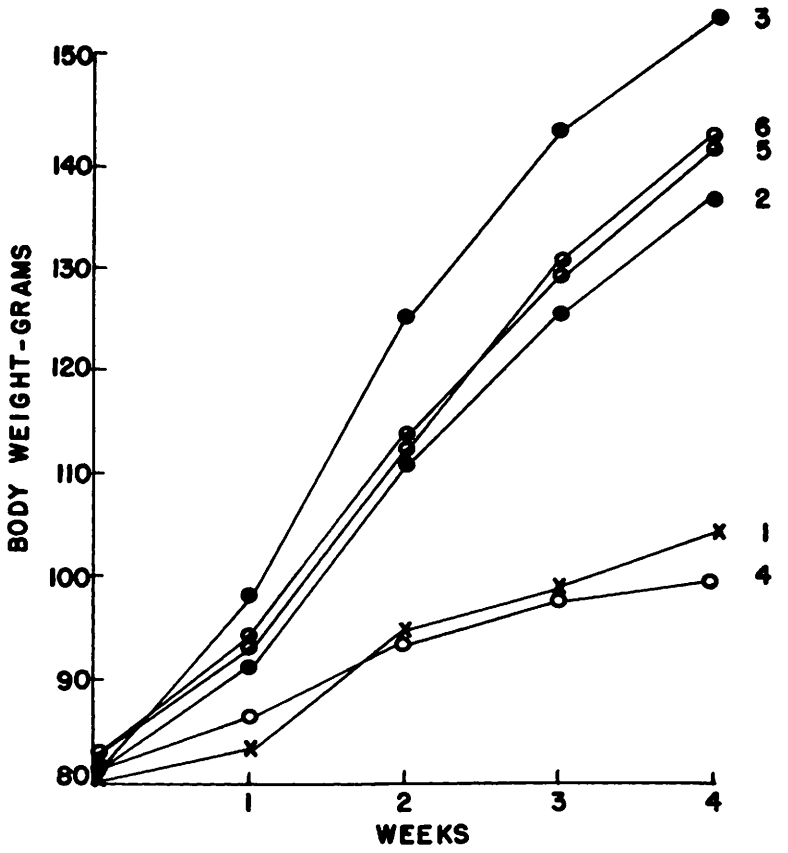

FIG. 4 Growth of pantothenic acid-deficient rats after dosage with pantothenic acid and/or L-lyxoflavin.

	Group	Dosage in γ per day
×——×	1	none
●——●	2, 3	calcium pantothenate, 35 and 70 γ, resp.
○——○	4	L-lyxoflavin, 20 γ
(Half closed circle) —— (Half closed circle)	5,6	calcium pantothenate, 35 γ + L-lyxoflavin, 7 and 20 γ, resp.

combination of 1.75 γ of lyxoflavin and 1.75 γ of riboflavin produced a similar growth response in a curative assay as 3.5 γ of riboflavin. No further weight gains were observed in groups receiving 2.5 or 10 γ of lyxoflavin in combination with 1.75 γ of riboflavin.

In order to determine whether the growth-promoting activity of lyxoflavin would be exhibited only in the presence of suboptimal levels of riboflavin in the diet, lyxoflavin was fed to rats receiving the same diet but containing optimal amounts of riboflavin and deficient in pantothenic acid. Lyxoflavin was fed alone and in combination with a suboptimal level of calcium pantothenate and compared to groups receiving calcium pantothenate alone (Fig. 4). Under these conditions L-lyxoflavin was inactive in promoting growth in pantothenic acid deficient rats and combinations of L. lyxoflavin and calcium pantothenate were no more effective than that level of calcium pantothenate alone.

Chick experiments. Bruins *et al.*(8) fed lyxoflavin to chicks receiving a ration lacking in unknown factors and found that it produced a small but consistent stimulation in weight at levels of 3 to 30 mg per kilo of ration. There were, however, no data given with comparable levels of riboflavin. Accordingly, we have repeated part of this chick work.

TABLE I. Comparative Effectiveness of L-Lyxoflavin and Riboflavin in Producing Weight Response in the Chick.

Supplement to basal ration, mg/kg	Total gain in 2 wk assay period g	Stand. error of mean
0	77	± 3.6
L-lyxoflavin, 3	72	± 3.8
L-lyxoflavin, 30	82	± 3.5
Riboflavin, 3	85	± 3.8
Riboflavin, 30	81	± 4.2

The ration of Bruins *et al.*(8) was supplemented with 2 levels of lyxoflavin and similarly with 2 levels of riboflavin. One-day-old, single comb, white Leghorn chicks were given the basal ration for 3 days at the end of which time runts and giants were culled and the remaining chicks divided according to weight into 5 groups of 20 chicks each. The chicks were housed in an electrically heated brooder for the 2-week assay period. The results are given in Table I. Small differences were obtained at the higher level of lyxoflavin and at both levels of riboflavin, showing that the small weight gains elicited with high levels of lyxoflavin can be obtained equally well with the same dosage of riboflavin. In any case, the differences from the control group are not statistically significant. However, it is interesting to note that the birds in these 3 groups had superior feathering in comparison to those in the groups receiving the unsupplemented basal or lower level of lyxoflavin which showed scratched and torn skin.

Discussion. From the results of the above experiments it appears that L-lyxoflavin has a limited capacity to replace riboflavin in the nutrition of *Lactobacillus casei* and the rat. Furthermore, for both *L. casei* and the rat, the activity of lyxoflavin appears to vary depending upon the relative levels of riboflavin and lyxoflavin. In this respect, it appears to resemble in activity the d- and l-arabityl analogs of riboflavin in *L. casei*(9) and d-xylityl and l-arabityl analogs in the rat(10,11). The activity of lyxoflavin when fed without riboflavin may vary depending on the stores of riboflavin in the organism or the presence of riboflavin in the diet or medium. Thus, in contrast to the riboflavin activity in the present experiments, lyxoflavin has been reported devoid of activity for the riboflavin deficient rat (7) and for *L. casei* in the absence of riboflavin(8). Recently Shorb(14) has demonstrated that L-lyxoflavin has some riboflavin activity for *Lactobacillus lactis* and confirmed the activity for *L. casei* in the presence of riboflavin.

Wahlstrom and Johnson(15) have described a differential assay procedure for mixtures of riboflavin and lyxoflavin involving the measurement of fluorescence after Florisil purification and microbiological assay with *L. casei*. It is surprising that these authors found no microbiological activity for lyxoflavin in mixtures with riboflavin. This finding is, of course, a necessary prerequisite for the application of the differential assay. Our findings, which have been confirmed by Bruins *et al.*(8) and Shorb(14), that in the presence of riboflavin lyxoflavin has activity for *L. casei* ranging from practically 100% at low levels to inhibition at high levels, would preclude the use of these strains of *L. casei* for this method of assay.

Emerson and Folkers(6,7) obtained a weight response with a daily administration of 150 γ of lyxoflavin in rats fed a soybean meal-dextrose diet containing 0.5% desiccated thyroid equivalent to that obtained when 10% liver or fish meal were added to the diet. Ershoff(16), on the other hand, noted that lyxoflavin was ineffective as an antithyrotoxic factor for the rat. Under his conditions, lyxoflavin failed to elicit a response when fed at levels of 30 and 100 mg per kilo of casein-sucrose diet containing 0.5% desiccated thyroid. At the higher level, the daily dosage of lyxoflavin would be expected to be greater than that administered by Emerson and Folkers(6,7). Liver, however, produced a weight response and prevented the glandular lesions observed in rats fed this thyroid-containing ration. It is apparent that liver counteracted the effect of thyroid powder when fed to rats receiving either a casein-sucrose(16) or soybean-meal dextrose ration(6,7), whereas lyxoflavin was only effective when fed with the soybean-meal dextrose ration(6,7). It is possible that under certain conditions, lyxoflavin may stimulate the synthesis of the liver factor by the intestinal flora. For example, it has

been shown(17) that riboflavin can partially replace vit. B_{12} in the nutrition of rats receiving a diet containing thyroxine. Hartman *et al.*(18) also noted that riboflavin can replace vit. B_{12} in the rat and demonstrated that its activity is due to increased synthesis of vit. B_{12} by the intestinal flora. A similar utilization of lyxoflavin, which has been shown to have riboflavin activity under certain conditions, may account for the growth effects observed.

Wahlstrom and Johnson(15) recently reported that lyxoflavin stimulated the growth of baby pigs fed a low fat basal ration containing 0.01% protamone, although the pigs did quite well on the basal ration devoid of any known lyxoflavin. These authors point out that lyxoflavin may exert its effect in a manner similar to that of antibiotics or surfactants rather than as a new member of the vit. B complex. In view of our evidence that riboflavin exerts the same effect as lyxoflavin in promoting growth of chicks on a diet deficient is unknown factors, it is possible that riboflavin would also be active in the rat(6,7) or the pig(15) under conditions where lyxoflavin has been found to stimulate growth. It appears desirable to test riboflavin in any circumstances where lyxoflavin has activity before drawing any conclusions regarding unique functions for lyxoflavin.

Summary. Lyxoflavin has been shown to possess a limited ability to replace riboflavin in the nutrition of *L. casei* and the rat. In chicks, large doses of lyxoflavin produce small weight gains, which, however, can be matched by equal doses of riboflavin. The present evidence does not warrant the classification of lyxoflavin as a new vitamin.

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Effect of Citrovorum Factor upon Tyrosine Metabolism in Clinical Scurvy.* (19777)

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Administration of tyrosine to guinea pigs (1-3), monkeys(4), infants(5,6) or adult men(7) with ascorbic acid deficiency results in the urinary excretion of abnormal quantities of hydroxyphenyl compounds, which decrease

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