THE EFFECTS OF LYSOLECITHIN ON THE GROWTH OF LACTOBACILLUS CASEI IN RELATION TO BIOTIN, PANTOTHENIC ACID, AND FAT-SOLUBLE MATERIALS WITH BIOTIN ACTIVITY

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Received for publication May 3, 1948

Williams and Fieger (1946) found that Lactobacillus casei could be grown continuously in a medium containing only traces of biotin if oleic acid was present at a suitable concentration. Trager (1947) observed a similar effect with a neutral oil (designated FSF) obtained from hydrolyzed horse plasma. This material, which had some biotin activity for chickens as well as for bacteria, permitted full growth of L. casei even in the presence of enough fresh egg white to inactivate completely any traces of biotin which might have been present in the medium. These results have been confirmed and extended (Williams and Fieger, 1947; Axelrod, Hofmann, and Daubert, 1947; Hofmann and Axelrod, 1947; Williams, Broquist, and Snell, 1947).

Lecithin exerts growth-stimulating effects on the lactic acid bacteria (Bauernfeind, Sotier, and Bowff, 1942; Strong and Carpenter, 1942) and can replace oleic acid for organisms requiring this material (Williams, Broquist, and Snell, 1947). It can also replace biotin in the growth of L. casei (Trager, unpublished). Since the effects of lecithin may be supposed to be a result of its oleic acid content, it seemed of interest to investigate the effects of lysolecithin, which differs from lecithin in that it does not contain an unsaturated fatty acid.

MATERIALS AND METHODS

Two samples of lysolecithin, both obtained from the Levene collection of the Rockefeller Institute, have been used. One, no. 1070, contained 3 to 5 per cent amino nitrogen, whereas the other, no. 1072, contained 5 to 10 per cent amino nitrogen. A stock solution of each, containing 2 mg per ml, was prepared in phosphate buffer of pH 7.4 and stored in the refrigerator. Preparation 1070 gave complete hemolysis of washed sheep red blood cells down to a concentration of 1:10,000, whereas preparation 1072 gave complete hemolysis at concentrations down to 1:50,000. The two preparations were, however, identical in their effects on the growth of L. casei. Since more of preparation 1070 was available, it was used for most of the experiments.

Stock cultures of L. casei were carried by weekly transfer in a medium consisting of 1 per cent yeast extract, 1 per cent glucose, 0.5 per cent peptone, and 1.5 per cent agar. The synthetic media used for the experiments and the method of inoculation were essentially those of Landy and Dicken (1942) slightly modified (Trager, 1947). The experimental cultures were incubated at 37 C for 4 days. Growth was measured by titration with 0.1 N sodium hydroxide.
RESULTS AND DISCUSSION

In a medium containing suboptimal amounts of biotin but an excess of all the other essential growth factors, lyssolecithin inhibits the growth of Lactobacillus casei. Increasing the concentration of biotin counteracts the inhibitory effect. The results of a typical experiment are shown in figure 1. Lyssolecithin and biotin, though structurally unrelated, behave toward each other like competitive metabolites, at least so far as the growth of L. casei is concerned. A number of instances of competition of this type between structurally dissimilar compounds have been previously reported (Woolley, 1947). It is possible to calculate a molar inhibition index (ratio of moles of inhibitor [lysolecithin] to moles of metab-

![Figure 1](http://jb.asm.org/)  

**Figure 1.** The inhibitory effect of lyssolecithin on the growth of Lactobacillus casei in the presence of different concentrations of biotin.

olite [biotin] which will just prevent growth) for the antagonism between lyssolecithin and biotin. This index has varied in different experiments between 50,000 and 100,000. For example, in the experiment shown in figure 1, 20 μg of lyssolecithin just prevented growth in the presence of 0.1 mμg of biotin, while 80 μg of lyssolecithin were effective against 0.4 mμg of biotin. In both cases the molar inhibition ratio is 100,000. In another experiment almost complete inhibition of growth was obtained by 16 μg of lyssolecithin in the presence of 0.1 mμg of biotin and by 30 μg in the presence of 0.2 mμg of biotin, the molar inhibition index being 75,000. However, if the biotin of the medium was replaced by various appropriate concentrations of either oleic acid (USP) or FSF from hydrolyzed horse plasma, lyssolecithin had a small growth-stimulating effect. This result is well illustrated by the experiment shown in figure 2. Note that whereas 50 or 60 μg of lyssolecithin almost completely prevented growth in the presence
of 0.3 \text{mg} of biotin, the same concentration or even ten times as high a concentration of lysolecithin stimulated growth in the presence of concentrations of FSF or oleic acid of roughly similar biotin activity. This experiment incidentally illustrates the fact that although FSF, like certain esters of oleic acid (Williams and Fieger, 1947), gives a response curve which almost parallels that with biotin, oleic acid gives an appreciably lower curve, probably because of its greater toxic-

![Figure 8](http://jb.asm.org/)  

*Figure 8.* The growth response of *Lactobacillus casei* to biotin, oleic acid, and FSF, and the effect of lysolecithin in the presence of each of these.

ity. The stimulatory effect of lysolecithin in the presence of FSF or oleic acid may be the result of a detoxification similar to that described for lecithin and some other materials (Kodicek and Worden, 1945).

Although oleic acid and certain other fatty compounds can fully replace biotin in the nutrition of some of the lactobacilli, they cannot replace riboflavin or pantothenic acid. In the absence of either of these growth factors, or in the presence of optimal concentrations of both, oleic acid and related materials have no growth-stimulating effect. But in the presence of suboptimal concentrations of either, they have a marked effect (Bauernfeind, Sotier, and Bowff, 1942; Strong...
and Carpenter, 1942). It was therefore not surprising to find that lysolecithin behaved toward calcium pantothenate, as well as toward biotin, as a competitive inhibitor. This could be clearly shown by using a medium with an excess of biotin and graded concentrations of calcium pantothenate and lysolecithin. In this case the molar inhibition index was only 500 to 1,000. If the biotin of the medium was replaced by either FSF or oleic acid at optimal concentration (an excess could not be used since the higher concentrations of these materials are toxic), the response curve to graded amounts of pantothenate was much steeper. Moreover, under these conditions, lysolecithin had, not an inhibitory effect, but a slight stimulatory one. These results are shown by the experiment of figure 3.

Any attempt at a complete explanation of the phenomena described in the present paper must await a better understanding of the relationship between biotin and the fatty materials which can replace it in the nutrition of certain organisms. It seems most reasonable to suppose that biotin functions in the synthesis of these fatty materials (Williams, Broquist, and Snell, 1947). Lysolecithin might then behave as a true competitive analogue and in some manner

Figure 3. The growth response of Lactobacillus casei to calcium pantothenate in media containing biotin or, in place of it, either oleic acid or FSF, and the effect of lysolecithin in each of these media.
block the synthesis. It could have no such effect if the products of the synthesis were supplied in the medium. In the light of the results with pantothenate and lysolecithin it would be necessary to extend the argument to assume that pantothenate also functions in the synthesis of the fatty materials. In any case it is interesting to note that lysolecithin has been observed to have a deleterious effect when fed to rats (Iwata, 1934). This effect was prevented by the inclusion of 3 per cent yeast in the diet.

SUMMARY

The growth of Lactobacillus casei could be completely prevented by the addition of low concentrations of lysolecithin to a medium which was complete except that it contained biotin in suboptimal amounts. With biotin concentrations up to 1 µg per tube, the amount of lysolecithin required to give complete inhibition varied directly with the concentration of biotin. If oleic acid or a fat-soluble biotin-active material from plasma was used in place of biotin, lysolecithin at concentrations up to ten times those found inhibitory with biotin had only a small growth-stimulating effect. In a medium containing excess biotin but suboptimal concentrations of pantothenic acid, growth of the organism was inhibited by appropriate concentrations of lysolecithin. Here again, if the biotin was replaced by an adequate concentration of oleic acid or the fat-soluble material from plasma, lysolecithin had a slight stimulatory effect rather than an inhibitory one.

REFERENCES