CLXXX. THE ACCESSORY FACTOR NECESSARY FOR THE GROWTH OF *NEMATOSPORA* *GOSSYPHII*.

II. THE RELATION OF THE ACCESSORY FACTOR TO "BIOS."

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IN view of the results recorded in the preceding communication it obviously became desirable to determine whether preparations known to possess "bios" activity towards yeast could promote the growth of *N. gossypii*, and, on the other hand, whether concentrates known to show activity in respect of this fungus could serve as a source of "bios."

A study of the literature regarding "bios" shows that there is still far from general agreement as to the nature of this substance, and even as to its existence. With regard to the latter point, the work of Copping [1929] seems to summarise the present position. As the result of a survey of the nutritional needs of a very large number of yeasts, as well as other organisms, she concluded that the yeasts differ widely in their requirements; some varieties she found were able to reproduce indefinitely on synthetic media, while others, including *S. cerevisiae*, were unable to grow normally unless supplied with a factor of the "bios" type.

The chemical nature of "bios" has been and still is a matter of considerable dispute; extremely active concentrates have been prepared, without throwing any light on the identity of the substance. Claims to have separated "bios" as a chemical entity have from time to time been made, but these results have not found general acceptance. The crystalline substance isolated by Eddy *et al.* [1924], and claimed to be a pure "bios," was found to be less active than a concentrate prepared by Narayanan, and admitted to be a mixture. The concentrate of Narayanan seems to be the most active preparation yet recorded, despite its admitted impurity.

The apparent similarity of the results recorded in the preceding paper and those reported by Eastcott [1928], made it desirable to examine further the relation between the active substances necessary for the growth of *N. gossypii* and of yeast. The results indicated definitely that the two factors are not identical.

EXPERIMENTAL.

(1) "*Bios*" activity of concentrates containing the accessory factor for *N. gossypii*.

The technique adopted in the experiments on the growth of yeast was that described by Narayanan [1930]. The medium used was that of Reader [1927], which was shown by Copping [1929] to be satisfactory. The strain of yeast employed was subcultured on wort-agar slopes, and cultures of 24–30 hours' growth were used for the tests. The inoculations of the sterile Reader's medium were so made that the original yeast-count of the culture was 2500 cells per cc. Adopting the system of Narayanan, this is shown in the table as a "count" of 0.01. The cultures were incubated at 23°, and a count was made after 30 hours; a multiplication of over a hundredfold in the number of cells within this period was taken to indicate "bios" activity.

The substances tested and the results obtained are summarised in Table I. The baryta-alcohol filtrate was prepared from lentil extract after removal of the substances precipitable by phosphotungstic acid, as described in the preceding communication.

Table I.

Substance tested					Amount per 25 cc. Reader's medium (g.)	Initial count	Count after 30 hrs.
Control (medium alone)...	...	...	...	...	—	0.01	0.01
Lentil concentrate	...	...	...	...	0.001	0.01	1
" "	...	...	...	...	0.005	0.01	2
Baryta-alcohol filtrate	...	...	...	...	0.001	0.01	0.01
" "	...	...	...	...	0.005	0.01	0.02
Inositol	...	...	...	...	0.005	0.01	0.05
Baryta-alcohol filtrate <i>plus</i> inositol	...	...	...	...	0.003 0.005	0.01	0.13
Marmite	...	...	...	...	0.005	0.01	0.01 (?)
" "	...	...	...	...	0.010	0.01	1.3
" "	...	...	...	...	0.015	0.01	3

It appeared from these results that the lentil concentrates possessed considerable "bios" activity, and were in fact rather richer in "bios" than yeast preparations (marmite). The two main fractions prepared from the lentil concentrate, inositol and the baryta-alcohol filtrate, were both devoid of activity, and the activity was not restored on re-combining them. This seemed curious, and indicated that the "bios" substance of the lentils had been lost at some stage of the fractionation. The loss may have occurred at the phosphotungstic acid stage, since Narayanan states that "bios" is precipitated by this reagent (although the majority of other workers do not find this to be the case). Another possibility was that the baryta-alcohol filtrate fraction contained traces of inhibitory substances; there was at any rate no substance present toxic to *N. gossypii*, and the question was not pursued further. It may be noted that inositol showed no activity in promoting yeast growth, this observation agreeing with those of Narayanan. The growth produced in the

presence of inositol amounted approximately to a fivefold increase, *i.e.* to only one-twentieth of that considered as evidence of true "bios" activity. The contention of Reader and of Narayanan, that this effect is parallel with that of mannitol on the growth of *S. corallinus*, seems plausible.

(2) *Growth of N. gossypii in the presence of substances of known "bios" activity.*

It has been shown in the preceding paper that marmite, a product of recognised "bios" activity, is a comparatively poor source of the accessory factor for *N. gossypii*; and further, that this lack of activity is due to a deficiency of inositol. Through the kindness of Prof. J. C. Drummond, we were able to test the effect of an extremely active sample of "bios" on the growth of the fungus. This material had been prepared from yeast and was obtained as a solution containing 0.6 % of organic matter, active in promoting a vigorous growth of yeast in doses of 0.2 cc. per 2 cc. salt-glucose medium (*i.e.* 0.0012 g. solid organic matter per cc.).

When tested in the usual way for the *Nematospora* factor, the preparation was found to be entirely inactive in doses up to 0.05 g. organic matter per 50 cc. medium. That no toxic substance was present was shown by the usual tests. Since the preparation of the "bios" concentrate had involved precipitation with basic lead acetate, any inositol originally present would have been removed at this stage; to this extent, therefore, the preparation corresponded to the baryta-alcohol filtrate fraction of the lentil concentrates, and its lack of activity for *N. gossypii* was to be expected. There still remained the possibility that this "bios" preparation contained the second *Nematospora* factor, *i.e.* would give growth of the fungus when in the presence of inositol. Unfortunately, lack of material made it impossible to test this point. Recent work on other lines has given definite indications that this is not the case, and that although concentrates of the accessory factors for *N. gossypii* may show "bios" activity, the factor responsible is quite distinct from those promoting growth of the fungus.

SUMMARY.

The growth-promoting accessory necessary for *N. gossypii* is found in association with "bios" among natural products, but the two substances are not identical. Inositol is devoid of true "bios" activity, but is essential for the growth of *Nematospora*.

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