

EXTRACT OF BROOD LAC AS A
SUPPLEMENTAL NUTRIENT FOR THE
PRODUCTION OF ANTIBIOTICS

THESE studies have been inspired by the finding that corn steep liquor¹ speeds up the formation and enhances the yield of penicillin and that organic complexes are essential for stepping up the formation of streptomycin. Waksman² has found that an "activity factor" is essential for the formation of the antibiotic; this factor can be synthesised by *Streptomyces griseus* but its addition favours the rapid production of the antibiotic.

Extracts of brood lac, which could easily be made available in abundant quantities, have been found to constitute a rich source not only the complex organic compounds but also a good source of the members of the B-complex. The overall growth-promoting potency of these extracts has been microbiologically assayed by Mande, Mistry and Sreenivasaya.³ It was, therefore, of interest to determine the overall efficiency of this extract as a fortifying supplement for increasing the rate of formation and the yield of penicillin and streptomycin by their respective micro-organisms.

Experiments on the formation of penicillin were carried with a medium which was composed as follows:—Sucrose 4 gms.; NaNO₃ 0.3 gm.; KH₂PO₄ 0.1 gm.; MgSO₄ 7H₂O 0.05 gm.; FeSO₄ 0.001 gm.; KCl 0.05 gm.; Distilled water 50 c.c.; pH 6.8.

Aliquots of this medium (2.5 c.c.) were fortified with graded dosages of the brood lac extract (dosages based on the nitrogen content of the extract) and the total volume made up in each case to 5 ml. with distilled water. The medium was sterilised twice at 10 lbs. for 30 minutes and inoculated with a 1 ml. spore suspension in normal saline of *P. notatum* N.C.T.C. 1540 and incubated at 28° C. for seven days. Other experimental details have been described at length in our previous communications.⁴ After the period of incubation, the cylinder-plate assay was carried out against a 24-hour old broth culture of *Staph. aureus* N.C.T.C. 2150 and the results are shown in the table below.

The results given in Table I show (1) that the growth of the fungus is proportional to the added amount of the supplemented lac extract; (2) at higher levels of the supplement an earlier sporulation of the fungus is included; (3) At 0.2 and 0.4 MgN levels there is a four-fold increase in the yield of penicillin as compared with the yield obtained with the un-supplemented basal medium; (4) higher concentrations of the supplement tend to lower the yield of penicillin apparently due to a toxic factor which becomes effective at higher levels of the supplement. This toxic effect has been observed by Mande, Mistry and Sreenivasaya in the course of their studies.

The brood lac extract was supplemented to the basal medium used for the production of

TABLE I

Basal medium no supplement	Basal medium + 0.2mgN/5 ml.	Basal medium + 0.4mgN/5 ml.	Basal medium + 0.6mgN/5 ml.	Basal medium + 0.8mgN/5 ml.	Basal medium + 1.0mgN/5 ml.
Total solids per 5 ml. 0.0 ..	4 mg	8 mg	12 mg	16 mg	20 mg
Rate of growth ++ ..	+++	++++	++++	+++++	+++++
Sporulation 4th day ..	3rd day	3rd day	2nd day	2nd day	2nd day
Initial pH 6.8 ..	6.8	6.8	6.8	6.8	6.8
pH after 7 days of fermenta- tion 6.2-6.4 ..	6.4-6.6	6.2-6.4	6.0-6.2	6.0-6.2	6.0-6.2
Activity against <i>Staph. aureus</i> expressed in Oxford units ml. 3.0 ..	11.0	11.0	9.5	9.2	6.2

TABLE II

	Basal medium--no supplement	Basal Medium + 0.2mg N	Basal Medium + 0.4 mg N	Basal Medium + 0.6 mg N	Basal Medium + 0.8 mg N
Total solids per ml. .		4 mg /5 ml.	8 mg /5 ml.	12 mg /5 ml.	16 mg /5 ml.
Rate of growth ..	+++	+++	+++	+++	+++
Sporulation ..	5th day	4th day	4th day	3rd day	3rd day
Initial pH ..	7.0	7.0	7.0	7.0	7.0
pH after 7 days of fermentation ..	10.2	9.8	9.8	9.8	10.4
Activity against <i>B. subtilis</i> ex- pressed in sq. mm. ..	416	416	491	528	616

the Streptomycin from *Actinomyces griseus*. The composition of the basal medium is as follows:—Glucose 1.0 gm.; NaCl 0.5 gm.; Casein hydrolysate 7.32 ml. (80 mg. of total N); K_2HPO_4 0.1 gm.; Distilled water 50 ml.; pH 7.0.

This basal medium was treated with graded doses of the extract (on nitrogen content of the extract). The total volume of the medium was made up to 5 ml. with distilled water.

A uniform suspension of the spores of *Actinomyces griseus* in normal saline (1 ml.) was inoculated into the medium previously sterilised twice at 10 lbs.—30 minutes. After seven days of incubation at 28° C., a "cylinder-plate" assay was carried against *B. subtilis* to determine the activity. The results are tabulated in Table II.

It will be seen from Table II that there is little difference in rate of growth. But the day of sporulation shifts a day earlier in cases which have received higher levels of the supplement. The rise in pH is marked throughout. The increase in antibiotic activity over the unsupplemented control is 50 per cent.

Attempts at a further purification of the brood lac extract, with a view to concentrate the active factor, are under progress.

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