

and 10 ml. 5 N hydrochloric acid added to end the enzymatic reaction. Phytate phosphorus was determined in the centrifugate. When yeast was added, it was first shaken for 1 hr. with 50 ml. 0.2 M. acetate buffer and the pH readjusted before adding enzyme and substrate. The yeast extract was made by shaking 20 gm. yeast with 150 ml. 0.2 N hydrochloric acid for 2 hr. The centrifugate was neutralized with sodium hydroxide and diluted so that 10 ml. is approximately equivalent to 1 gm. yeast. 10 ml. of the extract contained 5.5 mgm. inorganic phosphorus.

INFLUENCE OF DRIED YEAST ON PHYTASE ACTIVITY

| No. | Addition                     | Phytate P split (mgm.) | Inhibition (per cent) |
|-----|------------------------------|------------------------|-----------------------|
| 1   | None                         | 41                     | 0                     |
| 2   | 10 gm. fresh bakers' yeast   | 44                     | 10                    |
| 3   | 30 mgm. P as pot. phosphate  | 37                     | 10                    |
| 4   | 1 gm. dried yeast            | 31                     | 24                    |
| 5   | 3 " " "                      | 22                     | 46                    |
| 6   | 5 " " "                      | 10                     | 76                    |
| 7   | 10 ml. yeast extract         | 33                     | 20                    |
| 8   | 30 " " "                     | 24                     | 41                    |
| 9   | 50 " " "                     | 12                     | 71                    |
| 10  | No enzyme                    | 0                      | —                     |
| 11  | No enzyme; 3 gm. dried yeast | 0                      | —                     |

If the phytase activity of the fodder and of the bacteria in the intestinal canal is as highly inhibited by yeast as the above experiments seem to indicate, the results of the feeding experiments obtained in Reading may become more intelligible.

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<sup>1</sup> Braude, R., Kon, S. K., and White, E. G., *J. Com. Path.*, **53**, 161 (1943); **54**, 88 (1944).

### Isolation of Phloridzin from Apple Seeds

IN a recent communication<sup>1</sup>, the preparation of apple seed extracts capable of stimulating the development of unfertilized tomato ovaries was described. Treatment of the seeds with water at 100° C. for 15 minutes gave, on cooling, a crystalline substance which preliminary tests indicated was possibly glycosidic in nature. While not possessing the fructigenic activity shown by the aqueous filtrate, this compound disappeared at the stage when the extracts were no longer active, and consequently further investigation was indicated.

Hydrolysis with N/10 sulphuric acid gave glucose, characterized by the formation of the osazone and the β-penta-acetate, and a phenolic substance, m.p. 259–260° C., which gave elementary analytical results corresponding to the formula C<sub>15</sub>H<sub>14</sub>O<sub>6</sub>. A comparison of the phenol, its tetra-acetate and trimethyl ether<sup>2</sup> with authentic specimens kindly supplied by Prof. A. Robertson confirmed its identity as phloretin<sup>3</sup> and the glucoside as phloridzin<sup>4</sup>. This latter is known to occur in the bark (especially the root-bark) and leaves of apple trees, but has not hitherto been reported in the seeds. In this connexion the yields are extremely interesting, being as much as 8 per cent (of seed fresh weight) four weeks after petal-fall, and decreasing to less than 1 per cent after eight weeks; ten weeks after petal-fall, phloridzin could no longer be isolated.

Further work involving an examination of the relationship (if any) between phloridzin and the

hormone, and possible isolation of the latter, is proposed.

My thanks are due to Dr. L. C. Luckwill for supplying most of the glucoside and for carrying out the physiological tests.

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<sup>1</sup> Luckwill, L. C., *Nature*, **158**, 663 (1946).

<sup>2</sup> Johnson, F. R., and Robertson, A., *J. Chem. Soc.*, 21 (1930).

<sup>3</sup> Fischer, E., and Nouri, O., *Ber.*, **50**, 611 (1917).

<sup>4</sup> Muller, A., and Robertson, A., *J. Chem. Soc.*, 1170 (1933).

### Medium Suitable for the Cultivation of Meredith's Actinomycete

IN our study of the antibiotic properties of certain Actinomycetes<sup>1</sup>, it has been one of our endeavours to devise a cheap medium for the growth of these organisms which would contain a minimum of organic and inorganic substances likely to interfere with the extraction of the antibiotic. Of the many varieties of liquid and solid media tested, we have finally adopted the following:

|  |               |
|--|---------------|
| Autolysed yeast extract                      | 0.25 per cent |
| Glucose                                      | 0.5 per cent  |
| Distilled water                              | to 100 ml.    |
| The reaction adjusted to a pH of 6.0 to 7.0. |               |

Increasing the yeast extract content of the media beyond 0.25 per cent leads to a reduction in antibiotic production.

As for the yeast extract to be chosen, it would appear that any reasonably carefully prepared commercial brand is suitable. The three actually tried, 'Marmite', 'Gye' and 'Yeastrel', all gave the same degree of growth and antibiotic production.

Our medium has been tried also for the growth of *Actinomyces griseus*. It was found eminently suitable for this organism, yielding growth and production of antibiotic equal to that obtained by the media usually employed in the cultivation of this organism.

It may be of interest to record that the growth-liquor of our antibiotic-producing Actinomycete is highly active against a culture of *Cerospora nicotianae* Ell. and Ev., obtained from the Baarn Type Culture Collection, and claimed to be responsible for the spotting of the tobacco leaf.

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<sup>1</sup> Thaysen and Butlin, *Nature*, **156**, 781 (1945).

### Connective Tissue Cementing Substance

IN 1876, W. Flemming<sup>1</sup> described a cementing substance associated with the fibrils of loose connective tissue. This finding is of considerable importance in relation to modern conceptions of the chemical structure of collagen and other fibrous proteins.

There is reason to suppose that the long-chain molecules of which collagen fibres are composed are mainly held together by lateral bondings of an electrovalent character. It has been pointed out by the late Dr. Jordan Lloyd<sup>2</sup> that, since such bondings are readily