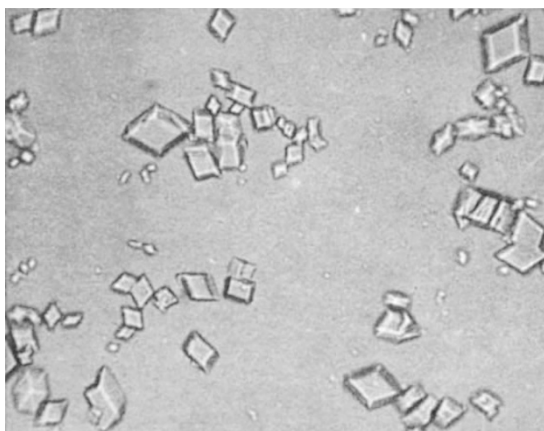


with a saturated solution of ammonium sulphate. The fractionation is repeated twice under approximately the same conditions. The last precipitate is now dissolved in the minimum quantity of water, and the slightly brown solution, adjusted to pH 7.2, is left in the cold. Crystals appear after two or three days, and, after a week, at least 50 per cent of the activity of the suspension is found in the crystalline material. This has been recrystallized (see photograph).



Crystals of  $\alpha$ -amylase from *A. oryzae*.  $\times 150$

A product the activity<sup>4</sup> of which was 2,400 mgm. maltose/mgm. nitrogen has been obtained. The total yield was approximately 15 per cent with a fourfold activity/nitrogen enrichment, based on the original extract. The purified enzyme behaves as a strictly homogeneous substance in the electrophoresis apparatus at all pH's investigated, namely, 4.7-8.6. Its isoelectric point lies at  $\sim$  pH 4.

The enzyme is stable in the cold between pH 4.7 and 7.8 and can be dialysed for long periods against aqueous or salt solutions without any loss of activity. Its activity is not dependent upon the presence of chloride ions as for the animal and bacterial amylases, nor does it need calcium ions as does malt  $\alpha$ -amylase.

The accompanying table compares some of the properties of taka-amylase with those of other crystalline amylases.

| Properties                     | Crystalline $\alpha$ -amylases of |                       |                    |              |                           |
|--------------------------------|-----------------------------------|-----------------------|--------------------|--------------|---------------------------|
|                                | Malt                              | <i>Asperg. oryzae</i> | <i>B. subtilis</i> | Pig pancreas | Human pancreas and saliva |
| Mgm. Maltose/mgm. N            | 2,350                             | 2,400                 | 3,600              | 4,000        | 6,200                     |
| Optimum pH                     | 4.7-5.4                           | 5-7                   | 5.3-6.8            | 6.9          | 6.9                       |
| Solubility (per cent)          | >15                               | >10                   | $\sim$ 6           | 0.3          | 0.3                       |
| Activation energy (cal./mol.)  | 7,050                             | 10,500                | 13,500*            | 13,500       | 13,500                    |
| Activation by Ca <sup>++</sup> | +                                 | -                     | -                  | -            | -                         |
| Activation by Cl <sup>-</sup>  | -                                 | -                     | +                  | +            | +                         |

\* At temperatures less than 20° C.

In conclusion, although the properties of crystalline taka-amylase are intermediate between those of malt and bacterial amylase, this enzyme should be considered separately due to the fact that its activity does not depend on the presence of any ion.

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ED. H. FISCHER  
R. DE MONTMOLLIN

Department of Organic Chemistry,  
University of Geneva.  
May 29.

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### 'Streptozyne', a Lytic Enzyme from Lactic Streptococci

It has already been shown that antibiotic-producing streptococci other than the lactic group (Lancefield's Group N) are rare. The difficulties of survey were increased by toxic metabolic by-products, lactic acid in particular giving false inhibitory reactions on agar<sup>1</sup>. During the later stages of this work (unpublished) neutralized culture fluids were used to overcome the inhibitory effects of lactic acid. A cylinder-plate method was used for assays with *Staphylococcus aureus* (491) as the test organism. Several strains of streptococci were isolated from silage, the neutralized culture fluids of which gave 20-mm. zones of inhibition, while the un-neutralized acid culture fluids were inactive. These cultures were proved serologically to belong to Group N (*Str. lactis*), yet the inhibitory substance produced is inactive in acid media and is heat-labile, characters distinguishing it from the main antibiotic, nisin, produced by *Str. lactis*<sup>2</sup>.

After centrifuging, both cells and supernatant were inactive and the inhibitory substance appeared to be mainly associated with the intact living cells. When growing cells were killed with nisin (200 units/ml.), they promptly became autolysed. Autolysis depended on pH (ceasing completely at pH 6 or below) and on the age of the culture. The activity of cultures more than 10 hr. old declined gradually, even when the pH was maintained by periodic neutralization at 6.5.

The autolytic nature of this enzyme clearly distinguishes it from lysozyme; it also appears to be more heat-labile. The lytic effect is not due to bacteriophage, since a cell-free culture filtrate was incapable of lysing living cells, but there was some activity against a heat-killed suspension. The name 'streptozyne' is proposed for this enzyme.

A. HIRSCH  
DOROTHY M. WHEATER

National Institute for Research  
in Dairying,  
Shinfield,  
Nr. Reading.  
May 25.

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