15° C., respectively. Above 27° C., there is very little growth and no crystal formation. Still cultures in flasks at 15° C. showed visual evidence of crystal formation in about 26 days; the 370 mgm. yield we report was obtained in 93 days. *D*-glucose and *d*-glutamic acid appear to be the best carbon and nitrogen sources for crystal formation. No sporulation by *NRRL* 2826 has yet been observed by us. Two other strains of *Pestalotia ramulosa* which do sporulate did not produce crystals.

Chemical studies are in progress.

CHESTER R. BENJAMIN*
FRANK H. STODOLA

Northern Utilization Research and
Development Division,
Agricultural Research Service,
U.S. Department of Agriculture,
Peoria,
Illinois.

* Present address: The National Fungus Collections, Plant Industry Station, Beltsville, Maryland.

1 Brewer, D., *Canad. J. Bot.*, **36**, 941 (1958).

Effect of Tetracycline Antibiotics on the Proteolysis of Fish Muscle

DURING recent experiments on the storage of herring over a 21-day period in normal ice and in ices containing chlortetracycline and oxytetracycline, an attempt was made to correlate changes in the bacterial flora with some of the changes which occur in the muscle.

Observations on the lower volatile fatty acids have already been reported[1], when it was found that the antibiotics did not affect their rate of production. This work has now been extended to the free amino-acids, estimated by the method of Moore, Spackman and Stein[2].

The results obtained showed that the changes occurring in the amino-acids followed a regular pattern. Proline, methionine, threonine, alanine, glutamic acid, lysine, valine, *iso*leucine and leucine showed a gradual increase up to around the fourteenth day, followed by a decrease. Phenylalanine, tyrosine and serine showed little change, while histidine, arginine and possibly glycine decreased steadily throughout the period.

In the cases where the amino-acid content changed over the period, there was always a larger residue in the treated, compared with the untreated, fish, this difference being greater at 14 days than at 7 days. Bacterial counts were lower on the skins of treated compared to untreated fish.

There are three possible explanations for these observations: (1) the activity of the selected bacterial flora may be greater than that of the flora on the untreated fish; (2) the selected flora are primarily proteolytic (*Pseudomonas* spp. and *Achromobacter* spp.), and even if their overall activity is not increased relative to the untreated fish, accumulation of amino-acids may occur due to the reduction of other groups which utilize them; (3) autolysis in the muscle may continue independently of the bacterial flora, amino-acids thus formed being utilized by bacteria. Reduction in the number of bacteria due to the presence of the antibiotics then leads to an accumulation of the acids in the treated fish.

There is reason to believe that the flora selected during antibiotic treatment is less active biochemically, particularly in the presence of the antibiotics[3–5]. Many enzyme systems are also depressed by antibiotics[6]. Thus it is unlikely that increased proteolysis occurs during antibiotic treatment, and it is therefore possible that the increased accumulation of the amino-acids in the treated fish was due to reason (3), the acids being produced autolytically, and their rate of accumulation governed by their assimilation rather than their production by bacteria. Furthermore, the observation that the increase in the amino-acids occurred during the first fourteen days of storage—before the stabilization of the dominant bacterial flora—again indicates that during the critical early stages of spoilage, proteolysis proceeds independently of the bacterial enzymes.

These results will be reported in greater detail elsewhere.

NIHAL N. DE SILVA
R. B. HUGHES*

Torry Research Station,
Department of Scientific and Industrial Research,
Aberdeen.

* Of the British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead.

1 Hughes, R. B., *J. Sci. Food Agric.*, **11**, 47 (1960).
2 Moore, S., Spackman, D. H., and Stein, W. H., *Anal. Chem.*, **30**, 1185 (1958).
3 Francois, A. C., and Michel, M., *C.R. Acad. Sci., Paris*, **240**, 124 (1955).
4 Michel, M., and Francois, A. C., *C.R. Acad. Sci., Paris*, **240**, 808 (1955).
5 Lerke, P. A., doctoral thesis, University of California (June 1958).
6 Eagle, H., and Saz, A. K., "Ann. Rev. Microbiol.", **9**, 173 (1958).

Determination of Acid Phosphatase Activity in Cells of Prostatic Tumours

A SIMPLE technique by which cell material can be easily secured from prostatic tumours with the aid of transrectal aspiration biopsy has recently been described[1]. The material thus obtained was shown to consist largely of plugs of epithelial cells with occasional admixture of blood. In the present work these tissue fragments were isolated for determination of their acid phosphatase activity.

The aspirate from the prostatic gland was suspended in 10 c.c. citrate buffer of *p*H 4·9 (ref. 2), containing 0·015 per cent 'Triton *X*-100' (ref. 3) and rendered hypertonic by addition of 2 per cent sodium chloride in order to minimize diffusion of the enzyme from the cells[4]. The large tissue fragments consisting of epithelial cells were sedimented by horizontal centrifugation for about 20 sec. at 500 r.p.m. In this series of experiments the sediment was washed twice more by re-suspension and centrifugation in the above-described medium. The isolated and washed cell material was finally suspended in 2 c.c. citrate buffer of *p*H 4·9 and stored in a refrigerator at 4° C. for 24 hr. The acid phosphatase activity of the cell-containing buffer solution was then estimated roughly by dropping 0·01 c.c. of the supernatant on to test paper specific for acid phosphatase, as previously described[5]. Taking as a guide the intensity of the coloured spot on the test paper, it was possible to select an appropriate incubation time (15–180 min.) for obtaining extinction for the final solution within the limits of the spectrophotometer used. In order to evaluate the enzymic activity quantitatively, the centrifuge tube containing the cell material in 2 c.c. citrate buffer of *p*H 4·9 was placed in a water-bath at 37° C. for 5 min. Substrate (0·2 c.c. of 1 per cent solution of disodium phenyl phosphate) was added to the tube. After a given time based on