Effect of Ions upon the Production of Vitamins in the Yeast Cells

In the course of an investigation of the nutritional requirements of insects in mineral elements, we were confronted with a peculiar instance of an effect exerted by salt ions upon the production of one of the vitamins. The wax moth *Galleria mellonella* can be reared on a medium composed of one part of yeast and two parts of wax. However, if in place of commercial bakers' yeast, yeast grown in the laboratory be used, the result will depend upon the composition of the culture medium.

It has been previously shown that yeast can be grown in a pure synthetic medium completely deprived of sodium and calcium salts, for example, in a medium of the following composition: 2 per cent purified dextrose, 0.1 per cent potassium dihydrogen phosphate, 0.3 per cent ammonium sulphate, 0.1 per cent magnesium sulphate (Rubinstein and Burlakowa¹). Although many strains of yeast (especially common bakers' or brewer's yeast) exhibit very poor growth in such media and can be markedly stimulated by the addition of sodium and calcium, some species of yeast, for example, *Torula utilis* or *Mycoderma casei*, develop fairly well in this sodium- and calcium-free culture medium.

However, larvae of the wax moth failed to develop if fed on such yeast mixed with wax in the usual proportion. The use of yeast grown in the presence of 0.1 per cent sodium chloride and 0.1 per cent calcium chloride, or even only of sodium chloride, was sufficient to render the medium adequate for the development of the insect, although there was no visible difference between the two yeast cultures. It should be emphasized that sodium ions must be present during the actual growth of the yeast: addition of sodium and calcium salts to the yeast - wax mixture proved entirely inefficient and did not enhance its nutritive value for *Galleria* in any way.

Development of the moth could be fully restored and proceeded at its usual rate if an aqueous or alcoholic extract of normal yeast was added to the deficient nutrient medium. The yeast extract could be autoclaved at 130° in a strongly alkaline solution without impairment of its growth-promoting activity. On the other hand, addition of normal yeast repeatedly washed with an excess of hot water was no longer efficient in stimulating the development of *Galleria* on the deficient food mixture. It can be inferred that the elimination of sodium ions prevents the production in the growing yeast of some water-soluble vitamin, which is supposed to be a thermostable component of the vitamin B group. A special test showed that this was not nicotinic acid, the importance of which for the developing wax moth was reported previously (Rubinstein and Shekun²).

It is thus possible at will to stimulate or prevent the production in the yeast cell of a growth-promoting factor (a thermostable component of the B vitamin group?) merely by changing the ionic composition of the nutrient medium. The exact nature of this factor has yet to be identified.

D. RUBINSTEIN.
L. SHEKUN.

Department of Biophysical Chemistry,
All-Union Institute of Experimental Medicine,
Moscow. Nov. 19.

¹ Rubinstein, D., and Burlakowa, H., *Biochem. Z.*, 271, 324 (1934).

² Rubinstein, D., and Shekun, L., *Nature*, 143, 1064 (1939).

Preservation of Ascorbic Acid in Vegetables by Hydrogen Sulphide during Air-Drying

DURING the drying of vegetables in a hot-air current, a loss in the ascorbic acid contents will generally be found. This is largely due to the action of oxidizing enzymes, which must therefore be destroyed if the losses are to be diminished. This can be done by treating the vegetables in hot water or steam (so-called scalding or blanching), but by this process the non-enzymic oxidation of the ascorbic acid is accelerated on account of the rise in temperature, and so the treatment must be followed immediately by drying.

Another way of destroying the enzymes is to introduce chemicals into the vegetables which are able to react with the prosthetic or protein part of the enzyme molecule. This has been tried by Morgan¹, who dried figs after treatment with sulphur dioxide, but the influence on the ascorbic acid retention was poor. Another way was described by Allen, Barker and Mapson², who recommended blanching in hot water containing sulphite. Further details were not given.

It has now been found that a pre-treatment of fresh vegetables with gaseous hydrogen sulphide has a marked effect on the retention of ascorbic acid contents during hot-air drying. In the accompanying table is shown the percentage retention of ascorbic acid in some vegetables, dried without any treatment and after treatment with concentrated gaseous hydrogen sulphide for an hour and blowing out excess of the gas with atmospheric air until the smell had disappeared.

Clearly, in all cases of materials treated with hydrogen sulphide the losses of ascorbic acid are very small compared with the losses in untreated materials.

Furthermore, it has been found that kale treated with hydrogen sulphide can be stored for 24 hours at 20° C. and then dried in a current

Vegetable	Temperature of air current	Ascorbic acid retained (per cent)	
		Untreated material	H ₂ S-treated material
Kale	90° C.	66.7 per cent	98.5 per cent
Cabbage, white	60	64.4	95.4
" summer	60	44.2	92.5
Lucerne	90	26.0	77.3
Parsley	90	77.4	96.1
Chervil	90	20.3	101.4

of hot air with very little loss of ascorbic acid. Thus the gas can be used to protect the ascorbic acid in vegetables during storage before drying.

It is not necessary to use concentrated hydrogen sulphide, for only 1 per cent of it in atmospheric air had the same effect. In the case of the treatment of kale, full protection with this mixture was obtained in 2 hours, but even after 15 minutes it was possible to dry the vegetable with a loss of 22 per cent against 72.2 per cent in untreated kale. The process was in this case carried out at 60° C., and at this temperature the loss was somewhat greater than that at 90° C.

The flavour, smell and colour of the vegetables are not changed by the treatment, and the rise in sulphur contents is not significant.

The ascorbic acid contents were estimated by reduction titration with 2,6-dichlorophenol-indophenol; and a biological control, carried out in the State Vitamin Laboratory in Copenhagen, showed that the reduction value in the materials was due to ascorbic acid.

In other experiments a good effect was found by blanching the vegetables before drying, but this effect was inferior to that of hydrogen sulphide treatment, and certain materials, such as white cabbage, were easily discoloured by the process.

ETTRUP PETERSEN.

Chemical Laboratory,
Atlas, Ltd.,
Copenhagen.

¹ Morgan, *Amer. J. Pub. Health*, 25, 328 (1935).

² Allen, Barker and Mapson, *Nature*, 150, 223 (1942).

Waterproofing Mechanism of an Insect Egg

RECENT work^{1,2} has shown that the cuticles of many insects are waterproofed by wax which occurs as a layer, approximately 0.25 μ thick, on the outermost surface of the cuticle.

A study of the eggshell of *Rhodnius prolixus* (Hemiptera) has revealed that the chorion, secreted by the cells of the follicle, consists of seven distinct layers; these appear to be modifications of a proteinaceous material. A cement is added to the external surface of the shell at oviposition. However, all these layers are freely permeable to water; they cannot account for the considerable resistance of the egg to desiccation.

It has been found that the waterproofing mechanism of the egg consists of a wax layer, approximately 0.25 μ thick, which covers the inner surface of the shell. This wax is apparently of similar composition to the cuticular waxes of insects³ and gives the egg a characteristic 'transition point' in its temperature/water-loss curve at 42.5° C. It is a product of the maturing oocyte and may be secreted on to the shell before or after the follicle cells have completed the chorion. Oocytes with half-formed chorions, stripped of their follicle cells, will waterproof themselves if incubated in osmotically balanced Ringer's solution at 25° C. for three days. Waterproofing, therefore, is independent of the formation of the chorion, and, probably, of follicular activity.

The waterproofing process is also independent of changes due to fertilization. Sterile eggs are waterproofed and fertilization produces a true fertilization membrane. This membrane is freely permeable to water; it lies between the wax layer and the developing embryo, and supports the wax across the inner openings of the micropyles.

A detailed account of these and other features of eggshell structure and permeability will appear elsewhere.

J. W. L. BEAMENT.

Agricultural Research Council Unit of Insect Physiology,
Department of Zoology,
Cambridge. Nov. 27.

¹ Wigglesworth, V. B., *J. Exp. Biol.*, 21, 97 (1945).

² Beament, J. W. L., *J. Exp. Biol.*, 21, 115 (1945).

Extrusion of Nucleic Acid from the Nuclei of Human Granulocytes

PETRI¹ described "kleine gestellte Kernprominenz", small, stalked, deeply staining buds attached to the contorted ring-like nucleus of the mature neutrophil leucocyte of the mouse. La Cour² showed that they arise from the deeply staining heterochromatic parts of the nucleus, that they are Feulgen-positive and so contain desoxyribose nucleic acid, and are ultimately split off and lie free in the cytoplasm.

Similar buds are present in human neutrophil and eosinophil leucocytes. Commencing as eminences on a heterochromatic body, they develop a stalk, which lengthens, thins, and ultimately ruptures, leaving the bud free in the cytoplasm. They develop from segmented

× 1,200.