Milk Proteins

I WISH to draw attention to some errors in the recent article by Winterburn and Phelps[1] on the significance of glycosylated proteins.

First, in the diagram of protein composition of cow's milk they include both α-casein and κ-casein. κ-Casein is in fact a component of the fraction originally known as α-casein. The α-casein can be separated into κ-casein and α_s-casein[2,3]. If Winterburn and Phelps have confused α-casein and α_s-casein, then "α-casein" should be replaced by "α_s-casein". It should not be classified as a glycoprotein as there are no reports that α_s-casein contains significant amounts of carbohydrates. On the other hand, if α-casein is used in the generally accepted sense, then it should be recognized that it contains carbohydrates only because of the presence of κ-casein. In this case κ-casein should not be allocated an additional space as this would mean that it was being included twice. In the light of current knowledge it is usual to consider κ-casein and α_s-casein as separate identities.

Furthermore, a proportion only of κ-casein is glycosylated[4,5], yet it is classified as a glycoprotein. α-Lactalbumin is similar in this respect as it contains glycosylated components. Therefore it is difficult to understand why this protein is not included in the same category as κ-casein.

It is also stated in the article that the liberation of a glycopeptide containing the majority of the carbohydrate destroys the micelle-stabilizing properties of the protein. This is true, but to put the matter in perspective it should also be pointed out that almost the same effect is achieved in molecules of κ-casein which are devoid of carbohydrates. Hence the implication that the carbohydrates have an essential role in the micelle-stabilizing properties of κ-casein is not valid. In fact, it is widely agreed that the κ-casein carbohydrates are not essential for either rennin action[6] or for the micelle-stabilizing power itself[4].

It may be that recognition of these errors does not greatly affect the conclusions of the authors, but nevertheless it is desirable that conclusions are based on a sound assessment of the facts.

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Uptake of Bacteria by Isolated Higher Plant Protoplasts

ISOLATED higher plant protoplasts can take up particles, by endocytosis, into vesicles in their cytoplasm. The uptake of tobacco mosaic virus, ferritin, and polystyrene latex spheres has been reported[1-4]. Particles up to 0.3 μm have been observed to be actively taken up[1]. The size of some vesicles as seen in thin sections of fixed and embedded isolated protoplasts, however, indicates that particles considerably larger than 0.3 μm could be accommodated in such vesicles in the cytoplasm. We have therefore investigated the possibility of uptake of bacteria into higher plant protoplasts, using *Rhizobium* and pea leaf protoplasts as our experimental system.

Fully expanded leaflets were excised from 5–7 week old pea plants (*Pisum sativum* var. Little Marvel), wetted by immersion in 1% sterile 'Teepol' (BDH Chemicals Ltd.) (3 min), and surface sterilized in 1.5% sodium hypochlorite solution (10 min). The sterilant was removed by 8 successive washes with sterile water. 1.5 g fresh weight of peeled leaflets[2] was incubated with 5 ml. of a mixture of 5% Meicelase (Meiji Seika Kaisha Ltd, Tokyo) with 5% Macerozyme (All Japan Biochemicals Co. Ltd., Nishinomiya) in 25% sucrose (*p*H 5.8) containing 200×10^6 *Rhizobium leguminosarum* (Rothamsted Culture Collection Cat. No. 1007). To prepare this mixture, bacteria were cultured in 100 ml. of liquid mannitol yeast-water medium[5] in 250 ml. Erlenmeyer flasks incubated at 25° C in the dark on a horizontal shaker (100 r.p.m.), and harvested by centrifugation 3 days after inoculation. Pelleted bacteria were re-suspended in water, an aliquot removed for cell density determination, and the volume adjusted so that 1 ml. of this suspension, when added to 4 ml. of the filter sterilized enzymes in 31.25% sucrose, gave the required bacterium density and a final plasmolyticum concentration of 25% sucrose. Leaf pieces were incubated for 20 h in this enzyme-bacterium mixture in the dark at 25° C. The released protoplasts were freed of leaf debris by straining through a fine wire gauze, and the protoplast-enzyme-bacterium suspension centrifuged at 225*g* for 5 min. The surface film of intact protoplasts was washed to remove enzymes and bacteria by re-suspending six times in 25% sucrose, then centrifuged at 225*g* for 5 min. Protoplasts were fixed for electron microscopy for 16 h at 22° C, in 3% glutaraldehyde in 0.1 M sodium phosphate buffer (*p*H 7.1) containing 25% sucrose. They were washed in 0.1 M sodium phosphate buffer, and post-fixed for 3 h at 2° C in 1% osmium tetroxide buffered with 0.1 sodium phosphate (*p*H 7.1). Thin sections were prepared as previously described[6].

Observations of ultrastructure showed the presence of *Rhizobium* localized within membrane bounded vesicles in the cytoplasm of the isolated pea leaf protoplasts (Fig. 1*a*). There were also bacteria in vesicles deep within the cytoplasm of many protoplasts and within transvacuolar cytoplasmic strands (Fig. 1*b* and *c*). Some burst protoplasts with bacteria still enclosed within vesicles were also present in the cytoplasmic debris. Morphologically the bacteria appeared unaffected by the enzyme mixture used to degrade the leaf cell walls, or the plasmolyticum, or by being enclosed within vesicles in the host cell cytoplasm. Constricted bacteria were present in some vesicles (Fig. 1*a*), but it is not clear whether they were dividing within the vesicles following uptake, or whether they had commenced division prior to uptake.

Pea leaf protoplasts take up bacteria only during enzymatic digestion of the surrounding cell wall. Uptake did not occur when peeled leaf pieces were incubated with the exposed mesophyll cells in contact with bacteria, nor when isolated protoplasts were incubated with *Rhizobium*. The uptake is thought to occur by engulfment of bacteria into vesicles formed by invagination of the plasmalemma during plasmolysis and concomitant degradation of the cell wall, rather than by a strictly endocytotic process. Since the membranes of the vesicles containing the bacteria are derived from the plasmalemma, they have an origin directly analogous to that of membranes enclosing bacteria which enter root nodule cells via the normal infection thread[7]. Approximately 5% of the protoplasts examined contained up to five bacteria per protoplast section, with two or three bacteria within some vesicles.

It seems likely that this plasmolytic uptake of *Rhizobium* by pea protoplasts during their isolation will occur in many microorganisms and protoplast systems. Such investigations may produce new endosymbiotic relationships. The symbiotic