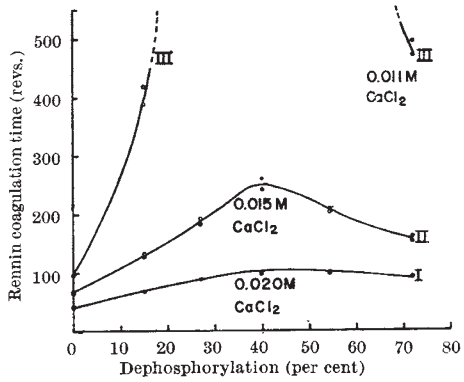

Fig. 1. The relation between percentage dephosphorylation of of bovine casein and the time required for clotting by rennin, at three concentrations of calcium. 10 ml. of casein solution was brought to the temperature of the bath (30° C.), and 1 ml. of rennin ($5{\cdot}6 \times 10^{-4}$ mgm. of rennin nitrogen) was added. Samples were rotated at 16·2 r.p.m. The concentrations of calcium given are those of the buffers against which the samples were dialysed

phorylated casein showed the same behaviour as that in Fig. 1 but gave slightly different values for the coagulation-times. The fact that the curves pass through a maximum suggests that more than one effect of dephosphorylation is present; it is likely that the increase in isoelectric point on dephosphorylation tends to produce shorter coagulation times.

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'Solubilization' of a Cytochrome *b* Component from *Micrococcus lysodeikticus*

In an earlier communication[1] we reported some preliminary observations on a cytochrome *b* component of *Micrococcus lysodeikticus*, strain N.C.T.C. 2665. The cytochrome was separated to some extent from the cytochromes *a* and *c* which were also present, but it remained attached to particles which were thrown down by centrifugation at 20,000*g*.

In further experiments, we have found that the cytochrome can be 'solubilized' by treatment of the particulate preparation with a 1 per cent solution of 'Cetrimide' (B.P.). Centrifugation at 20,000*g* at this stage separated a small deposit which contained some cytochrome *c*. Further purification of the cytochrome *b* component was achieved by fractional precipitation from the supernatant by addition of ammonium sulphate. Between 20–40 per cent (w/v) ammonium sulphate, the small quantity of residual cytochrome *c* was precipitated. The cytochrome *b* component came down as a red, gelatinous precipitate at ammonium sulphate concentrations of 40–60 per cent (w/v). This material was apparently soluble in 0·04 *M* phosphate buffer at *p*H 7. The solution obtained was dialysed for 24 hr. against running tap-water, and during this time precipitation of a yellow pigment occurred, the absorption spectrum of which suggested that it might be a carotenoid. The maxima in petroleum ether, 415 mμ, 435 mμ and 465 mμ, corresponded to the wave-lengths recently reported[2,3]. The precipitate was filtered off and the filtrate was allowed to stand in the cold. More of the yellow pigment separated over a period of 48 hr., but no further precipitation occurred during the subsequent 72 hr.

The remaining solution was examined spectrophotometrically, after reduction by addition of solid sodium dithionite, and peaks corresponding to a cytochrome of the *b*-group were found. The absorption maxima of this component were at 558 mμ, a small peak at approximately 525 mμ and a Soret peak at 529–530 mμ; there was no evidence of residual cytochrome *c*. It appears, therefore, that the component should be regarded as a cytochrome b_1. The maximum at 558 mμ differs slightly from that found earlier with cruder preparations in glycerol, which showed a 'flat' peak extending from 560 mμ to 563 mμ. These observations have been repeated, and the slight difference has been confirmed. Whether it is due to the presence of an extra component in the crude preparations or to a slight shift due to different optical or physico-chemical conditions is not yet certain. Because of the residual light-scattering with the cruder materials, the readings were necessarily less precise than with the clear solutions which were later available.

The solutions obtained by the method described still contained some yellow carotenoid pigment which could not be extracted with petroleum ether. The pigment was, however, extractable with chloroform or butanol, but the cytochrome b_1 was damaged by this treatment.

These observations suggest that at least some of the carotenoid is conjugated with a protein, and while it is possible that this might be the protein moiety of cytochrome b_1 itself, we have no direct evidence for this.

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