

the amount of strontium-85 transferred from the human gastro-intestinal tract to blood and whole body (and presumably bone) by a factor in the range 8-10.4.

Sodium alginate is non-toxic at the level used in this experiment, and selectively inhibits the uptake of radiostrontium without significantly interfering with the uptake of calcium by the body^{1,2}. The ability to inhibit selectively the uptake of radiostrontium from the gastro-intestinal tract would seem to be of definite value in the event of contamination of food or water with radiostrontium. The effect of food in the stomach at the time of administration of sodium alginate, however, has not as yet been worked out. After an accidental inhalation of radiostrontium, quite a large proportion of material may be cleared from the respiratory tract to the gut; in this event there may also be a place for alginate therapy.

Our present programme of experiments is designed to assess the effect of sodium alginate administered before, with or after an intake of radiostrontium, and also to assess the optimum doses and type of sodium alginate. It is also proposed to see whether the degree of inhibition is affected by the contents of the stomach.

A low viscosity form of sodium alginate ('Manuocol SS/LD', supplied by Alginate Industries, Ltd.) was used in this experiment.

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BIOLOGY

Production of Carotenoid by a Green Alga

THE first observations on carotenoid production by the chlorophycean alga *Dictyococcus cinnabarinus* were made by Wenzinger¹, Haag² and recently Kol³. From experiments carried out on static culture and for periods of a few months of growth, these authors reported that when sugars were present in the medium the cultures changed colour from green to red. The production of redness, they observed, was due to the prevalence of carotenoids in the chlorophyll formed.

Starting from these observations we decided to investigate the behaviour of *Dictyococcus cinnabarinus* and the eventual carotenoid production in submerged culture using different sugars, with and without addition of carbon dioxide, and in different conditions of temperature and light.

The experiments were performed in 500-ml. Erlenmeyer flasks containing 100 ml. of culture broth, on a rotatory shaker duly thermostated and illuminated. Illumination was supplied by fluorescent tubes ('warm white' type) which gave a light intensity of 3,500-4,000 lux. The strain used was *Dictyococcus cinnabarinus* 280 ('Kol-F. Chodat') (obtained from the algal collection of the Botanical Institute of the University of Geneva) and as culture broth, Detmer medium, diluted three times, was used. In the experiments with addition of carbon dioxide, a mixture of

air containing 3 per cent carbon dioxide was supplied to each flask at a rate of 0.5 v/v medium/min.

Each experiment lasted about 2 weeks and the following sugars were tested: glucose, fructose, galactose, lactose and sucrose, at a concentration of 2 per cent w/v of the culture medium.

200,000 cells/ml. grown in a seed flask containing Detmer/3 medium without sugar were inoculated into the 'fermentation' flask. It must be emphasized that in the absence of sugars the culture remained green, while in the presence of sugars, namely glucose, the culture changed colour gradually from green to yellow, to pink up to an intense orange colour by the end of the fermentation, which indicated accumulation of carotenoids.

The experiments showed that in the presence of glucose a good growth is obtained with a sugar utilization of 40-50 per cent, while other sugars are not utilized and the growth is quite poor; that supply of carbon dioxide does not interfere with the utilization of carbohydrates. Furthermore, the results have shown that the optimum for inoculum is 200,000 cells/ml. of green culture, each cell having a diameter of 5 μ , and in the red culture 10-12 to 20-25 μ (the latter are aplanosporangia, that is, mother cells in sporogony); that the optimum temperature is 25°; and that the best growth is obtained when 12 h of light is alternated with 12 h of dark.

For pigment determination, the cells collected by centrifugation were ground in a mortar with Na₂CO₃ and 'Celite', adding gradually 80 per cent aqueous acetone; the acetone solution was then extracted with light petroleum. The absorption spectrum of the ethereal extract was determined between 400 and 700 m μ using a Cary spectrophotometer.

Thin-layer chromatography was used for the preliminary characterization of the pigments. Amounts sufficient for their identification were obtained by column chromatography on 'Florisil' or alumina. Elution was effected with a gradient of acetone in light petroleum (b.p. 40°-70°). Coloured fractions belonging to each band were pooled, evaporated and examined in the spectrophotometer.

Six fractions of colour ranging from yellow to red were separated and identified as follows:

(1) β -Carotene (epiphasic); 5-6 per cent of the total; m μ 478,450 (425) in light petroleum, m μ 515,485 (458) in carbon disulphide—plates yellow or orange—yellow—m.p. 174°.

(2) Echinenone (90 per cent epiphasic); m μ 456-458 in light petroleum, m μ 488 in carbon disulphide; by reduction with LiAlH₄ gives isoerythroxanthin.

(3) Unidentified (epiphasic); m μ 462 in light petroleum, m μ 500 in carbon disulphide.

(4) Unidentified (epiphasic); m μ 470 in light petroleum.

(5) Xanthophyll (hypophasic); traces—m μ 471, 441, 420 in light petroleum.

(6) Unidentified (hypophasic); traces.

The spectra of fractions I and II can be superimposed on those taken with pure samples.

It is interesting to emphasize finally that in the cultural conditions which we describe, no chlorophyll could be detected; echinenone does not seem to have been previously demonstrated in the Chlorophyceae.

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