According to Soeder[4], when the division and sporulation processes are delayed or inhibited generally larger cells are originated and these contain an enormous amount of reserve substances. Griffiths[5] has shown that *Chlorella* cultivated in a medium with glucose has its division inhibited and its volume increased, and that its dry weight is considerably greater than when grown in an inorganic medium. These accumulated reserve substances are those which, as I have shown[1], cause a considerable proliferation of autospores in the giant cells, and these, on being carried to inorganic medium and placed in conditions of autotrophism, break the wall of the mother cell and cause in the culture a growth which is notably superior to normal growth.

It is therefore advisable, whenever it is desired to accelerate the growth of a *Chlorella pyrenoidosa* culture and obtain an abundant mass of this in a short time, to use medium *A* inoculated with giant cells.

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[1] Rodríguez-López, M., *Nature*, **199**, 506 (1963).
[2] Stegmann, G., *Z. Botanik*, **35**, 385 (1940).
[3] Noack, K., Pirson, A., and Stegmann, G., *Naturwiss.*, **28**, 172 (1940).
[4] Soeder, C. J., *Flora*, **148**, 489 (1960).
[5] Griffiths, D. J., *Ann. Bot.*, **27**, 493 (1963).
[6] Letovský, R., and Klásterská, I., *Fol. Microbiol.*, **6**, 115 (1961).

MICROBIOLOGY

Toxic Action of Hæmolytic *Escherichia coli* isolated from Pigs

Certain hæmolytic strains of *E. coli* are associated with œdema disease and gastro-enteritis of young pigs. Timoney reproduced œdema disease in pigs by injecting supernatant material of the centrifuged intestinal contents from pigs which had died of this disease[8]. Since then, Erskine *et al.*, Sojka *et al.* and Sweeney *et al.* have successfully reproduced the disease by injecting bacterial extracts prepared from hæmolytic strains of *E. coli*[2,4,6]. These reports suggest that œdema disease is caused by absorption of a toxic substance produced by specific strains of hæmolytic *E. coli*.

In contrast to the 'toxæmia hypothesis', Lemcke *et al.* observed severe anaphylactic reaction following injections of polyvalent *E. coli* antiserum into pigs that had shown symptoms of œdema disease and concluded that œdema disease was produced due to an antigen–antibody reaction and therefore this disease is a specific anaphylaxis[3]. Further evidence in favour of this 'anaphylactic hypothesis' on œdema disease and gastro-enteritis complex was presented by Buxton and Thomlinson from their observations on passive cutaneous anaphylactic tests in guinea pigs and by Thomlinson and Buxton from reversed anaphylactic experiments in pigs[1,7].

The work recorded here was carried out to test the validity of each of these two hypotheses and to seek a possible reconciliation. Bacterial extracts of a hæmolytic strain of *E. coli*-0141: *K85a,c* (*B*), and a non-hæmolytic strain —*Mac* 429 isolated from a pig were prepared by the method of Erskine *et al.* with minor modifications in that the cells were washed twice in 0·85 per cent sodium chloride solution, and disintegrated by sonic oscillation for 30 min[2]. Total protein content was determined by measuring the amount of precipitate produced with trichloroacetic acid[5].

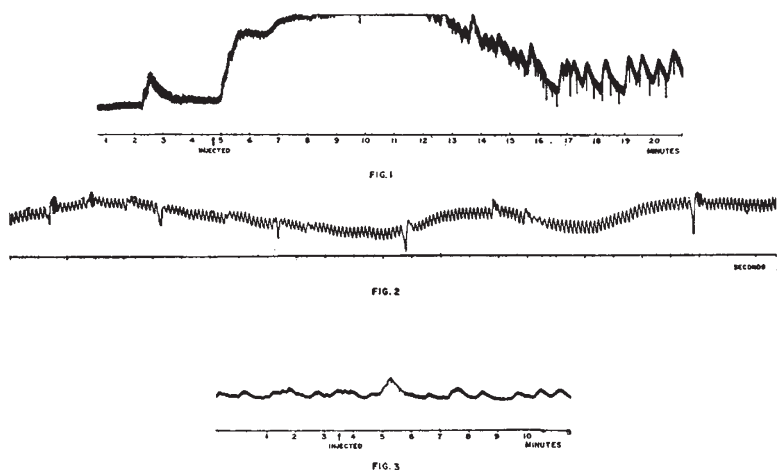

Fig. 1. Effect of an intravenous injection of 0·5 ml. extract of hæmolytic *E. coli*-0141: *K85a,c* (*B*). Speed of the paper 20 mm/min

Fig. 2. Difficulty in expirations, deep and prolonged inspirations accompanied by convulsions are recorded on this graph by running the paper at 20 mm/sec

Fig. 3. Effect of an intravenous injection of 0·6 ml. extract of non-hæmolytic *E. coli*. Speed of the paper 20 mm/min

Each extract was tested on a separate young, healthy, non-sensitized, female guinea pig weighing about 300 g. After anæsthetizing the animal with ether inhalation, its uterus was exposed and the severed right horn was attached to a transducer of an electronic polygraph recorder. About 0·5 ml. of the bacterial extract adjusted to contain 7·0 mg of protein per ml. was inoculated into one of the mesenteric veins.

The reaction of the animal to an injection of hæmolytic *E. coli* extract was observed within 15 sec by a sharp contraction of the uterus and respiratory distress (Fig. 1), the uterus remained contracted for at least 11 min, the rate of respiration increased and the duration of inspirations and expirations became shorter. After this 11-min period of violent reaction, the animal showed signs of respiratory failure with intermittent gasping inspirations and convulsions (Fig. 2) and died within ½ h of the injection.

A lag of 1·5 min followed an injection of the non-hæmolytic *E. coli* extract when the uterus contracted also coincident with respiratory distress (Fig. 3). However, in contrast to the action of the hæmolytic *E. coli*, the reaction lasted for only 1 min and the animal recovered.

The results of these experiments indicate that hæmolytic *E. coli* produce a potent toxin that has a marked effect on non-sensitized guinea pigs. Extracts of non-hæmolytic *E. coli* have a less toxic effect, but whether this difference in potency is due to a different type of toxin produced by the two strains or different amounts of the same toxin is not known. All these data support the 'toxæmia hypothesis'.

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[1] Buxton, A., and Thomlinson, J. R., *Res. Vet. Sci.*, **2**, 73 (1961).
[2] Erskine, R. G., Sojka, W. J., and Lloyd, M. K., *Vet. Rec.*, **69**, 301 (1957).
[3] Lemcke, R. M., Bellis, D. B., and Hirsch, A., *Vet. Rec.*, **69**, 601 (1957).
[4] Sojka, W. J., Erskine, R. G., and Lloyd, M. K., *Vet. Rec.*, **69**, 293 (1957).
[5] Stadtman, E. R., Novelli, G. D., and Lipmann, F., *J. Biol. Chem.*, **191**, 365 (1951).
[6] Sweeney, E. J., Sojka, W. J., and Lloyd, M. K., *Res. Vet. Sci.*, **1**, 260 (1960).
[7] Thomlinson, J. R., and Buxton, A., *Immunology*, **6**, 126 (1963).
[8] Timoney, J. F., *Vet. Rec.*, **62**, 748 (1950).