protein in *Agrobacterium tumefaciens* B_6 is linked to the peptidoglycan by covalent bonds different from those in *Escherichia coli*.

About 40% of the purified walls is accounted for by typical peptidoglycan components (table). There is a molar excess of glucosamine over muramic acid, which is probably in the form of a polysaccharide. The latter very likely contains also galactosamine and neutral sugars, most of which (80%) was identified as D-galactose (with β-D-galactose dehydrogenase, according to Finch et al.[10]). These may be components of a polysaccharide covalently linked to peptidoglycan. The polysaccharide is different from lipopolysaccharide as the latter is not known to be covalently linked to peptidoglycan[11]. Moreover, lipopolysaccharide prepared from *Agrobacterium tumefaciens* by the hot phenol method[5] does not contain peptidoglycan constituents.

10 P. R. Finch, R. Yuen, H. Schachter and M. A. Moscarello, Analyt. Biochem. *31*, 296 (1969).
11 V. Braun and K. Hantke, A. Rev. Biochem. *43*, 89 (1974).

Cell number and cell doubling times during the development of carrot embryoids in suspension culture

G. S. Warren[1] and M. W. Fowler

Department of Biochemistry, The University, Sheffield S10 2TN (England), 4 August 1977

Summary. A time course for the appearance of different stages in embryogenesis in carrot cell cultures is presented, together with the data on cell doubling times. Removal of auxin appears to dramatically increase the rate of cell division, particularly in early embryogenesis.

There is now good evidence from a number of plant cell cultures that single cells on the surface of small meristematic groups of cells may be induced to form embryoids[2]. Such a phenomenon is usually promoted by decreasing the auxin content of the culture medium, following which a series of rapid cell divisions ensues with the eventual formation of a bipolar embryoid structure. The process of embryoid development encompasses 3 characteristic, morphologically distinct structures usually distinguished as the globular, heart and torpedo stages[3,4].

Table 1. Cell number and time of appearance in the culture of different embryoid stages

Stage (dimensions in mm)	Cell number	Time for appearance (h)
Young globular (diameter 0.15)	70–115 (peripheral cell No. 16–19)	90–100
Globular (diameter 0.2–0.25)	411–607 (peripheral cell No. 29–33)	138–145
Heart (width 0.25) (length 0.3)	900–1300	160–170
Young torpedo (width 0.3) (length 0.5)	2000–3000	190–200

3 separate experiments were performed.

Table 2. Cell growth rate in embryoids at different developmental stages

Interval	Developmental stage	Average doubling time (h)
0– 95	Initial – young globular	14.5*
90–142	Enlargement of globular	21
140–165	Globular – heart	22
165–195	Heart – young torpedo	25

*Assuming single cell origin and no lag period prior to initiation.

During studies into the biochemistry of embryo development in carrot cultures we noted that high rates of cell division were achieved during the early phases of the developmental process. In this communication we report on the time scale of appearance of the different embryoid structures and on the rates of cell division at various points in the process.

Material and methods. Carrot cells were grown in suspension culture in Murashige and Skoog medium supplemented with sucrose (0.073 M), zeatin (10^{-7} M) and 2,4-D[5] (4.52×10^{-7} M) as described previously[3]. Cultures of high embryogenic potential were obtained by inoculating 5 ml of 21-day-old, washed, cells (obtained by filtration through 1 mm diameter glass beads) into 25 ml of Murashige & Skoog medium minus 2,4-D.

The time scale of embryoid development was studied by following the appearance of the different stages in embryogenic cultures initiated as described above. All the embryoids considered were free floating in nature, since cell-group bound embryoids, especially the globular stages, are very difficult to observe. It is known that embryogenic suspensions established from freshly isolated callus and grown in an auxin-containing medium often have slowly developing embryoids which advance rapidly once the auxin level is decreased[6]. To avoid such 'preformed' embryoids the stock embryogenic suspensions used had been subcultured 4 times (every 21 days) and did not contain any microscopically identifiable embryoids. Cultures which had been subcultured considerably more than 4 times showed slower embryoid formation. This could be due to increased sensitivity to auxin carried over from the stock suspension[6] or may represent in part, an increasing lag period during which the cells achieve the metabolic state required for the onset of embryogenesis.

1 Acknowledgment. GSW thanks the Science Research Council U. K. for a post graduate research studentship.
2 H. E. Street and L. Withers, in: Tissue Culture and Plant Science, p. 71. Academic Press, London 1974.
3 G. S. Warren and M. W. Fowler, Plant Sci. Lett. *9*, 71 (1977).
4 A. A. McWilliam, S. M. Smith and H. E. Street, Ann. Bot. *38*, 243 (1974).
5 Abbreviation. 2,4-D, = 2,4-dichlorophenoxyacetic acid.
6 S. M. Smith and H. E. Street, Ann. Bot. *38*, 223 (1974).

The first embryoid-like structures to appear in the cultures were usually abnormally large and often showed no signs of cotyledon formation. This type of structure was disregarded as were rhizogenic structures when determining the time of appearance of normal embryoids. Cell counts were performed on these abnormal embryoids. The data indicate a faster cell doubling time than that occurring during 'normal' development. This abnormal growth is possibly due to the initial lack of some conditioning factor from the medium. Some support for this view comes from an experiment in which a sample of globular embryoids separated as eptically[3], was transferred to fresh Murashige and Skoog medium lacking 2,4-D. Large abnormal embryoid structures were produced.

To obtain cell counts for the very young globular stage we used a geometrical method which assumed that the structures were spherical and that the cells packed within the embryoid approximated to cubes all of a similar size. From a determination of the peripheral cell number of a cross-section we were then able to calculate the number of cells in the embryoid. Cell counts for later stages in embryoid development were obtained by more conventional means. Individual cells were freed from the embryoids by treatment with 1% cellulase in acetate buffer pH 4.6 for 3 h at 37 °C. The separated cells were then suspended in 50% glycerol and counted using a haemocytometer slide.

Results and discussion. Rates of cell division (i.e. cell doubling times) were calculated assuming that embryoids develop from single cells[4]. Thus, average cell doubling times are tiven by h/D where h is the time required for appearance of the embryoid stage and D is equal to the number of doublings in cell number. D is defined by the expression $\log N - \log N_0/\log_2$ where N = the cell number after h hours and N_0 is the original cell number. The values obtained provide a minimum estimate for the maximum rate of cell division and assume that all the cells in the embryoid are dividing and at a similar rate. The lack of polarized growth in the young globular stage points to this 'ideal' situation.

Table 1 shows the average number of cells in each embryoid stage together with the time from the initiation of a culture to when examples of each stage may first be observed. In table 2 we have presented the average cell doubling times for each stage in embryoid development.

The initial development of embryoids in culture appears to be much more rapid than occurs in vivo. E.g. in cotton[7] the initial cell doubling time is 20–22 h as compared with 14–15 h in carrot cultures. This may reflect the modification to, or absence of, important control systems in culture as compared with the in vivo situation. The formation of large abnormal embryoids is probably a further manifestation of this, representing a situation yet more remote from the controlled environment of the ovule.

Values for the doubling time of small meristematic (embryogenic) cells of carrot growing in a nondifferentiating stage (td 65 h, calculated from Jones[8]) indicate that the removal of auxin from the culture medium results in a dramatic reduction in doubling time for cells destined to develop into embryoids (see table 2 for comparison). Data of Bayliss[9] also indicate that doubling times for carrot cells growing in the absence of auxin are much reduced, however it is not clear to what extent embryoids were included in the cell counts.

7 E. G. Pollock and W. A. Jensen, Am. J. Bot. *51*, 915 (1964).
8 L. H. Jones, Ann. Bot. *38*, 1077 (1974).
9 M. W. Bayliss, Exp. Cell Res. *92*, 31 (1975).

Effect of beta-adrenergic blockade on intramuscular triglyceride mobilization during exercise[1]

B. Stankiewicz-Choroszucha and J. Górski

Department of Physiology, Institute of Physiology and Pathology, Medical School, Białystok (Poland), 1 August 1977

Summary. It was shown in the rat that mobilization of intramuscular triglyceride during exercise is controlled by the adrenergic system.

It was repeatedly shown that intramuscular triglycerides are mobilized during exercise[2–8]. However, the mechanism controlling this process has not been understood yet. In the present work it was found that the adrenergic system is responsible for activation of intramuscular triglyceride breakdown during exercise.

Materials and methods. The experiments were carried out on male Wistar rats weighing 200–250 g, fed ad libitum with commercial pellet diet for rodents. The animals were divided into 2 groups: I the control group, II the propranolol-treated group. Propranolol (Inderal, Galenika) in a dose 2.5 mg/kg was injected s.c. 15 min before exercise. Half of each group was subjected to the exercise, while the other half was kept at rest and deprived of the food at the same time. The exercising rats were loaded with a weight equivalent to 1% of their b. wt attached to base of the tail and then they were forced to swim in a metal barrel 58 cm in diameter filled to a level of 40 cm with water at temperature 33–35 °C. 6 rats were made to swim simultaneously. It was found in preliminary experiments that the control rats were able to swim for not much more than 5 h, and the propranolol-treated rats for not much more than 3 h. According to these data, to avoid stressfull drowning, rats were subjected to exhaustive swimming which was ceased after 5 h in the

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2 K. M. Baldwin, J. S. Reitman, R. L. Terjung, W. W. Winder and J. O. Holloszy, Am. J. Physiol. *225*, 1045 (1973).
3 J. K. Barclay and W. N. Stainsby, Am. J. Physiol. *223*, 115 (1972).
4 L. A. Carlson, L. G. Ekelund and S. O. Fröberg, Eur. J. clin. Invest. *1*, 248 (1971).
5 S. O. Fröberg, Metabolism *20*, 714 (1971).
6 S. O. Fröberg and F. Mossfeldt, Acta Physiol. Scand. *82*, 167 (1971).
7 J. S. Reitman, K. M. Baldwin and J. O. Holloszy, Proc. Soc. exp. Biol. Med. *142*, 628 (1973).
8 D. G. Therriault, G. A. Beller, J. A. Smoake and L. H. Hartley, J. Lipid Res. *14*, 54 (1973).