relationship of the three classes. Studies of additional cestodian species are needed, however, before a definite conclusion can be reached. For the moment we can only conclude that the sperm tails of all platyhelminths studied by electron microscopy show a 9 + 1 pattern in the axial fibre bundle. It is interesting that the 9 + 2 pattern occurs in the axial fibre bundles in the extensions of flame and epithelial cells in the turbellarians[3,4] and the cestodes[6], the only classes of the platyhelminths so far investigated.

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Chemical Composition of the Cell Wall of *Caryophanon latum*

THE "giant micro-organism" *Caryophanon latum* was first described by Peshkoff as the "missing link" between the blue-green algae and the bactoria. He isolated the micro-organism from cow manure near Moscow, noting its unusual morphology including "its long rod forms containing a varying number of nuclei"[1]. Others have since isolated this organism in England and in the United States[2,3]. Pringsheim and Robinow have described this micro-organism. *Caryophanon latum* Peshkoff as "a very large Gram negative, peritrichously flagellated bacterium of unusual structural complexity"[2]. On the other hand, Provost and Doetsch reported the organism to be a Gram positive bacterium, susceptible to egg-white lysozyme and able to form protoplasts readily[4]. The work described here was designed to obtain further evidence as to the bacterial nature of the micro-organism by analysing the isolated and purified cell walls of *Caryophanon latum* for its constituent amino sugars, amino acids and monosaccharides.

The method of analysis was that of Becker *et al.*[5] except that the organism was grown on cow dung extract agar, then gently rinsed into a beaker with 95 per cent ethanol, and the cell disruption was accomplished with a 'Heat Systems Sonifer'.

Our results showed large amounts of glucosamine, muramic acid, alanine, glutamic acid and lysine, typical of cell walls of Gram positive bacteria. Diaminopimelic acid, which often appears in place of lysine in the cell wall mucopeptide, was absent. No monosaccharides were found. These results provide further evidence of the bacterial nature of *Caryophanon latum*. Although the finding of a small number of amino-acids in the cell wall indicates a Gram positive nature, we found the organism to stain Gram negative.

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HAEMATOLOGY

Erythropoietic Response of Bone Marrow Cells cultivated in Diffusion Chambers

IT is well known that cultivation of bone marrow by traditional *in vitro* methods does not sustain normal morphogenesis for prolonged periods of time[1–4], and although synthesis of DNA and haem and some erythropoietic activity have been demonstrated *in vitro*[5–9], these processes are maintained only for extremely short periods.

An alternative approach to the *in vitro* method is the technique of cultivating haematopoietic cells in diffusion chambers in the peritoneal cavity of host animals. With this method it has been demonstrated[10–12] that mouse marrow cells divide, differentiate and undergo maturation in a much more normal fashion for a considerably longer period than in strictly *in vitro* systems. Attempts to demonstrate morphologically the effect of the humoral agent, erythropoietin, on erythropoiesis in chambers by myself and other investigators[13,14] have, however, been unsuccessful. In view of the ability to demonstrate morphologically erythroblasts in unstimulated marrow cultures[10], two questions arose as to why marrow in diffusion chambers did not respond to erythropoietin. Had cells in the diffusion chambers become refractory to erythropoietin, or were the methods utilized to reveal a possible effect adequate? In an attempt to resolve these questions, experiments were devised in which modified diffusion chambers and a non-morphological end point for demonstrating erythropoiesis were used.

In the first experiment a well-type (solid bottom) diffusion chamber was used. 15×10^6 cells from a pool of *C57/bl*-6 mouse marrow were placed in chambers containing 0·1 μc. of iron-59 and 0·1 ml. of either saline, normal serum or serum rich in erythropoietin. The latter was obtained from mice which had been bled 12 h earlier. Chambers sealed by membranes with a pore of 0·3μ diameter were placed into the peritoneal cavity of normal mice. At intervals after implantation, chambers were taken from animals and cells were removed by treatment with hyaluronidase. Cells were washed three times with sterile saline to eliminate unincorporated iron-59 and were assayed for cellular incorporation of iron-59 in a well-type scintillation counter. The results obtained are summarized in Table 1, and are expressed in terms of incorporation of iron-59 as a percentage (10^5) of the original amount of iron-59 in the chamber. Values for the saline and normal serum were essentially the same and are presented as pooled controls. Within 24 h there was seven times as much incorporation of iron-59 in those cells cultivated in serum rich in erythropoietin as in the control group. By day 3 incorporation of iron-59 in the cultures rich in erythropoietin reached a maximum value approximately four times greater than on day 1 and fifteen times greater than the controls. A drop in the incorporation of the isotope was noted between days 3 and 5 and between days 7 and 9. The value on day 9 is essentially the same as that on day 1 and still represents a fivefold increase over the controls.

The data obtained indicate that precursors of erythrocyte are present in diffusion chambers which are capable of responding to an erythropoietic stimulus. The failure to demonstrate a cumulative increase in incorporation of iron in the group with serum rich in erythropoietin could result from the utilization of erythropoietin by erythroid precursors which has been suggested[15], or from a depletion

Table 1. INCORPORATION OF IRON-59

	Days after implantation				
	1	3	5	7	9
Serum rich in erythropoietin	29·0 (6)*	100·7 (6)	39·5 (6)	47·3 (6)	24·5 (5)
Controls	4·2 (12)	6·6 (12)	8·8 (12)	8·7 (12)	5·0 (12)

The percentage of iron-59 in each chamber is multiplied by 10^5.
* Figures in parentheses denote the numbers of animals used.