different concentrations of phosphate ions were studied. The solution of K_2HPO_4 was autoclaved separately to avoid precipitation in the basal medium. All the culture flasks were incubated under fluorescent light with an intensity of 1.5×10^5 erg/cm²/sec at a temperature of 30 ± 2°C. Growth of the alga was estimated on dry weight basis. Nitrogen contents of the alga as well as of culture filtrates were determined by semi-micro-Kjeldahl method. A selenate catalyst mixture was used during digestion and ammonia distilled in a Markham still.

The Table shows dry weights and nitrogen contents of *C. majus* at different pH values and phosphate-ion concentrations. A perusal of the data shows that the alga favoured a relatively high pH for its maximum growth, whereas percentage of total nitrogen fixed was highest at pH 8.0. Also, growth and nitrogen fixation were improved by increasing concentration of phosphate ions upto a certain level. Maximum nitrogen fixation on per cent basis was recorded up to phosphate concentration of 100 mg/l only.

The data obtained were subjected to statistical analysis. It was found that pH of the culture medium affected the algal growth significantly but no significant effect of different pH values on nitrogen fixation was observed. However, different concentrations of phosphate ions and their interactions with pH of the basal medium were found to be quite significant for growth and total nitrogen fixed by the alga.

Of the different kinds of algae found in paddy fields of India, the blue-greens are most abundant. Many of them are nitrogen fixers and play an important role in the nitrogen economy of soils. It is generally recognized that the nitrogen-fixing blue-green algae liberate appreciable quantities of fixed nitrogen into the medium during healthy growth. Most of this extracellular nitrogen is in the form of peptides, whereas free amino acids are present only in small amounts (FOGG[2]). Therefore, the real importance of nitrogen fixation by the blue-green algae in nature lies in the fact that fixed nitrogen becomes available for growth of the associated non-nitrogen-fixing plants. In fact, STEWART[3] has found evidence of a direct transfer of previously fixed nitrogen by a species of *Nostoc* to the associated non-nitrogen-fixing plants.

Nitrogen fixation by blue-gree algae is maximum only in the slightly alkaline range and any deviation on either side of this range depresses nitrogen fixation significantly. The fixation falls off markedly below and above the pH range of 7.0–8.0. FAY and FOGG[4] studied the effects of hydrogen-ion concentration on *Chlorogloea fritschii* and found no differential effects of pH on growth and nitrogen fixation. COBB and MYERS[5] also did not find any significant effect of pH on nitrogen fixation by *Anabaena cylindrica*. STEWART[6] has suggested that the effect of pH is probably on metabolism in general and not specifically on nitrogen fixation.

While considering the uptake of phosphate ions, pH of the medium is very important as it may alter the rate of phosphate uptake either by a direct effect on permeability of the cell membrane or by changing ionic form of the phosphate (EPSTEIN[7]). Our results indicate that at relatively low concentrations of exogenously supplied phosphate ions or even in a medium entirely devoid of them, growth and amounts of nitrogen fixed by *C. majus* were quite considerable. But higher concentrations of phosphate ions were not found conducive to the process of nitrogen fixation, the exact reason of which is not clearly understood.

Zusammenfassung. An der auf den indischen Reisfeldern heimischen blaugrünen Alge *Cylindrospermum majus* wurde der Einfluss von Änderungen der pH- und PO^3_4-Konzentration auf Wachstum und Stickstoff-Fixation untersucht. Während pH-Änderungen sich nur auf das Wachstum auswirkten, hatte Erhöhung der PO^3_4-Konzentration bis zu einem gewissen Bereich (bei konstantem pH) sowohl signifikante Wachstumssteigerung als auch Stickstoffeinbau zur Folge. Die Bedeutung der Stickstoff-fixierenden niederen Algen als Stickstoffquelle für andere Pflanzen wird diskutiert.

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The Glair Glands and Oosetae of *Austropotamobius pallipes* (Lereboullet)

The origin and function of the glair exuded by the crayfish just prior to egg laying is obscure. In this connection a number of studies have been made of spawning in the European[1], Australian and American crayfishes, but not of *A. pallipes*, the endemic British species.

In mid-September, sexually mature females of *A. pallipes* are conspicuous by the presence of creamy-white patches on the pleura, and sterna of the abdomen, and on the pleopods and uropods, but never on the telson. A closer examination of these cream coloured areas, using the scanning electronmicroscope, discloses the presence of numerous pores in the overlying integument (Figure 1a). These pores are grouped together in roughly circular patches, presenting the appearance of 'pepper-pot' tops (Figures 1b, c and d). Observations in the field show that it is through these pores that the glair is exuded (Figure 1e), and the cream colouration is due to the very large groups of glair glands underneath the areas of perforated integument. On the pleopod the pores occur in much smaller groups (Figure 1f).

In *A. pallipes* the distribution pattern of the pores is constant; they are very numerous on the anterior faces of the pleura, less so on the anterior faces of the protopodites, and first segments of the endopodites and

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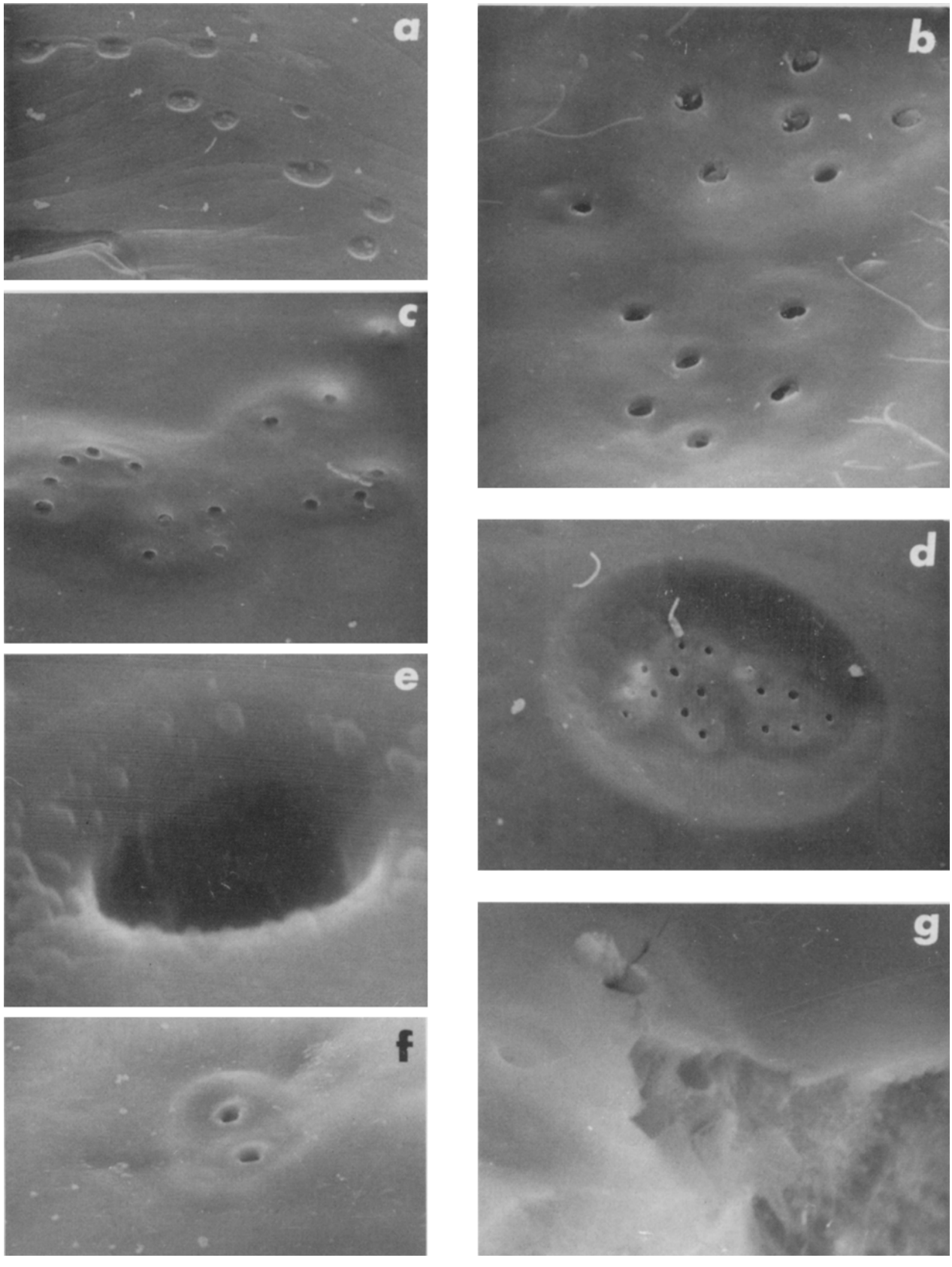

Fig. 1. a) The pleural surface of abdominal segment 3, showing the glair gland pores. ×73. b) A group of pores from the pleuron of segment 4. ×2.2 K. c) A group of pores on the sternum of segment 4. ×1.08K. d) Pores in the sternum of segment 3, near the base of a pleopod. e) A single pore of a glair gland on the pleuron. ×22K. f) Typical pore group on the endopodite of a pleopod. ×2.15K. g) Sagittal cut through the integument showing the pore and its attendant duct and gland. ×3.05K.

exopodites of the pleopods. There are large numbers of pores on the sterna of the abdomen, particularly around the bases of the pleopods and uropods. It is interesting to note that all the pores face anteriorly – facing the openings of the two oviducts. This siting of the pores is particularly significant when considering the arched condition of the abdomen during spawning – forming the egg chamber. The openings of the glair glands are quite characteristic and differ from the pores of the integumental glands, which never occur as groups. A hand section through a pore region, viewed under the scanner, shows clearly, well developed ducts leading into the glair glands (Figure 1g). Transverse sections through the pore region (Figure 2a) shows that the ducts emerging from the glair glands merge to form a roughly spherical chamber within the integument and it is in these chambers that glair is stored until exuded. The ducts ramify through the entire gland (Figure 2b), leading finally into the integumentary chambers. It is this mass of glair, in the large numbers of chambers within the ventral integument, that gives the creamy colour to the female abdomen in September. Glair glands and pores first appear in the females of *A. pallipes* in the second September of their lives. Pores appear in the integument after the final moult before spawning. So these pores and glands could be looked upon as belatedly appearing secondary sexual characteristics. Also developed early in the life of the female are the oosetae, setae specialized for egg attachment. These oosetae (Figures 2c and d) are found on the pleopods and sterna of females near the glair glands and their openings, increasing in number as the crayfishes grow larger.

Proximally, the oosetae are smooth (Figure 2e), with a pronounced groove in the shaft; it may be that part of the glair moves up the shaft to play some role in the attachment of the eggs. Distally the oosetae are flat in section, bearing very fine setules (Figure 2d) and it is these setules which become intimately attached to the eggs. After egg laying, the glands persist until late July becoming inconspicuous following an early August moult, which takes place when the hatchlings have become totally independent. Soon after the glands start developing again in preparation for another spawning.

Zusammenfassung. In der Deckhaut des sexuell gereiften Weibchens von *A. pallipes* treten Porengruppen auf. Diese Poren überlagern die Schleimdrüsen, die während des Laichens grosse Mengen Schleim produzieren. Die Poren und Drüsen befinden sich auf dem Unterleib und den Pleopoden; die Oosetae, die zur Eiablagerung dienen, befinden sich ebenfalls an diesen Stellen. Dies sind sekundäre sexuelle Charakteristika, die eng mit dem Legen und der Ablage der Eier verbunden sind.

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The Effect of Kryptopyrrole on the Porphyrin Auxotrophic Strains of *Bacillus subtilis*

Kryptopyrrole (2,4-dimethyl-3-ethylpyrrole) increases the level of porphyrin synthesis of *Bacillus subtilis* strain 168, and significantly increases the quantity of coproporphyrin III excreted by the bacterium[1]. The stimulating effect of exogenous delta-aminolaevulinic acid (ALA) on the haem synthesis of several bacteria is well known[2], and the enhanced amount of coproporphyrin III excreted by *B. subtilis*, in addition to the accumulation of uroporphyrin III, is also significant. With regard to the metabolism of kryptopyrrole by *B. subtilis*, some porphyrin auxotrophic strains with an enzymatic block in the first 2 steps of the porphyrin biosynthetic pathway were tested as to their growth on solid medium containing kryptopyrrole.

Bacteria: *Bacillus subtilis* strain 168 trpC2 and hemA1 (lacking ALA-synthetase)[3] and hemB1 (lacking ALA-dehydrase)[4] were used as test microorganisms. Media: YP (yeast extract peptone) medium[5], GGM as a minimal medium[6], supplemented with tryptophan (50 µg/ml) and different concentrations (1, 5, 10 µg/ml) of kryptopyrrole. In some experiments the GGM medium was also supplemented with cysteine (50 µg/ml) and bovine albumin (0.5 mg/ml). Both freshly-prepared and 2-day-old kryptopyrrole solutions were used. The inoculated plates were incubated at 37 °C for 48 h.

Our experiments made so far indicate that neither freshly-prepared nor old kryptopyrrole solution can support the growth of strains hemA1 and hemB1. The question arises whether these bacteria are unable to utilize kryptopyrrole as pyrrole source or whether the kryptopyrrole is unable to penetrate into the cells, similarly to porphobilinogen[4]. The fact that kryptopyrrole increases the haem synthesis of prototrophic *Bacillus subtilis*[1] may be explained in that kryptopyrrole disturbs the bioregulation of the haem synthesis pathway, perhaps via complex formation[7]. binding the iron necessary for haem synthesis.

Zusammenfassung. Nachweis, dass Kryptopyrrol zwar die Porphyrinsynthese in *Bacillus subtilis* Wildtyp stimuliert, nicht aber das Wachstum von Porphyrin-Mangelmutanten dieses Bakteriums ermöglichen kann.

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