remarked that 'generic level of relationship of *V. cholerae* and *V. parahaemolyticus* is considered dubious'. Hence a similar polyphasic taxonomy of *V. parahaemolyticus* in relation to members of *Pasteurella* and its allied genera seems relevant to settle the precise taxonomic position of the organism under present study.

[11] The authors wish to convey their gratefulness to Professor J. B. CHATTERJEA, School of Tropical Medicine, Calcutta, for his kind permission to publish this paper.

Zusammenfassung: Durch normale Färbungstechnik wird das Vorhandensein der Kapseln in *V. parahaemolytiens* gezeigt.

B. D. CHATTERJEE and K. N. NEOGY[11]

Department of Bacteriology, School of Tropical Medicine, Calcutta 12 (India), 22 February 1971.

Restoration of Declining Morphogenetic Capacity in Long Term Tissue Cultures of *Daucus carota* by Kinetin

A common problem in the culturing of plant tissues has been the eventual loss of morphogenetic response over prolonged periods of time[1-4]. No report is known of the restoration of the initially vigorous response seen in younger cultures.

For the past several years liquid suspension cultures of *Daucus carota* (wild carrot) have been subcultured in a basal medium containing 2×10^{-6} *M* 2,4-dichlorophenoxyacetic acid (2,4-D). Such tissue grows actively but exhibits little organization. With recently isolated strains, transfer of auxin grown tissue to medium devoid of auxin will initiate extensive organization and development of adventive embryos[5]. The first embryos will appear after about three to four days of growth with almost total conversion after 1 week to 10 days.

During subculturing over long periods of time, the tissue which once had a strong capacity for embryo formation shows a progressive decrease in its ability to produce embryos. There is a twofold increase in time needed for initial appearance of the embryos and the number produced is considerably diminished. When an embryo which does develop in such aged cultures is transferred back to an auxin medium it produces a typical callus culture. Transfer of this tissue to an auxin-free medium results in the vigorous embryogenic response characteristic of recently explanted tissue.

Knowledge of the control of organization exerted by auxins and kinetin on cultured tobacco tissues[6] led us to study the effects of these substances on embryogenesis in carrot cultures. Kinetin and 2,4-D were supplied, at varying concentrations, to cells from long term subcultures. Cells were thoroughly washed to reduce the carryover of materials from previous culture. The results were evaluated in terms of dry weight and the number of embryos produced. Embryo number was determined by dispersing the tissue fragments in a tightly packed monolayer and counting the number of embryos per unit of area which had reached the heart stage of development.

The application of kinetin to recently isolated cultures did not enhance embryogenesis whereas older cultures exhibiting reduced embryogenesis showed a strong response as exemplified in Table I. Kinetin at 10^{-7} *M* caused a 3-fold increase in embryo number over the control, without any effect on growth as measured by dry weight. A 10-fold increase in the kinetin level had no effect on either embryo number or dry weight. At 10^{-5} *M* kinetin growth is considerably diminished and embryo production is totally prevented.

In recently isolated cultures exogenous auxins have not been required to obtain optimum embryogenesis. Table I shows that the addition of 10^{-6} *M* 2,4-D enhances embryo number to a slight degree. More striking is the response evoked by the addition of both 10^{-6} *M* 2,4-D and 10^{-7} *M* kinetin. The results of these studies can be compared with the normal embryogenic response of a recently isolated strain seen in Table II.

Table I. Effect of kinetin and 2,4-D on growth and embryogenesis in long term suspension cultures of wild carrot

Kinetin (*M*)	2,4-D (*M*)	Embryo number	Dry weight (mg)
10^{-5}	10^{-6}	0 ± 0.0	1.3
10^{-6}	10^{-6}	3.8 ± 0.6	13.0
10^{-7}	10^{-6}	16.5 ± 1.4	13.2
10^{-5}	10^{-5}	0 ± 0.0	2.0
10^{-6}	10^{-5}	0 ± 0.0	11.0
10^{-7}	10^{-5}	0.8 ± 0.3	11.6
10^{-5}	—	0 ± 0.0	0.5
10^{-6}	—	3.5 ± 0.6	12.0
10^{-7}	—	10.1 ± 0.9	12.5
—	10^{-5}	0 ± 0.0	13.6
—	10^{-6}	6.5 ± 1.0	13.1
—	—	3.1 ± 0.9	13.0

The embryo number represents the 95% confidence intervals with $N = 40$. Period of growth was 11 days. Dry weight data represent the average of triplicated 5 ml cultures.

Table II. Growth and embryo development in recently isolated tissues of wild carrot

Treatment	Embryo number	Dry weight (mg)
MS (auxin-free)	15.9 ± 4.5	12.0
2×10^{-5}*M* 2,4-D	0.0 ± 0.0	12.3

The culture period was 11 days. Embryo number represents the 95% confidence intervals with $N = 30$.

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Further support for the idea of cytokinin involvement in adventive embryony came from tests carried out with a 1-year-old strain of tissue which had its origin as an embryo isolated from a 5-year-old strain of diminished embryogenic capacity and treated as described above. In the first months of culture embryogenesis in this new strain was as intense as that of freshly explanted material. Kinetin had no demonstrable effect on embryogenesis at this time. After 1 year of subculturing, the new strain had lost much of its capacity to produce embryos. Kinetin supplied at a concentration of 10^{-7} M restored the original high level of embryogenesis. The addition of 2,4-D alone or together with kinetin had no effect on this strain.

The fact that a vigorous capacity for embryogenesis is retained by the cells of embryos derived from long-term subcultures with decreased capacity suggests that long-term cultures become genetically heterogenous. Reports of genetic instability in other long-term tissue cultures have emphasized chromosomal changes as a factor in the eventual loss of the capacity for organ formation[7,8]. Chromosome numbers were not determined in the present material at the time of the hormone experiments. However, all embryos observed so far have been diploid.

It appears that one manifestation of long-term cumulative changes may be a change in the cytokinin-auxin status of the tissue. Either the endogenous levels of these hormones are altered or the sensitivity of the tissue to these hormones is changed. The lack of enhancement of embryogenesis in young isolates by exogenously added hormones suggests that optimum endogenous concentrations prevail at this stage. Substrains of cultured tobacco pith tissue, no longer requiring exogenously supplied cytokinin have arisen spontaneously[9]. The gradual loss of requirement for exogenous auxins (habituation) has been reported frequently. Some of these altered strains have been shown to produce auxins and cytokinins endogenously[10,11]. We have worked with one substrain of wild carrot tissue which gradually developed an absolute requirement for exogenous auxin for growth. Direct analysis of endogenous concentrations of cytokinins and auxins will be required to establish the nature of the changes which have altered the embryogenic capacity of our cultures.

Our work indicates the involvement of cytokinins in the embryogenic process and provides some insight into the nature of declining morphogenetic capacity in long term cultures of plant tissues[12].

Résumé. Les cultures de tissu de *Daucus carota* L. récemment isolées possèdent une capacité embryogénique considérable. L'intensité de cette réponse morphologique décline graduellement dans les cultures subséquentes. La kinétine et, à un moindre degré, l'auxine 2,4-D rétablissent la capacité embryogénique originale dans de vieilles cultures. Les changements de l'état hormonal au cours du vieillissement des cultures pourraient être impliqués dans ces manifestations.

Z. S. Wochok[13] and D. F. Wetherell

Osborn Memorial Laboratories, Yale University, New Haven (Connecticut 06520, USA), and Biological Sciences Group, University of Connecticut, Storrs (Connecticut 06268, USA), 4 June 1971.

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[13] Present address: Z.S. Wochok, Dept. of Biology, Univ. of Alabama, P.O. Box 1927, University (Alabama 35486, USA).

Sex-Specific Pheromones in a Termite

The postflight behaviour of termites strongly suggests that sex pheromones are used during pairing[1]. In some species, tergal gland products appear to be involved in releasing tandem behaviour[2], and in *Kalotermes flavicollis*, the sternal gland of the female and tergal glands of the male secrete a specific attractant for the other sex[3]. The sternal gland also produces a trail pheromone during nymphal stages in several termites[4]. In this note, we report evidence for the secretion of sex-specific pheromones by the sternal gland of both sexes in *Zootermopsis nevadensis*.

Material and method. The alates developed in a laboratory colony[5]. Their abdomens and those of nymphs[6] were dissected into several parts which were separately extracted in hexane to a final concentration of 10 termite parts/ml. Batches of 5 males, or 5 females, were placed in a square arena (625 cm^2) and were given a choice between 2 filter paper squares either impregnated with 0.1 ml of the test extract, or with 0.1 ml of pure hexane. The number of times the termites touched either filter paper was counted during the first 5 min of the test.

The trail pheromone activity of the extracts of the sternites I-V and adjacent epidermis of the alates and nymphs was tested, using an apparatus similar to the one described by Moore[7]. The choice was given to 5 termites, either nymphs or female or male dealates, between 2 S-shaped artificial trails, 40 cm long, joined together to produce a figure of 8. On each trail, an amount of solvent equivalent to the extract of sternites I-V from one termite, or the same amount of pure solvent, was streaked. The number of termites which followed at least 30 cm of either of the trails was determined.

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