Section A 11

The primitive cell system of *Entamoeba invadens* as related to cell differentiation

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Our knowledge of the cell system of Entamoebae is limited. The existence of different cell classes is described, however only morphological parameters are employed. Cell classes are defined as magna and minuta form, or small and big clones, respectively. Relationships between cell classes are practically unknown.

By growing amoebae in a new culture medium (see section G III) populations exhibiting spontaneous encystment as well as populations unable to produce cysts were obtained. Populations not exhibiting spontaneous encystment could be experimentally stimulated to encyst. Encystment was brought about by treatment with an encystment medium (EM), in the presence of $10^{-3}$ g. *Aerobacter* cells logarithmically grown. Sodium phosphate buffer pH 5.5, 50 mOsm/l was used as EM. The mixture of amoebae and bacteria was centrifuged and incubated at $28^\circ$C.

A method for detection of different cell classes in populations not exhibiting spontaneous encystment was found. Amoebae were stimulated with EM containing the DNA inhibitor hydroxylimur (HU concentration was 400 mM). By treating with EM-HH homogeneous as well as heterogeneous populations could be detected. We found cells able to begin the cyst wall synthesis in EM-HH and cells unable to do so. Cysts yielded in EM-HH were abnormal. Cells unable to produce the cyst wall survived between 24 and 30 hr. No injuries could be detected when cells treated for 3 to 5 hr were transferred to a new culture medium.
With respect to their abilities for encystment following cell classes were identified:
1. a cell class programmed for sequential encystment, spontaneously encysting during amoebic culturing,
2. a "covered differentiated" cell class, able to synthesize the cyst wall in EM-HU and
3. an undifferentiated cell class, unable to synthesize the cyst wall in EM-HU.

Amoebic populations were further investigated with respect to encystment competence and cell behaviour after division. Stimuli inducing overt differentiation (encystment) were detected. Interrelations between cell classes were studied.
A culture method for study of the cell system in *Entamoeba invadens*.

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Undoubtedly, axenic methods are elegant culturing procedure and the absence of contaminants is good for biochemical investigations. How good however for studying the cell system in *Entamoebae* remains questionable on account of the absence of certain features of the natural habitat (e.g. food-bacteria, bacterial metabolites, redox potentials determined by bacteria). For this reason we returned to the bacterial association and developed a culture medium able to support amoebic growth by inhibiting bacterial propagation.

The basic medium

Some weeks before utilization a 10% beef serum solution was autoclaved. A beneficial sedimentation occurred at room temperature. The supernatant was diluted to a basic medium with sodium monophosphate—sodium diphosphate buffer (pH 6.0, 1200mOsm/l=0.4M) and distilled water, both previously autoclaved. Dilution was made in a 5:2:3 ratio.

Preconditioning

Preconditioning occurred at 37°C with *Serratia marcescens*. Incubation was stopped 48 hrs. later and bacteria removed by centrifugation at 5000 rpm.

Enrichment

An enrichment supplement was freshly prepared by mixing beef serum with 22% meat extract and 60% glucose (both autoclaved stock solutions). The ratio was 50:15:5.
An antibiotic mixture containing 1 g. Streptomycin and 25 mg. Erycinum, dissolved in 6 ml. water, was added to 70 ml. enrichment supplement. Finally, the supplement containing antibiotics was added to the preconditioned medium in the ratio 1:3. This complex medium was then used for amoebic growth.

**Refrigeration**
The medium could be kept for a longer time at -20°C. Gentle thawing at room temperature was required to avoid precipitation.

**Bacteria for feeding**
*Aerobacter aerogenes* was successfully used as particulate food. Since inhibited by streptomycin and erycinum, it remained metabolically active during amoebic culturing. Experience showed that bacteria killed by heating did not support amoebic growth.

In order to yield bacteria able to induce phagocytosis, *Aerobacter* was grown at 37°C in a medium containing beef serum. The same basic medium described above was used after enrichment with Bacto Nutrient Broth (concentration of BNB 2%). Bacteria grown in pure BNB were not ingested by amoebae. Logarithmically grown bacteria were centrifuged and collected in phosphate buffer pH 6.0, 250 mOsm/l. Cells kept for several days at 4°C could also be used.

**Amoebic growth**
Amoebae and bacteria were inoculated together and centrifuged immediately at the bottom of the culture vessel. Cultivation occurred at 28°C. Amoebae removed bacteria from sediment. The medium remained permanently transparent. Superinfections producing precipitation were therefore easy to detect. Not more than $10^{-4}$ g. bacteria/ml. culture medium could be employed. At higher concentration undesirable opacity occurred.

We used: *Entamoeba invadens* (strain TR 2 nr.1), *Aerobacter aerogenes* (strain AH$_8$) and *Serratia marcescens* from the Hygiene Institute Tübingen, beef serum (ORKP 44/45 from Behringwerke, Marburg), meat extract (E. Merck, Darmstadt) Erycinum (Schering, Berlin).

Dear Mr. Wagner,

Many thanks for your letter of 14/1/1974 and the submission of the title of your (and your coworkers') very interesting short communication in section AM and §3.

May I expect, as far as it is possible on your side, the abstract of your presenting paper - being not longer than 300 words - already during the month of February (deadline is 30th March).

Submission of abstract:

(Section)

(Title)

Surname, first name, address

Text of the abstract (not more than 300 words)

I hope that you have received at the end of December 1973 the third announcement with further informations.

With best regards,

Yours sincerely

[Signature]

KONGRESSZENTRUM - MESSEGELANDE MÜNCHEN
25 - 31 August 1974
Permanent address: D 8000 München 34, P. O. Box 55, Federal Republic of Germany

Sehr geehrter Kongressteilnehmer,

wir danken Ihnen für die verbindliche Anmeldung zu dem oben genannten Kongress.

Ihr Teilnehmerausweis sowie Ihre weiteren Kongressunterlagen liegen für Sie im Kongressbüro ab Sonntag, 25. August 1974, 16.00 Uhr, bereit. Das Kongressbüro ist ab 26. August täglich von 8.00 Uhr bis 18.00 Uhr geöffnet.

Noch ein kurzer Hinweis: Sollte im Ausnahmefall die Überweisung Ihrer Teilnehmergebühr erst so kurz vor der Tagung erfolgen, daß ein rechtzeitiges Eintreffen des Betrages nicht mehr gewährleistet ist, so können wir Ihnen den Teilnehmerausweis nur gegen Vorlage Ihres Einzahlungsbeleges aushändigen.

Wir freuen uns, Sie in München begrüßen zu können und wünschen Ihnen einen angenehmen Aufenthalt.

Mit freundlichen Grüßen

Kongress Sekretariat
ICOPA III

i.A. L. Hagn