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[1] Fredericq, P., *C.R. Soc. Biol., Paris*, **148**, 399 (1954).
[2] Hamon, Y., *C.R. Acad. Sci., Paris*, **242**, 2064 (1956).
[3] Amati, P., and Ozeki, H., *Abstr. Eighth Intern. Congr. Microbiol.*, A51, 26 (Montreal, 1962).
[4] Ozeki, H., Stocker, B. A. D., and Smith, S. M., *J. Gen. Microbiol.*, **28**, 671 (1962).
[5] Coetzee, J. N., *Nature*, **197**, 515 (1963).
[6] Hirota, Y., and Iijima, T., *Nature*, **180**, 655 (1957).
[7] Stocker, B. A. D., Smith, S. M., and Ozeki, H., *J. Gen. Microbiol.*, **30**, 201 (1963).

A Factor required for Growth Initiation of *Pasteurella tularensis*

Previous investigations[1,2] established the essential requirements of *Pasteurella tularensis* (*P. tularensis*) for growth in both complex and chemically defined media. However, the media described sustained luxurious growth of *P. tularensis* when relatively large numbers of cells (about 10^6 to 5×10^6/ml. medium) were used for inoculation. Attempts to reduce the size of the inoculum by supplementing the media with a variety of vitamins, tissue extracts and other preparations noted for their growth-promoting ability, were by and large unsuccessful. The nutritional fastidiousness of *P. tularensis* appeared particularly puzzling in view of the notoriously extreme infectivity of this micro-organism, which is reputed to be capable of multiplying *in vivo* out of a few cells[3]. Furthermore, both highly virulent and completely avirulent strains exhibited the same pattern of growth requirements.

A clue for at least a partial resolution of this paradox was provided by an observation incidental on another line of investigation concerned with the exceptional susceptibility of low tonicity exhibited by *P. tularensis*. It was noted that a brief exposure of *P. tularensis* cells to a hypotonic environment resulted in a disruption of their permeability barrier, as evidenced by the leakage of intracellular constituents and the concomitant drastic decline of viability[4]. The viability of the so injured cells was found to be largely restored in the presence of a concentrate prepared from the suspending medium containing the material which leaked out of the osmotically shocked cell suspension ('leakage material'). Moreover, culture media fortified with the leakage material acquired the ability to support growth of the intact organism from apparently single-cell inocula, thus permitting the performance of viable counts which were otherwise not feasible with the strains tested by us.

The substance responsible for the growth initiating effect was found to be elaborated by the proliferating *P. tularensis* cells and to diffuse out in the course of growth into the surrounding medium. The active factor proved to be of low molecular weight, and owing to its relative stability and resistance to various chemical and physical treatments could be readily concentrated and extensively purified[5]. Work designed to procure sufficient quantities of the purified material for chemical analysis and biochemical studies is now in progress.

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[1] Mager, J., Traub, A., and Grossovicz, N., *Nature*, **174**, 747 (1954).
[2] Traub, A., Mager, J., and Grossovicz, N., *J. Bact.*, **70**, 60 (1954).
[3] Foshay, L., *J. Infect. Dis.*, **51**, 280 (1932).
[4] Mager, J., *Biochim. Biophys. Acta*, **36**, 529 (1959).
[5] Halman, M., and Mager, J. (to be published).

CYTOLOGY

A Yeast Mitogenic Factor Active on Human Peripheral Leucocytes in Culture

We have observed that the addition of zymosan-treated sera to short-term human peripheral leucocyte cultures leads to a slight but significant increase in mitotic frequency. Inactivation of serum complement by zymosan[1] as an important factor in the increase in mitotic frequency was ruled out, and it could be shown that an active mitogenic factor was extracted from zymosan during treatment of the sera. It was found that a more active mitogenic factor could be extracted directly from whole frozen yeast (*Saccharomyces cerevisiae*) and this extract was then used exclusively. The method of extraction consisted of overnight incubation at 37° of equal volumes of saline washed cells and 0·2 N potassium hydroxide, followed by centrifugation at 2,000 r.p.m. for 30 min. The resulting supernatant was neutralized with perchloric acid, and then passed through a 'Sephadex' DEAE column which had been washed with 0·2 M *tris* buffer at *p*H 7·1. Before use, the yeast extract (*YAF*) was sterilized in boiling water for 30 min. Leucocyte suspensions were obtained by dextran sedimentation (2·5 ml. 6 per cent dextran per 10 ml. heparinized blood at 37°). Colchicine (0·02 μg/ml. culture) was added 8–10 h before collecting and chromosome preparations were made according to the method of Hungerford *et al.*[2].

The mitogenic factor in the crude 0·2 N potassium hydroxide extract is non-dialysable, acid labile (1 N hydrochloric acid for 20 h at room temperature), heat resistant, and is not retained by either an anion or a cation exchange column. The crude extract contains protein[3], phosphorus[4], and hexose[5] in approximately equimolecular amounts. It is resistant to digestion by ribonuclease, sensitive to digestion by alkaline phosphatase and lysozyme, and partially sensitive to trypsin.

The frequency of mitoses observed in more than 40 experiments varied from 0·4 to 3·0 per cent; the mitotic index always being significantly higher than that observed in control cultures, and lower than in cultures treated with phytohæmagglutinin (PHA). The peak of mitotic activity occurred between the fifth and sixth day of culture, although it was possible to observe occasional metaphases in cultures incubated for 20 days. Titration experiments with the same preparation of yeast extract always showed a limited region of linear mitotic response to increasing *YAF* concentration, followed at higher *YAF* levels by inhibition of mitoses and occasionally toxicity. It is possible that some of the responses of cultures to higher *YAF* levels might be due to impurities in the crude extract. This question can only be resolved when a purer preparation of the active factor is available.

An additive mitogenic effect was obtained when PHA and *YAF* were added to the same culture. This suggested that *YAF* was acting on a cell population different from that which was sensitive to PHA. To characterize this difference further, a comparative examination of DNA replication was undertaken in *YAF*, PHA, and *YAF* plus PHA treated cultures. Tritiated thymidine (Schwarz 1·5 c./mM, 0·2 μc./ml) was added at the beginning of the cultures and left until collected, at which time slides were prepared for autoradiography (Kodak *NTB* 3 emulsion). After exposure for one week, the slides were developed, stained, and then scored for labelled and unlabelled metaphases.

Table 1. Percentage of Metaphases with Varying Grain Counts in Different Cultures

Cultures	No. of grains per metaphase					Total metaphases counted
	0	1–10	10–20	20–30	30–over	
PHA*	0	25	12	22	41	59
YAF†	67	23	7	2	1	105
YAF+PHA	6	15	25	21	33	33

* PHA, phytohæmagglutinin. † *YAF*, yeast extract.