something peculiar to the kidney alone, for the ability to stimulate supernumerary growth is not confined to renal tissue.

The data on the anatomical distribution of the inducing factor are instructive. From these and previous experiments, some type of a pattern is beginning to emerge. Many of the typical components of extremities (skin, muscle, nerve and resorbing tail) are essentially lacking in inductive ability. Cartilage has moderate ability, but this may be a tissue specific induction. Of the internal organs tested, liver has slight inductive powers, but this capacity is highly developed in kidney, urinary bladder and intestine in the region of the cloaca. On the basis of this distribution as well as the data on the development of inducing capacity in kidney, it would be of interest to further test 2 hypotheses. First, there may be some common developmental association of tissues possessing inductive ability, or second, the fact that this capacity seems to be most concentrated in excretory organs but not altogether lacking in other tissues may indicate a concentration of a substance produced throughout the body. These possibilities are currently being tested[13].

Выводы. Индуктивная способность почки лягушки увеличтвается в течении онтогенеза. У тканей мочевыводящих путей и кишки есть большая индуктивная способность но у других тканей незначительная индуктивная способность.

B. M. CARLSON

Department of Anatomy, University of Michigan, Ann Arbor (Michigan 48104, USA), 29 April 1968.

[13] This work was supported by a Rackham Grant from the University of Michigan.

Ultrastructural Changes in the Yeast *Candida lipolytica* Caused by Penetration of Hydrocarbons into the Cell

A number of papers have been devoted to the first stages of degradation of hydrocarbons by yeasts or by other microorganisms[1] but no experimentally founded explanation exists with regard to the contact of the microbial enzymes with the hydrocarbon.

In our first paper we described the fact that during cultivation of the yeast *Candida lipolytica* on hexadecane or on gas oil the hydrocarbons pass through the cell wall and are concentrated on the cytoplasmic membrane[2].

[1] A. C. VAN DER LINDEN and G. J. E. THIJSSE, Adv. Enzymol. *25*, 469 (1965).

[2] J. LUDVÍK, V. MUNK and M. DOSTÁLEK, Proc. int. Symp. Yeast, Bratislava, in press.

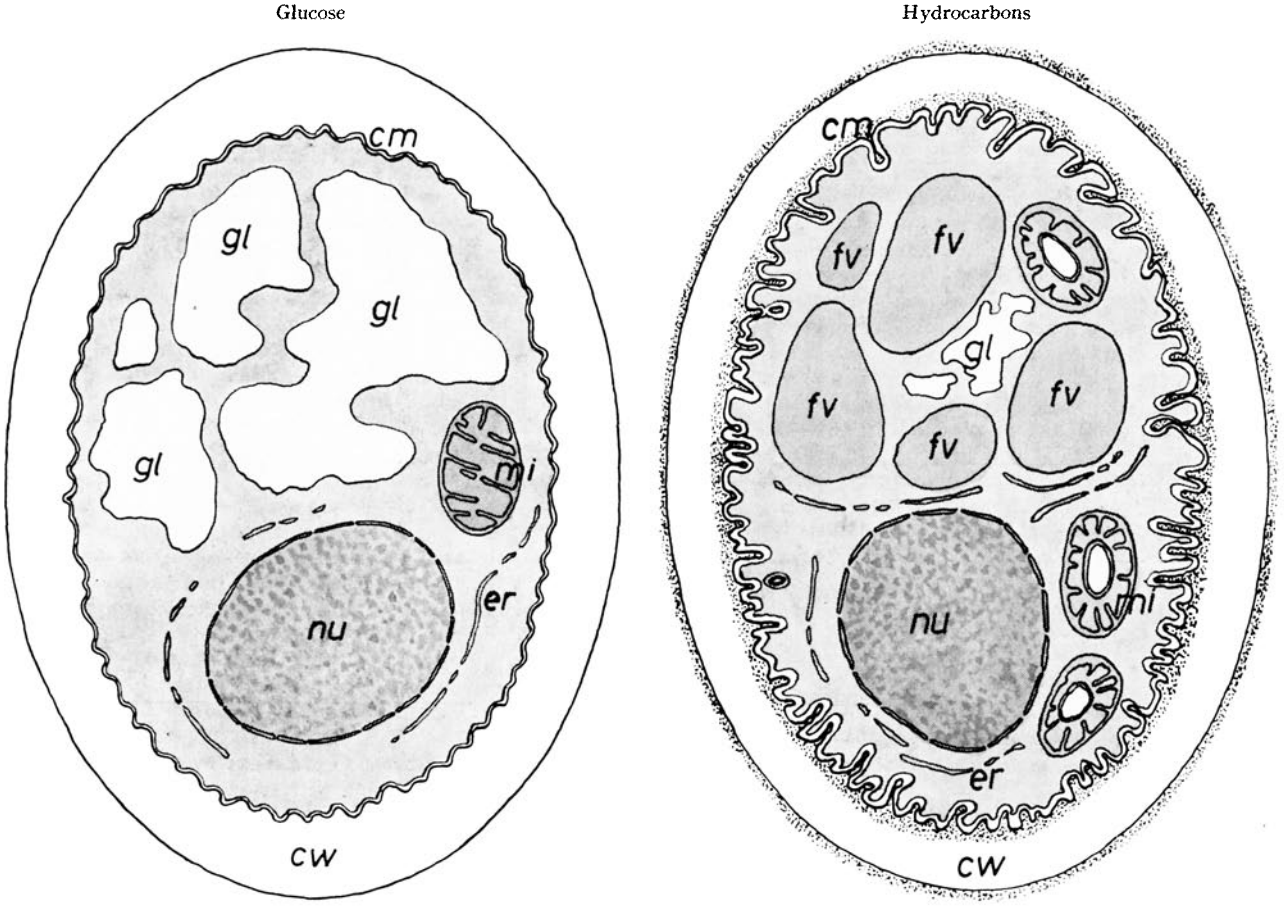

Fig. 1. Schematic picture of an ultrathin section of *Candida lipolytica* grown on glucose or hydrocarbon medium. cm, cytoplasmic membrane; cw, cell wall; er, endoplasmic reticulum; fv, fat vacuoles; gl, glycogen; mi, mitochondria; nu, nucleus.

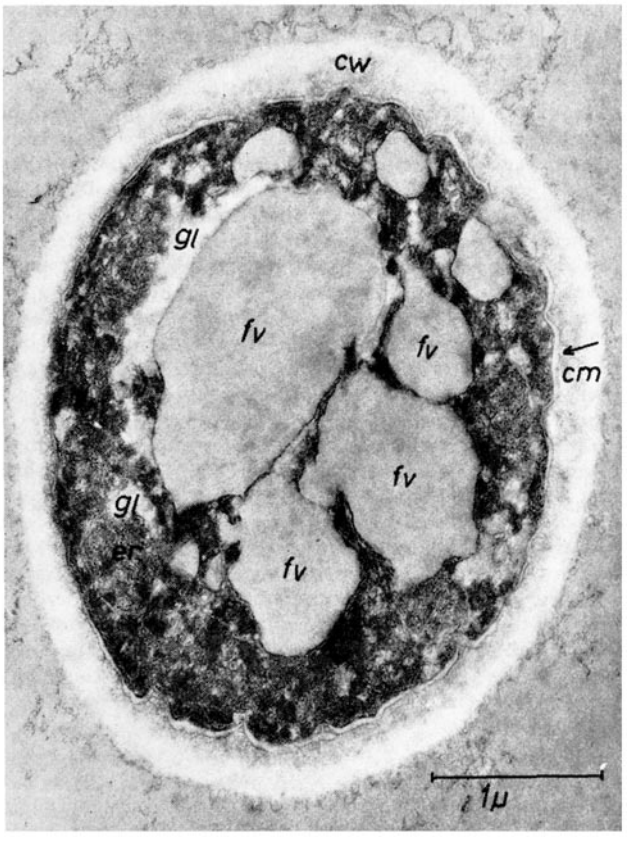

Fig. 2. Ultrathin section of *C. lipolytica* grown on hexadecane medium with addition of vanadium naphthenate.

Later we could show that spheroplasts of *C. lipolytica* cells lose their ability to oxidize hydrocarbons, although they oxidize glucose with the same activity as intact cells[3].

Our present studies dealt with morphological changes of yeast cells grown on a hydrocarbon substrate.

We used a culture of *C. lipolytica* grown in an inorganic medium with an addition of gas oil or hexadecane as the only source of carbon. To examine the penetration of the hydrocarbon by electron microscopy, we added 0.1% vanadium or nickel naphthenate. These compounds are soluble in hydrocarbons and at the concentration used they do not inhibit yeast growth. For control we had the same yeast culture grown on glucose in an inorganic medium. After 24 or 48 h of growth the culture was centrifuged and washed with an isotonic buffer to remove the remaining substrate and fixed by a method modified from LUFT[4]. After 10 min of fixation with cold 2% $KMnO_4$ in acetate-veronal buffer we resuspended the yeast in cold 2% uranyl nitrate or in 1% uranyl acetate. The solution was repeatedly replaced until no change in its colour could be observed. The fixed suspension of yeast cells was washed several times with the acetate-veronal buffer (pH 6.0) containing 1.5% sucrose and embedded in 2% agar. The agar blocks were dehydrated through an alcohol series and embedded in Vestopal.

Ultrathin sections were prepared in a LKB ultramicrotome and contrasted with uranyl acetate and lead citrate according to REYNOLDS[5]. For examination a Tesla BS 413 electron microscope was used.

[3] O. VOLFOVÁ, V. MUNK and M. DOSTÁLEK, Experientia *23*, 1005 (1967).
[4] J. H. LUFT, J. biophys. biochem. Cytol. *2*, 799 (1956).
[5] E. S. REYNOLDS, J. biophys. biochem. Cytol. *17*, 208 (1963).

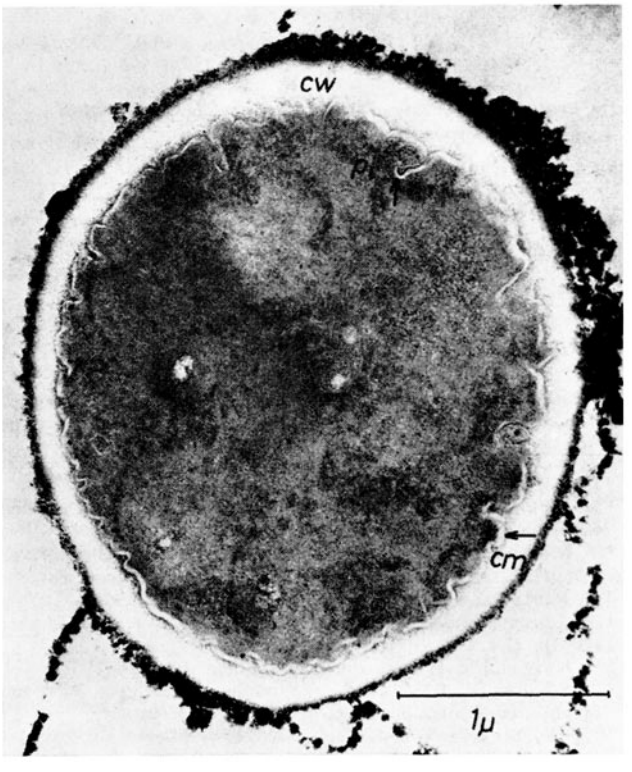

Fig. 3. Ultrathin section of *C. lipolytica* grown on gas oil medium.

Fig. 4. Ultrathin section of *C. lipolytica* grown on hexadecane medium with addition of nickel naphthenate.

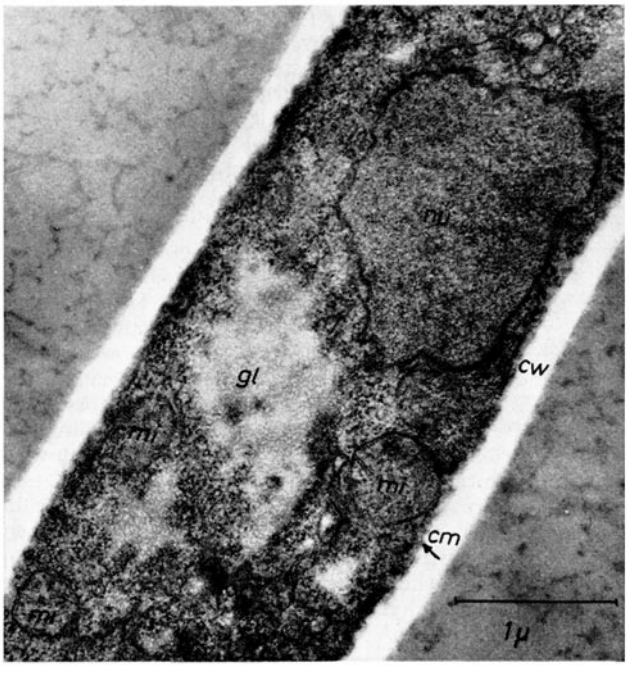

Fig. 5. Ultrathin section of *C. lipolytica* cultivated on glucose medium.

The principal differences in the ultrastructure of yeast cells grown on glucose or on hydrocarbons can be summarized into 8 features, shown schematically in Figure 1. (1) The surface of the yeast cell wall after growth on hydrocarbons is covered with a thin layer of hydrocarbons which penetrate through the cell wall to the cell membrane. The accumulation of hydrocarbons is especially marked in yeast cells grown on gas oil which never disappears completely from the growth medium (Figure 3). The mechanism of penetration could not be analyzed in full detail but it appears that ultrafine pores are involved. Hydrocarbons accumulate on the surface of the cytoplasmic membrane. (2) The cytoplasmic membrane of cells grown on hydrocarbons is always thicker and clearly visible and contains deep invaginations and digital projections which represented an increase of the surface of the cytoplasmic membrane. Pinocytotic vesicles were frequently observed at the ends of deep invaginations, suggesting the possibility of an active translocation of hydrocarbons into the cytoplasm (Figures 2 and 3). (3) Yeast cells grown on hydrocarbons contain more abundant endoplasmic reticulum (Figure 2). (4) Cells grown in media with hydrocarbons contain more fat vacuoles than do cells grown in a glucose-containing medium (Figures 2 and 5). (5) Yeast cells grown on hydrocarbons have more mitochondria which contain frequently an intramitochondrial vacuole (Figure 4). (6) The cell wall of these yeasts is thinner than in cells grown on glucose (Figures 3 and 5). (7) The cytoplasm of cells grown on hydrocarbons is more electron-dense and contains more ribosomes. (8) Cells grown on glucose contain numerous glycogen granules (Figure 5) whereas the hydrocarbon grown cells contain less polysaccharide and more fat vacuoles.

Our observations support the view that hydrocarbons penetrate through the cell wall of *C. lipolytica*, are concentrated at the surface of the cytoplasmic membrane and bring about numerous morphological changes of the cell. The cytoplasmic membrane seems to play an important role in the metabolism of hydrocarbons and in their transport into the cell. The question remains whether the hydrocarbons are oxidized at the cytoplasmic membrane or whether they penetrate by pinocytosis into the cytoplasm to be oxidized there by enzymes associated with the membraneous system of the cytoplasm.

Zusammenfassung. Durch Lösung von 0,1% Vanadium- oder Nickel-Naphthenat in Kohlenwasserstoffen kann deren Durchtritt in die Zelle der Hefe *Candida lipolytica* elektronenmikroskopisch verfolgt werden. Die Kohlenwasserstoffe durchdringen die Zellwand, reichern sich an der Zytoplasmamembran an und verursachen im Zellinnern reiche Veränderungen. Diese beweisen meistenteils die Schlüsselaufgabe der Zytoplasmamembran, den direkten Kontakt der Kohlenwasserstoffe mit den Oxydationsenzymen zu vermitteln.

J. LUDVÍK, V. MUNK and M. DOSTÁLEK

Laboratory of Electron Microscopy and Department of Technical Microbiology, Institute of Microbiology, Czechoslovak Academy of Science, Praha 4 (Czechoslovakia), 12 February 1968.

DISPUTANDUM

Peptide Antibiotic Biosynthesis: A New Approach

It is now well established that the biosynthesis of peptide antibiotics is independent of the ribosomal RNA-requiring processes operating in protein synthesis. A wealth of evidence from studies with tyrocidines[1], gramicidin S[2-4], polymyxins[5], bacitracin[2-6], actinomycins[7-8], and U-22324[9], together with a retraction[10] of earlier contra-indicatory findings for gramicidin S, leaves no doubt that a purely enzymatic process is involved. A recent cell-free

[1] B. MACH, E. REICH and E. L. TATUM, Biochemistry, N.Y. *50*, 175 (1963).

[2] T. S. EIKHOM and S. LALAND, Biochim. biophys. Acta *100*, 451 (1965), and references therein.

[3] M. YUKIOKA, Y. TSUKAMOTO, Y.SAITO, T. TSUJI, S. OTANI and S. OTANI, Biochem. biophys. Res. Commun. *19*, 204 (1965).

[4] T. L. BERG, L. O. FROHOLM and S. G. LALAND, Biochem. J. *96*, 43 (1965).

[5] H. PAULUS and E. GRAY, J. biol. Chem. *239*, 865 (1964).

[6] N. CORNELL and J. E. SNOKE, Biochim. biophys. Acta *91*, 533 (1964).

[7] E. KATZ and H. WEISSBACH, J. biol. Chem. *238*, 666 (1963).

[8] T. YOSHIDA, A. B. MAUGER, B. WITKOP and E. KATZ, Biochem. biophys. Res. Commun. *25*, 66 (1966).

[9] E.g. F. REUSSER, J. biol. Chem. *242*, 243 (1967).

[10] N. V. BHAGAVAN, P. M. RAO, L. W. POLLARD, R. K. RAO, T. WINNICK and J. B. HALL, Biochemistry *5*, 3844 (1966).