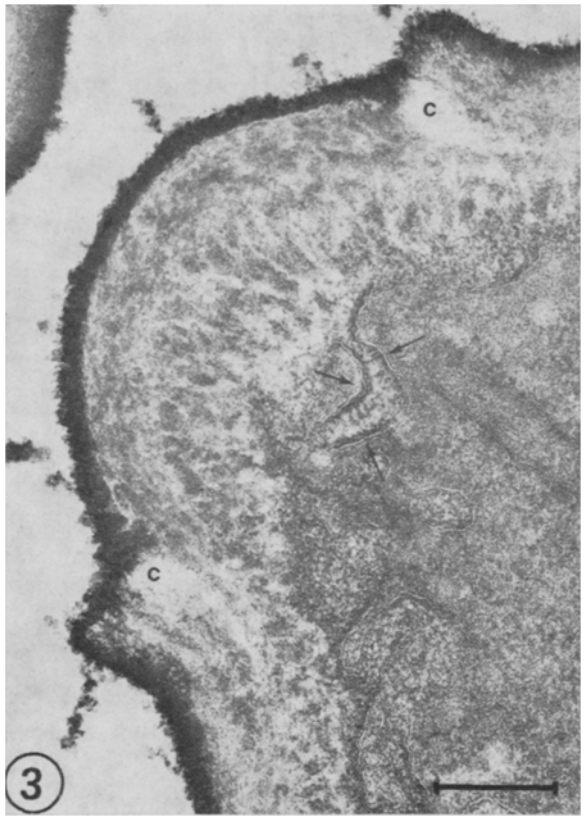

Fig. 3. A 'bud scar' in a cell of *S. cerevisiae* strain 4098. The electron-lucid annular region indicated with c is thought to consist essentially of chitin. The arrows point to the plasmalemma which circumscribes wall material.

When TAO method of fixation is used, the inner broad region of the wall is seen rich in granular and fibrous components arranged in 2 dixstinct layers (the 2nd and the 3rd described here). Furthermore, as the data reported in this and previous papers show[2,4], some significant differences in the wall architecture are found between the 2 yeasts, the main being concerned with the absence in the wall of *S. cerevisiae* of the finely fibrous outermost layer noted instead in *C. albicans* wall[2].

That cell surface could be chemically different in these yeasts was already inferred from studies on protoplasts

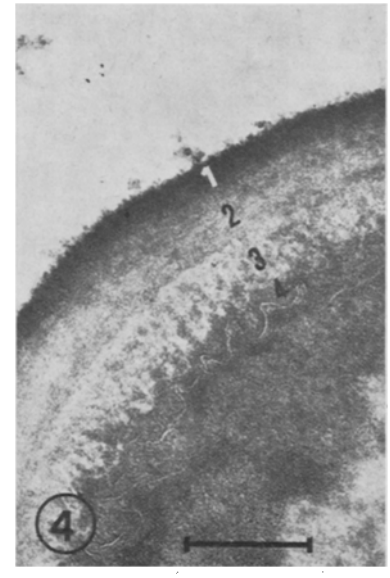

Fig. 4. Four layers of the wall in *S. cerevisiae* strain 9696 are sequentially numbered. For a description see text.

formation[9]. The different degree of preservation of the intracytoplasmic membranes in the two organisms is also indirect evidence for some difference in wall structure in view of the well established importance of the 'penetrability' of the yeast wall toward osmium fixatives in order to achieve a good preservation of internal organelles.

Riassunto. Usando fissativi contenenti TAPO é stata migliorata la visualizzazione dei componenti strutturali della parete cellulare di *Saccharomyces cerevisiae*; questa é stata paragonato con quella osservata precedentemente in *Candida albicans*.

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[10] Acknowledgment. The author thanks Mr. B. Pasquetti and Mr. M. Mari for their excellent technical assistence.

Detection of Encephalomyocarditis Virus Infection in Animal Cells by Gas Liquid Chromatography

The outstanding resolving power and extreme sensitivity of gas chromatography (GC) techniques has been employed for the detection of very small amounts of microbial products[1–4]. Reiner[5,6], Simmonds[7] and others used GC techniques for the identification and characterization of several microorganisms (Clostridia, Salmonellae, etc). Mitruka, Alexander and Carmichael[8,9] and Sinyak et al.[10] could detect by this technique viral infections in cells and animals.

On the assumption that viral infection brings about some specific metabolic changes in the infected cell, one would expect specific and different chromatograms from infected and uninfected cells. Such chromatograms could serve as 'fingerprints' for easy identification of a specific

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virus-host system. In the present work, an attempt has been made to apply GC technique for the rapid and early diagnosis of viral infection of cells, and to ascertain whether changes in cellular metabolism following such an infection are specific to the infecting virus or to the host cell.

Materials and methods. The cells used for the experiments described in the present work were BHK-21 (hamster cell line), L-929 (mouse cell line) and chick embryo fibroblasts (CEF). These were grown in Eagle's medium containing 10% calf serum as monolayers in Petri dishes. For infection, RNA viruses were used: Newcastle disease virus (NDV)-enveloped, and encephalomyocarditis (EMC) – naked. The infectivity of the viruses was determined as plaque forming units (PFU). Growth curves of the virus were constructed in order to determine proper sampling sequence and intervals for GC. Results were plotted as PFU/cell vs. time in hours. Time of cytopathic effects (CPE) was noted in each case. After removal of the medium, the cells were infected with each virus at a multiplicity of infection of about 10 PFU/cell. Following an incubation at 37 °C for 50 min, prewarmed medium was added to the cultures. Supernatant samples from infected and uninfected cultures were taken at hourly intervals up to 12 h after infection, then at 2 h intervals till 24 h, and then at 28, 32 and 48 h. At the same intervals, cell homogenates were prepared from parallel cultures. After centrifugation at 2000 rpm for 15 min, the samples were frozen at −70 °C and stored in deep freezer till analysis.

For chromatography the samples were brought to room temperature, acidified to pH 2 with 1–2 drops of HCl (5 *N*) and 2 ml of HCl. KCl buffer at pH 2, extracted with 3 × 5 ml of absolute ether (Frutarum), the combined extracts dried over anhydrous Na_2SO_4, concentrated with rotary evaporator at room temperature to about 2–3 ml, and a volume of 1–2 µl injected into the gas chromatograph under the conditions specified below.

The chromatograph was Hewlett-Packard Model 700, equipped with flame ionization detector, oven temperature 100 °C, injection temperature 160 °C detector temperature 140 °C; carrier (He) 30 ml/min; column of stainless steel 6 ft × 1/8 inch, filled with 10% carbowax 20 *M* on chromosorb W AW DMCS 60/80. The instrument was operated near its maximum sensitivity.

Results and discussion. Consistent and significant results were obtained only with cells infected with EMC virus. Chromatograms of supernatants from the 3 types of cells infected with that virus, as well as those from homogenates of infected cells, displayed 3 peaks with retention times of 2.2, 6.8 and 7.9 min (Figure 1). The first peak (2.2 min) also appeared in chromatograms of uninfected homogenates. This may constitute an indication for some damage to the cell membrane during infection associated with release of a normal metabolite in to the medium. The time of appearance of this peak coincides indeed with the beginning of CPE (Figure 2). The other two peaks, with retention times of 6.8 and 7.9 min, appear in all tested cells infected with EMC virus, but not in uninfected cultures. We assume therefore that they represent metabolites generated in the host cells as a result of the viral infection, and may serve as a 'fingerprint' identification of this infection. In extracts of NDV-infected cells (supernatants and homogenates) no peaks could be ascribed definitely and specifically to virus in any of the host-virus systems used.

It is to be emphasized that our approach, as well as that of previous authors[8–10], was an empirical one. It was hoped that at least one specific peak would be detected in the chromatograms of infected cells. The presence, or absence of a certain metabolite or its derivative in the injected sample depends to a large extent on the chemical treatment of the sample prior to chromatography (extraction, silylation, etc.). It is conceivable that chemical

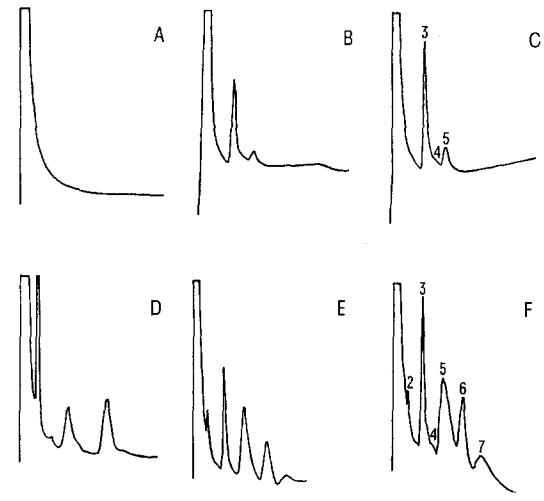

Fig. 1. Gas liquid chromatograms of ether extracts of supernatants of BHK-21 cells infected with EMC virus. Conditions for extraction and chromatography, see text. Numbers indicate chromatographic peaks. A) Uninfected controls, 6 h after infection; B–F) Cultures infected with EMC virus at times 0, 2, 3, 5 and 6 h post-infection respectively.

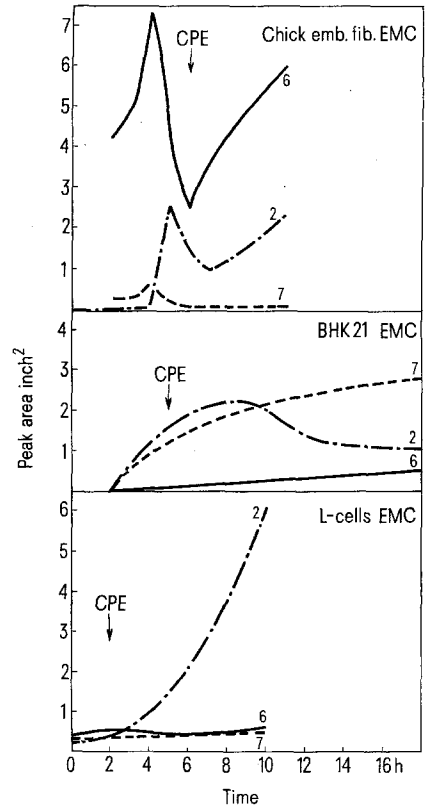

Fig. 2. Changes in concentration of metabolites detected by gas chromatography as peaks 2, 6 and 7 during the course of infection of cells by EMC virus. The peak area was calculated as length × breadth at half height. Arrow indicates the time of appearance of cytopathic effects in culture.

treatment different from the one employed by us (ether extraction) would reveal specific peaks in cells infected by viruses other than EMC. So far we have no information as to the identity, or chemical character of the metabolite represented by the peaks discussed above, apart from their ether solubility.

Résumé. Des extraits, par l'éther, de cellules BHK 21,L929 et de fibroblastes d'embryon de poulet, infectés par le virus EMC contiennent des métabolites spécifiques qui peuvent être décelés par chromatographie en vapeur sous forme de pics ayant des temps de retention de 6,8 et 7,9 min.

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COGITATIONES

Information and Evolution

In an article on the use of information in the description of biological systems and of their evolution, Mayo[1] raises several points concerning our treatment of this subject[2,3]. We described a terrestrial system whose input consists of a flow of solar information i_s; a fraction i_b enters the biosphere, while the remainder i_{nb} does not. If the biospheric and nonbiospheric information lifetimes are τ_b and τ_{nb}, the corresponding information contents will be, under 'quasi steady-state', $I_b \approx i_b\ \tau_b$ and $I_{nb} \approx i_{nb}\ \tau_{nb}$. The essence of this approach is the conclusion that the biosphere will evolve toward a higher I_b, both through changes that increase τ_b, and through the increase of the fraction i_b of the information flow. Since $\tau_{nb} \ll \tau_b$, the total information content and the average information lifetime of the overall system will also be increasing.

The conclusion that a system of information content I which receives an input i flowing with a dwell time $\tau \approx 1/i$ will evolve toward longer τ's and larger I's is based on the following argument. Irreversibilities are the only way in which information is dissipated; if irreversibilities are reduced, information lifetime is extended. Changes in biospheric components that are 'good' and lead to better efficiency and improved survival, always reduce irreversibilities; spread of such 'improvements' leads toward longer τ's and larger I's. The statement that 'improved' forms will survive and spread is indeed a tautology, as pointed out by Mayo; the essence of the argument, however, lies in the fact that such 'improvements' always reduce irreversibilities. This is true whether the 'improvement' is the reduction of friction in an engine, a change in the foot skeleton of an animal that allows movement at less effort, or the development of a smoother societal organization that reduces tensions, conflict, or loss of life and property.

In the case, for instance, of a sketelon alteration that reduces the effort necessary for a particular movement, this activity will require the transformation of a smaller amount of energy from a high-grade chemical form (contained, e.g., in energy-rich ATP molecules) into a low-grade form (ultimately heat, after taking such forms as the instantaneous potential or kinetic energy of the limb of the animal). Thus, the skeleton alteration reduces the rate at which the irreversible transformation of high-grade energy into heat occurs.

The manner in which the replacement of a species I_1 by an improved species I_2 leads to a net increase in τ and I has been discussed elsewhere[4]. Assume that the 2 species compete for a fixed information input i_{12}, and that the improvement is the capability of species 2 to maintain its body temperature at a lower cost in high-grade (i.e., information-rich) energy; this may result from better fur coverage or, perhaps, from the use of clothing. Since the transformation of chemical energy into heat is an irreversibility, its reduction results in a lifetime τ_2 for the information that enters species 2 that is longer than τ_1. Before the appearance of species 2, the information content of this niche was $I_1 \approx i_{12}\ \tau_1$. After species 2 will replace species 1, the new information content will be $I_2 \approx i_{12}\ \tau_2 > I_1$. The inequality $I_2 > I_1$ implies that for the same input i_{12} the new species will be able to maintain a higher population than the old one.

Mayo states that one can hardly argue that the biospheric information increases when the last eggs of a species are eaten by predators. The information increase that we are discussing occurs in essence while the species declines from its peak population (e.g., several million) to near extinction (e.g., several thousand), and not when the last member dies; and it is represented by the information content of the new species (one or several) that replaced the old species in its niche. We are not considering, therefore, the type of informational transaction that occured in 1861 when the eggs of the last dodos were inadvertently eaten by pigs or dogs; the corresponding change in biospheric information content would have been practically identical if chicken eggs had been eaten instead. The biospheric information change due to the disappearance of the dodos is rather the difference between the information content of their population when they were stably entrenched in their ecological niche (i.e., when they were equal or better than any existing competition), and the information content of the species that replaced them in that particular niche, feeding on and being preyed upon by about the same species. If, as usually happens, the disappearance of the dodos was part of a rearrangement of niches within a wider ecological domain, the entire domain that was affected must be considered in assessing the change in biospheric content.

With respect to the definition of information content, Mayo states that such a quantity cannot be measured, and is in doubt as to how it is defined. Here, information is meant in its thermodynamic sense. In this context, the information in the DNA of an organism is a small fraction of the total content. In thermodynamics, one defines the information of a distribution of particles with respect to their most probable distribution[5]; the information content of a structured organism is defined by considering the distribution of its components versus their

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