After a short exposure period, compartment cells begin to show a blue fluorescence with a maximum at 470–480 nm. Again the fluorescence is associated with inner membranes, but does not fade significantly with time. Under excitation the entire orange pigment cell shows a stable fluorescence at 520±5 nm. No fine structure is observed within the cell at 1200-fold magnification.

The fluorescent properties of various blood cell types can be used to explore several problems: cell development, phylogenic positions of various species and probing the chemical processes occurring within the cell and at the vacuolar membrane. It has been suggested from an examination of the cell types in the blood of *P. mamillata,* a species closely related to *A. ceratodes,* that the vacuolized compartment cell evolves into the vanadocyte, and the orange pigment cell is unrelated to the vanadocyte[10]. This study suggests that the 3 types of cells in *A. ceratodes* are related. Vanadocytes show an interconversion of the 590 and 525 nm bands upon irradiation, suggesting a chemical interrelation of the compounds these bands represent. The presence of the 525–590 nm system suggests an evolutionary relationship between the vanadocyte and the orange pigment cells which have a fluorescent band at 520 nm. Note that the true maximum of the vanadocyte 525 nm band falls at a shorter wavelength; approximately 520 nm. In some vanadocytes a 470-480 nm band occurs suggesting a tenuous relationship with the compartment cell which has such a band after short irradiation.

With respect to phylogenic position, some agreement is observed in the fluorescent properties of the comparment cells observed in the bloods of *A. ceratodes* and *P. mamillata*[13], however, not between vanadocytes of the 2 species. This observation is surprising as both *P. mammillata* and *A. ceratodes* belong to the same family (Ascidiidae), and have nearly identical blood cell morphologies. The blood of another member of the family Ascidiidae, *A. nigra,* is reported to possess no visible fluorescence upon UV-irradiation[9].

It has been suggested that the difference in behavior noted between vanadocytes of *Ciona intestinalis* (fluorescent) and *P. mamillata* (not fluorescent) is due to the quenching effect of highly-concentrated vanadium(III) in the latter relative to the former[13]. This chemical explanation is not supported by the results of this study, since *A. ceratodes* vanadocytes have approximately the same level of vanadium[3] as those of *P. mammilata* and fluoresce.

Some aspects of the compounds responsible for the fluorescent behavior can also be discussed. The orange pigment cells have been suggested as depositories for purines and related compounds[14], and purines, pteridines and their derivatives have been reported in the ascidians *Microcosmus polymorphus*[15] and *Ascidiella aspersa*[16]. The UV-spectral and fluorescent properties of bloodcell extracts of *A. ceratodes* are consistent with this view.

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2 W.R. Biggs and J.H. Swinehart, in: Metal Ions in Biological Systems, p. 142 Ed. H. Sigel. M. Dekker, New York and references cited therein 1976.
3 J.H. Swinehart, W.R. Biggs, D.J. Halko and N.S. Schroeder, Biol. Bull. *146,* 302 (1974).
4 W.C. George, J. Morph. Physiol. *49,* 385 (1930).
5 W.C. George, Q.J. Microsc. Sci. *81,* 391 (1939).
6 J.F. Fulton, Acta zool., Stockh. *1,* 391 (1920).
7 W. Andrew, Q.J. Microsc. Sci. *102,* 89 (1961).
8 J.A. Vallee, Bull. Sth. Calif. Acad. Sci. *66,* 23 (1967).
9 K. Kustin, D.S. Levine, G.C. McLeod and W.A. Curby, Biol. Bull. *150,* 426 (1976).
10 R. Endean, Q. J. Microsc. Soc. *101,* 177 (1960).
11 D.A. Wegg, J. exp. Biol. *16,* 499 (1939).
12 R.M.K. Carlson, Proc. nat. Acad. Sci., USA *72,* 2217 (1975).
13 DeVincentii and W. Rüdiger, Experienta *23,* 245 (1967).
14 I. Goodbody, in: Advances in Marine Biology, vol. 12, p. 1. Ed. F.S. Russel and M. Yonge, 1974.
15 P. Karrer, C. Manunta and R. Schwyzer, Helv. chim. Acta *31,* 1214 (1948).
16 A. Monzikoff, Archs. Zool. exp. gén. *114,* 603 (1973).

Computer-aided biochemical system analysis in open systems with environment simulation

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Summary. A computer-aided arrangement is described which allows kinetic and regulative studies with enzymes, organelles and cells in an open system. This is demonstrated with some simple examples.

Organisms and their subunits are open systems. Their steady state properties can only be recognized by measurement of input and output. The character of a flowing equilibrium is visualized by applying external perturbation and monitoring the subsequent return to the equilibrium[1,2]. In this way the typical time-function of a regulative system is realized. Due to the complexity of metabolic systems the application of computers is indispensable. Simulation of environmental factors is performed by programming the computer to a number of time functions governing the addition of chemical substances, which interfere with the system on various levels of biochemical regulation.

Methods. To begin with a simple case, we take as an example an enzyme or several enzymes intercorrelated by reaction (figure 1). The enzymatic solution is circulated through the reaction chamber (RC) of a measuring unit (e.g. the cuvette of a photometer) and a microfilter (e.g. hollow fibre type laid in a vacuum chamber VC) by means of a peristaltic pump (P_2). Particles of low molecular weight (including the reaction products) are filtered through the microfilter by means of a vacuum (VAC) suction pump. The loss in volume is restored with substrate by another peristaltic pump (P_1). Volume regulation is performed by a level indicator (LI) and a magnetic valve (MV) which con-

nects the VC to the suction pump. Molecular modulators (activators, inhibitors) are supplied by dosing pumps (DP_1, DP_2) to the RC.

A magnetic stirrer (MS) in the RC provides fast mixing of the solution. All units of the apparatus are controlled by a computer (DIETZ 621/8) and an interface. If the biochemical systems involve more complicated structures (organelles or cells), they are contained in a cell cage (CC) formed by ultrafilter membranes, which is located in the RC in such a way that the measuring beam for optical methods is not perturbed. Measurement of the products is performed either in the same manner as mentioned above by enzymatic reactions or by applying other methods of continuous registration.

Since the supply of substrate and molecular modulators is processed by the computer, a hybrid connexion of a mathematical model with the experimental system is possible.

Also dynamic correlations between perturbation of the system and its measured reaction as a function of time can be examined on various levels of steady state. Thus mutual influences of environmental factors and biochemical systems can be studied.

Results and discussion. The abilities of the experimental setup will be demonstrated using the reaction

$$\text{Pyruvat} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{L(+) LDH}} \text{L(+) lactate} + \text{NAD}^+$$

$$(\text{K} = 2.76^{-12}\,\text{M at pH} = 7.0 \text{ at } 25\,^\circ\text{C}).$$

The absorption at $= 340$ nm of NADH can be used to measure the pyruvate concentration. The time dependence of the concentration is an indicator for enzymatic processing of the substrate[3].

Figure 2, a shows the change in the equilibrium concentration when the pump rate of P_1 is changed. Stable equilibria are possible as well as transient.

The computer-aided dosing technique allows application of periodic, aperiodic, or stochastic test-functions as required

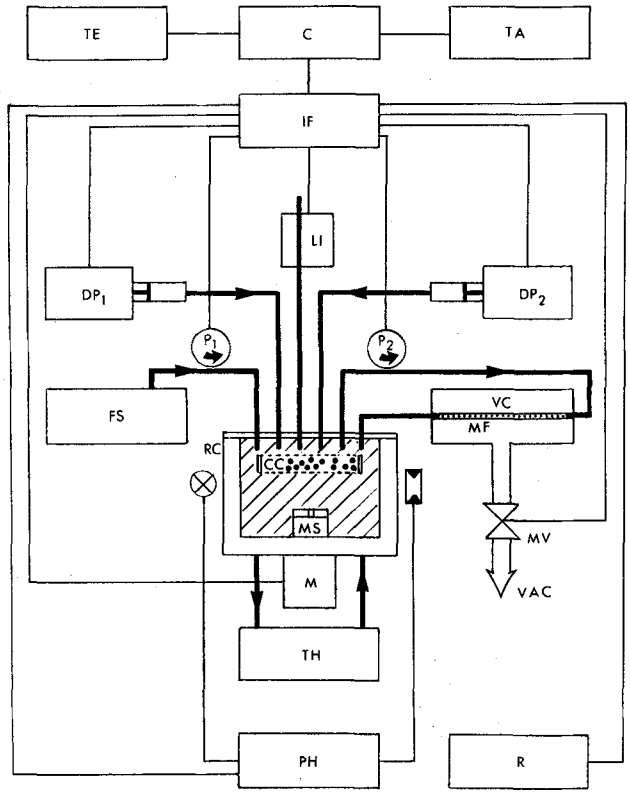

Fig. 1. Blockdiagram of the experimental setup: TE, terminal; C, computer; TA, tape; IF, interface; LI, level indicator; DP_1,DP_2, dosing pumps; P_1,P_2, peristaltic pumps; FS, feeding substrate; VC, vacuum chamber; MF, microfilter (Hollow filter type); RC, reaction chamber; CC, cell cage with cell suspension; MS, magnetic stirrer; MV, magnetic valve; M, stirrer motor; VAC, vacuum by suction pump; TH, thermostat; PH, photometer; R, registration.

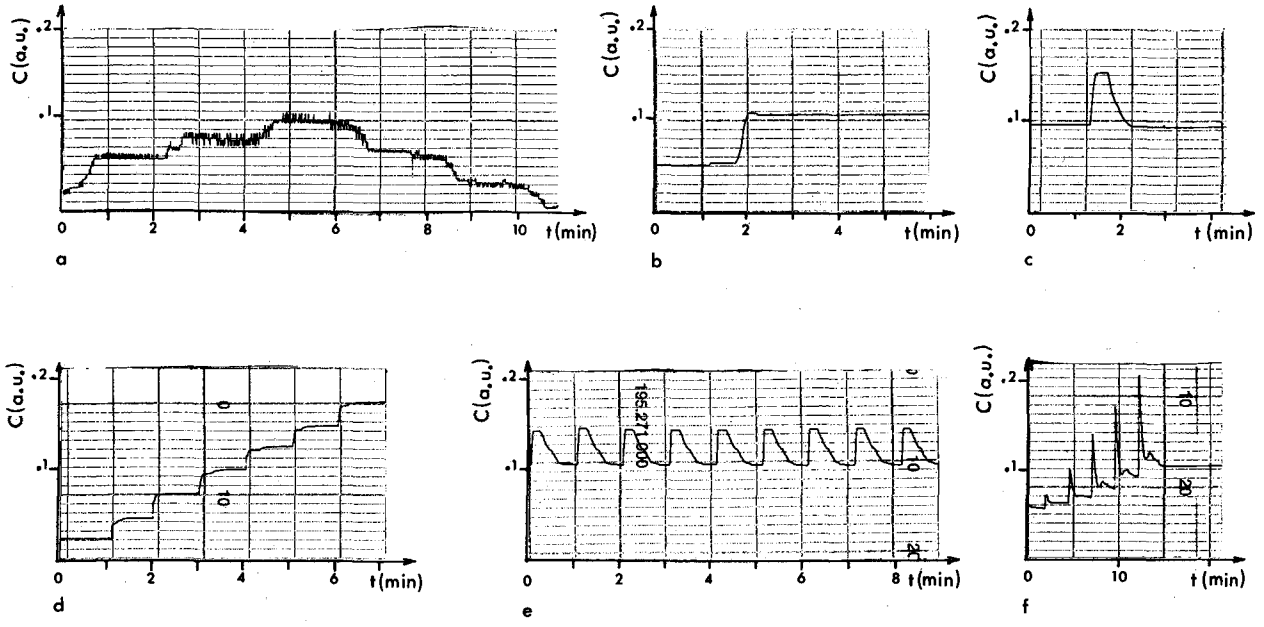

Fig. 2. *a* Change in the equilibrium concentration of the reaction product (C in arbitrary units) versus time, when the pump rate of P_1 is changed. *b* Product concentration C versus time following a rectangular ascent (b) and a pulse (c) in substrate supply. *e* Typical differences between closed and open systems in product concentration C. After a pulsed substrate supply in the closed system (d) the product concentration increases in accumulating manner. The same pulse sequence applied to an open system (e) shows that product concentration raises after each pulse, but afterwards it approaches the equilibrium value again. *f* Linearly increasing substrate pulses in an open system show the linearity of the arrangement.

by the regulation theory[4]. Figure 2, b shows the reaction of the system following a rectangular ascent and figure 2, c the reaction following a pulse in substrate supply.
Figure 2, d and e show typical differences between closed and open systems. After a pulsed substrate supply in the closed system (figure 2, d) the extinction decreases accordingly, which means a rise in product concentration in an accumulative manner. Figure 2, e shows the same pulse sequence in an open system.
After each pulse the concentration of product rises, but afterwards it approaches the equilibrium value again. Figure 2, f shows the linearity of the arrangement by applying linearly increasing quantities of substrate.
The results show that the arrangement renders accurately various flowing equilibria in a short time interval. This enables one to detect the optimal and limiting states of equilibria by simulation. As distinguished from a closed system, self regulation of an open system by feedback of recognized data is provided. Especially in more complex systems like allosteric enzymes or organelles and cells the validity of this procedure is evident[2]. The figures also show that the accuracy and reproducibility of the arrangement are sufficient. Results concerning further experiments with allosteric enzymes and cells will be reported subsequently.

1 B. Chance, R.W. Estabrook and A. Gosch, Proc. nat. Acad. Sci. *51*, 1244 (1964).
2 B. Hess, Nova Acta Leopoldina *33*, 195 (1968).
3 H.U. Bergmeyer, Methoden der enzymatischen Analyse, vol. 1. Verlag Chemie, Weinheim 1967.
4 W. Oppelt, Handbuch technischer Vorgänge. Verlag Chemie, Weinheim 1960.

Ciliary locomotion in squid hatching

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Summary. Squid hatchlings are shown to use their transitory set of integumental cilia as a locomotory organ when they cross the gelatinous envelopes of the eggs.

All cephalopod hatchlings use a special gland to open the egg case. This 'organ of Hoyle' is known to produce an enzyme, and to stock it until it is released to dissolve the egg case locally[2]. Rather little is known of the auxiliary equipment that hatchlings use in working themselves out through the hatch opening.

Clearly the structure and functioning of the entire hatching equipment must be correlated with the nature of the egg case, which is a variable feature among cephalopod orders or sub-orders. Thus, in the incirrate octopods, the embryo is surrounded only by the so-called chorion and during hatching is assisted (if it is not using its arms) by a set of integumental hard structures; these are the 'Kölliker tufts' in their closed state[3].

Among the decapods, the sepioids enclose individual eggs in gelatinous envelopes, which they wrap spirally around the chorion[4]. As the chorion increases considerably in size during embryonic development, the surrounding gelatinous layers are stretched and they become thinner. Thus the hatchling has to cross only 1 compound envelope. The teuthoids or squids, on the other hand, enclose several to very many eggs in one and the same envelope, which they wind on in the fashion of a spiral staircase[5]. In *Loligo vulgaris* and some other loliginid species, the chorion swells so strongly, and the outer envelopes become so thin that at the end of embryonic development the eggs show up as densely packed vesicles facing the open water with their bulging outer wall. Nevertheless, the greater part of the chorion is surrounded by the gelatinous layers separating one egg from another, so that the hatching animal may not immediately emerge into the open water. In other loliginids, such as *Alloteuthis,* the gelatinous envelope is still very thick all around the eggs at the end of embryonic development. This occurs even in *Loligo* egg masses infested by capitellid polychaetes[6].

The question then is: how do the hatchlings cross gelatinous masses when funnel jetting does not have a locomotory effect? The answer is that the kinocilia of the animal's integument, which beat headwards, give the animal backward locomotion in the jelly that is liquified by the enzyme emanating from the hatching gland. The amount of enzyme available is sufficient to make long ducts in the envelopes. To test this, animals were taken from their chorion when they were ready to hatch; thus the hatching gland remained intact. In a pipette with a tip sufficiently wide for a smooth passage, each animal was taken up and then injected, tail first, into the envelopes of an egg mass. Regularly the following events were then observed.
After a period of immobility lasting between a few sec and 1 min or more, the animal stretches out its mantle tip. This movement is known to initiate normal hatching[7]. It apparently causes the rupture of the apex of the gland cells, which liberates the enzyme. Immediately after the first 1 or 2 stretching movements, the animal begins to glide along, tail first, and steadily advances until it emerges at the surface of the egg mass (figure 1). There it immediately begins to swim by jetting. Inside the gelatinous envelopes, sporadic mantle contractions have no locomotory effect at all.
The cilia providing locomotion have been known before (apparently non-motile cilia are also present in the integument[8]). Ranzi[9] has described the currents of the perivitellinic fluid that are generated by the kinocilia of the embryo and its outer yolk sac. They all beat in anterior direction. The length and external structure of these cilia varies over different areas of the surface, and their arrangement differs particularly between the mantle and the head[10]. The dorsal and the ventral surfaces of the mantle of *Loligo* hatchlings show longitudinal strips of densely set cilia continuous over several cells (figures 2 and 3). Live observations show that in moribund hatchlings these cilia continue to beat steadily when the longer cilia of the separate ciliary cells on the head have already ceased to beat.
Although the mantle cilia, and particularly those arranged in the longitudinal strips, would appear to be the most effective in locomotion, the cilia of the head also have a part in it, as can be seen when the mantle of a hatching animal has broken through the surface of the egg mass (figure 1, e, f). Possibly these longer cilia are more effective in a fluid medium, whereas the shorter cilia of the mantle may have their greatest propulsive effect in a more viscous