PHASE PLANE ANALYSIS OF PERIODIC 'ISOZYME PATTERN' CHANGES IN CULTURED CELLS

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Summary: Previously reported studies, involving kinetic analytical methods, have indicated that cold shock can induce periodic variations in the lactic dehydrogenase and aldolase isozyme patterns of cultured cells. The possibility of coupling between the two isozyme systems is now examined principally by means of phase plane plots of the data.

In a previous communication, concerned primarily with the possible significance of cellular periodicities to the problems of differentiation, oncogenesis and ageing, supporting experimental evidence was given which demonstrated the existence of oscillatory variations in cells subjected to cold shock. Thus using kinetic methods of analysis, results were obtained which have been provisionally taken to indicate that rhythmic changes can be induced in both the lactic dehydrogenase (LDH) and aldolase isozyme patterns. Furthermore, using different cell lines, it was shown that phasing differences could occur between these two oscillations. The purpose of this article is to consider the phase plane analyses of the data obtained in preliminary experiments on monolayer cultures of BHK-C13 and BHK-PYY hamster kidney fibroblasts which have been subjected to cold shock.

The culture conditions were as described previously[1], any exceptions being noted in the captions. Basically, a number of miniature cultures were established and simult-

aneously subjected to short term cold shocks. At varying times before, during and after this period, cells in individual cultures were extracted[1] and assayed for activity toward four substrates, pyruvate (P), α-ketobutyrate (B), fructose-1:6-diphosphate (FDP) and fructose-1-phosphate (FMP). The B/P, FDP/FMP activity ratios have been provisionally taken as a measure of the LDH and aldolase isozyme patterns, respectively[2,3]. It is possible that the variations we observe are partly or totally due to activation or inhibition rather than synthesis or degradation of particular isozymes. At this stage we therefore prefer to consider that the activity ratios indicate the effective rather than the actual isozyme patterns of the cell sample.

Since LDH and aldolase are enzymes associated with the glycolytic pathway (in which oscillatory variations in substrate and co-enzyme levels have also been observed[4-7]), one might expect the isozyme periodicities to be coupled in some way. However, the 'in-phase' and 'anti-phase' states previously described[1] indicate that the B/P and FDP/FMP ratio variations are not rigidly associated in the time domain. This is confirmed by additional data in which no definite temporal relationship was found to exist (e.g. expt.608 Fig.1b). This argues against coupling. On the other hand, the phase plane presentation of the data suggests that the ratio values may, in fact, be related in some way. Thus plots of the B/P ratio against the corresponding FDP/FMP value for the same sample, gives a simple curve of -ve or +ve slope when the oscillations are, respectively, anti-phase (Fig. 1a) or in-phase (cf. Fig.2a). This is to be expected for any pair of oscillations, coupled or not, which are of the same wave form and frequency, constant amplitude and in these phase states. However, in two experiments on PYY cells, where sufficient data is available, the results suggest that two such phase trajectories existed, the systems 'jumping' from one to the other at

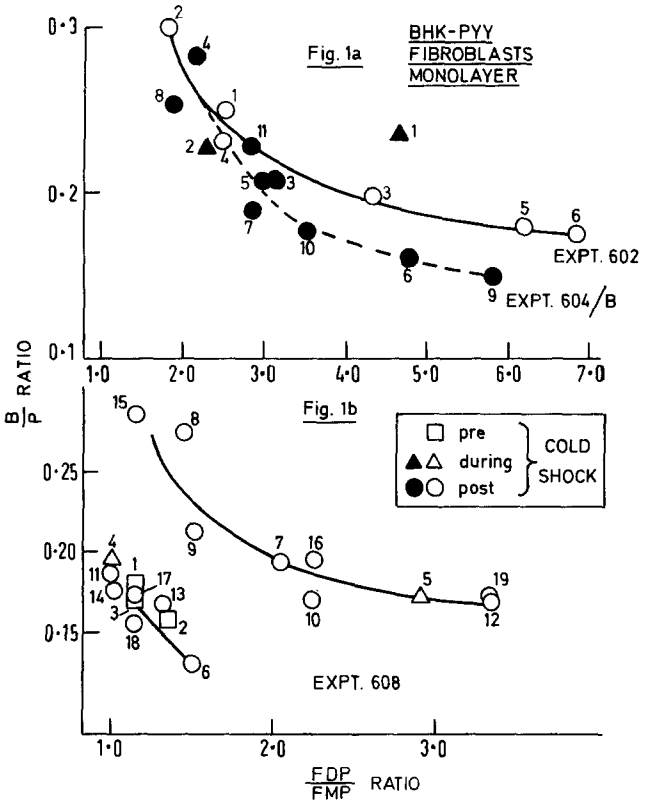

Fig.1. Phase plane plots for oscillations in PYY cells

Cells were grown in bottles 2.5cm dia. x 5cm. high. At a suitable time the cultures were subjected to a rapid decrease in temperature. At varying times before, during or after this period, individual cultures were washed and the cells extracted as described in ref.1. The enzymic activities were determined by the initial rate of oxidation of NADH, using the method of Yasin and Bergel (2) for LDH, with pyruvate (P) and α-ketobutyrate (B) as substrates. For aldolase, the assay conditions were: (final concns.) 50mM tris/HCl, pH 7.4; 0.1mM NADH (Boehringer); 1/1500 dilutions of a mixture of triose isomerase/α-glycerophosphate dehydrogenase (10 mg/ml susp.in ammonium sulphate-Sigma) and either 5mM fructose-1:6-diphosphate (FDP-Sigma) or 20mM fructose-1-phosphate (FMP-Sigma)-sodium salts - as substrate. The corresponding values of the activity ratios B/P and FDP/FMP are plotted against each other. The temporal order of the samples is denoted by the figures adjacent to the points. The spacing is, in general, hourly except during the shock periods. The time of sub-culturing is taken as the reference zero time.

Fig.1a. expt. 602: inoculum: 120,000 cells/2ml medium. cold shock: 1 hr. at 4 °C, 7 hrs post sub-culturing. Initial point at 10 hrs.; period covered 13 hrs. Expt. 604: 120,000 cells/2ml: cold shock: 1 hr at 4 °C, 17 hrs post subculture. Initial point 17.25 hrs. period covered 15 hrs. Fig.1b. 150,000 cells/2ml. cold shock: 2 hr. at 20°C, 7 hr post subcultures. Initial point 4 hrs. period covered 18 hr.

different times (Fig.1b). This interpretation could prove to be incorrect in the light of more frequent sampling but it may be noted that 'jump' phenomena are not unknown in other fields[8-13].

Different phase plane characteristics have been observed in other experiments involving C13 cells. In one case, variations were observed prior to the cold shock such that the initial values give a straight line phase plot of +ve slope; this relationship was disturbed by the temperature drop but in a temporary way, the original trajectory seemingly being restored (Fig.2a). In contrast, the second set

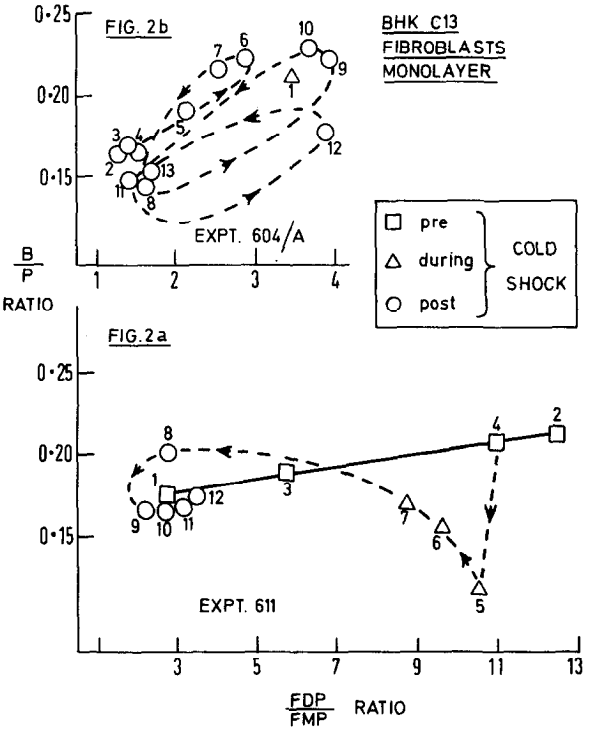

Fig.2. Phase plane plots for oscillations in C13 cells

Conditions as described in caption to Fig. 1
Fig.2a. expt.611: 100,000 cells/2ml. cold shock: 1 hr at 4 °C 23 hrs post-sub-culturing. Initial point at 20 hrs. period covered 8 hrs.
Fig. 2b. expt. 604/A: details as for expt. 604B, Fig.1, The pyruvate activity variations in all these experiments were generally similar to those described in ref.14.

of results are consistent with the relationship being progressively modified, with no evidence of an approach to a stable trajectory during the period of observation. This latter curve is reminiscent of phase plane plots of Volterra equations containing hereditary factors[8], the kind of situation to be expected in cellular systems, under some conditions at least (cf. 16).

The possible relationship between the ratio values for two experiments was also examined by plotting their ratio against time (Fig.3). The graphs so obtained suggest that the systems were in an 'expanding' state, apparently initiated at the start of the shock period in the C13 cells but earlier in the PYY culture. It is probably significant that marked periodicity was observed in a control culture of PYY cells, but the extrapolated 'zero' time for the PYY experiment does

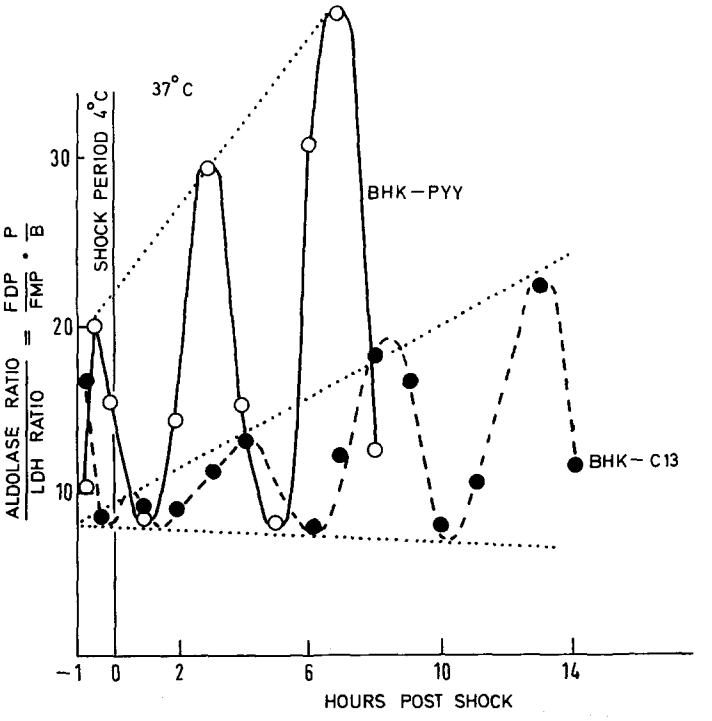

Fig.3. Plot of the ratio of the activity ratios against time
The experimental details are those given in Figs. 1,2 for experiments 604/A, 604/B.

not appear to correspond to the time of sub-culturing.

The question arises as to whether curves of the above forms are likely where the variations in the parameters are completely independent or if they are to be expected only when the isozyme patterns are inter-related in some manner, albeit indirect. In one experiment involving a double shock, the distribution of points in the phase plane was apparently random. It thus seems likely that the two systems are coupled in some circumstances but that they can be dissociated.

The interpretations reported here can only be considered as preliminary attempts to gain an insight into the dynamic behaviour of cells existing under what is, essentially, normal conditions. At this stage it is still uncertain if the periodicities exist in the undisturbed, individual cells; as already mentioned, we have detected activity and activity ratio variations in cell cultures (including human embryonic lung fibroblasts at second passage) which have not been deliberately shocked. In many instances these have been of high frequency and high amplitude[14]. It can be argued that in these cases, the sub-culturing procedure constituted an initiating disturbance (also responsible for rhythmic mitosis etc.?) and, where applied, the cold shock caused re-phasing or re-initiation.

The results presented support the concept outlined earlier[1]. Thus the data of Fig.2a can be considered to depict the 'inertia' presented by interacting cellular periodicities toward temporal re-organisation, while that given in Fig. 2b is consistent with an unstable response likely to have produced cell death or temporal re-organisation (i.e. transformation). Unfortunately no other information is available by which to judge the biological outcome of the experiments but it seems clear that further, more detailed studies along these lines, could provide invaluable information regarding the nature of dynamic cellular processes.

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