# High-Energy Laser Stimulation of Creatine Kinase Activity in Tissue Cultures

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The creatine kinase (CK) activity of a primary culture of chick myoblasts has long been known as a sensitive indicator of the effect of different environmental factors. However, in detailed studies of the behavior of this model system a remarkable uniformity has been revealed in the sequelae of treatment of competent cells with diverse chemical agents: an increase in the specific activity of CK has been noted along with a reliable and marked decrease of the content of water-extractable protein in the culture [7], phenomena which require a rational explanation. In a previous study [4], in which similar effects of creatine phosphate were assessed and profound qualitative changes were shown to attend the process, we suggested that the shifts observed exhibit a nonspecificity similar to the biochemical shock phenomena [1]. Since the validity of such a statement may be verified according to the nature of the response to an equally nonspecific but deliberately stimulating manipulation, we decided to study the corresponding effect of the light of copper vapor laser (CL); its biotropic effect has previously been well documented in clinical investigations and in *in vitro* experiments [2,3]. For comparison we followed up the response of the neuroblast culture in parallel.

## MATERIALS AND METHODS

Tissues of the brain and hind limbs of 11-day chick embryos were used as the initial material. A monolayer myoblast culture was obtained as described previously [4]. A preliminarily trypsinized nerve tissue culture was grown on a nutrient medium containing 60% medium 199, 30% bovine serum, 9.7% fetal serum, and 0.3% glucose. The myoblast culture was irradiated on day 3, and the neuroblast culture on day 8. The CL radiation was delivered through the bottom of Petri dishes with a scattered beam (0.51+0.58 μ) of an MD-102 apparatus with a mean radiation power of 3 W (the power density at the object irradiated being 200 mW/cm$^2$). The total doses used per entire dish were 3 and 15 J. After irradiation, the culture was exposed for 2 h to normal conditions of growth and was then removed from the surface of the dishes with EDTA solution. The cell suspension was homogenized for 30 sec and centrifuged for 20 min at 15,000 g. The level of CK activity was measured in the supernatant [6], and the total protein content was also determined after Lowry. The data were processed by the method of paired comparisons; the reliability of differences of the means was assessed using Student's *t* test.

## RESULTS

The results obtained are presented in Table 1. Evidently, under the influence of CL radiation the level of the biochemical parameters measured was reliably elevated. At the same time, as the photoload increased, all deviations detected became more marked: the increment of the water-extractable protein content and of the total and specific CK activity in the myoblast culture was about 10-20,

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TABLE 1. Biochemical Indexes of Tissue Cultures Irradiated with CL in Stimulating Doses ($M \pm m$, $n = 12$)

| Dose, J/cm² | Myoblasts | | | Neuroblasts | | |
|---|---|---|---|---|---|---|
| | P | TA | SA | P | TA | SA |
| 0 | 1.17±0.04 | 0.06±0.01 | 0.05±0.01 | 1.37±0.04 | 0.11±0.01 | 0.08±0.01 |
| 0.2 | 1.31±0.01* | 0.13±0.01* | 0.11±0.01* | 1.64±0.02* | 0.21±0.01* | 0.14±0.01* |
| 1.0 | 1.40±0.02* | 0.33±0.02* | 0.25±0.02* | 1.82±0.04* | 0.34±0.01* | 0.30±0.03* |

**Note.** P: water extractable protein content, mg/ml; TA: total CK activity, mmol/min×ml; SA: specific CK activity per mg protein; *) $p<0.05$ vs. the control.

200-550, and 200-450%, respectively. In the case of neuroblasts the same parameters were 20-30, 200-300, and 200-350%, respectively.

Although these parallels are arbitrary, it should be noted that there are no fundamental differences between the responses of the two types of culture; the trends exhibited are of a similar nature. This circumstance does not just reflect that the follow-up period is well chosen in each case, but also underlines the role of CK per se in the development of model objects which are so dissimilar [9]. Evidently, from the biological viewpoint, the trend of the response to such a nonspecific trophic effect as CL radiation [5] is precisely the same. Certain quantitative differences between the numerical results, when interpreted in terms of biostimulation, suggest that the total increase in protein synthesis is more marked in neuroblasts; in turn, the slightly smaller increase in the CK level is in accord with the notion that a prominent role in the development and function of nervous tissue is played by other specialized protein systems [8]. In addition to general considerations, the marked correlation between the relative changes in the total and specific CK activity speaks in favor of the "biosynthetic" hypothesis. We are inclined to assume that the increase of the protein pool and CK activation are closely connected.

Our findings concerning the mechanism of formation of the dynamics observed may be regarded otherwise: an objective characteristic of a biostimulation-induced response is presented, and it is shown to manifest itself on the part of the competent culture as an increase in the water-extractable protein content, as well as a marked elevation of not only the specific but also the total CK activity. Since this dramatically differs from the picture observed in the myoblast culture exposed to chemical agents (where fundamentally different trends are noted, including a drop of the water-extractable protein level), the hypothesis about the stress nature of such "chemical shifts" [4] is evidently true. The most probable explanation is that the loads employed were too high, and this may be elucidated by markedly reducing the test doses of biologically active compounds.

The positive results of such studies could also be of practical importance with regard to the use of the myoblast culture as an indicator (marker) system. It is worthy of note that its behavior not only makes it possible to distinguish between the injurious and stimulating effects, but also easy to do so, owing to the qualitatively specific response to these effects, by routine determination of the protein content, without having to resort to more complicated techniques (for example, the determination of enzyme activity). In addition, such an approach is cost-effective; however, its sensitivity and limitations are naturally still to be determined.

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