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Letter to the Editor: Abnormal Growth Kinetics and 5′-Nucleotidase Activities in Cultured Skin Fibroblasts from Patients with Duchenne Muscular Dystrophy

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In a letter to the editor, Richard L. Wertz and Allen D. Roses report that, in contrast to our results, they did not find any differences between Duchenne muscular dystrophy (DMD) and normal skin fibroblasts with regard to growth kinetics and 5′-nucleotidase activities. Moreover they suggest that our data might be due to selective technical cell damage or other artifacts.

We would therefore like to comment on their critical remarks. First, we did not maintain that there is a premature aging in DMD cells, but concluded from our results that DMD fibroblasts behave *similar* to prematurely aging cells, what ever the reason for this behavior would be. Secondly, H. P. Rodemann from Germany (5) found a diminished *in vitro* lifespan in DMD skin fibroblasts, which is in accordance with our results and thus confirming the possibility of an intrinsic disorder in DMD fibroblasts.

Our cultivation methods were performed according to standard techniques (6), so we did not repeat the procedures in detail. It is well known, that there is an inverse correlation between donor age and lifespan of cultured fibroblasts. Figure 1 of Wertz and Roses clearly shows, that there is however no difference in lifespan between the fifth and the fifteenth year of the donors. Because both our patients and matched controls were all aged between 4–14 years, our differences between DMD and control cells are certainly not due to different ages.

The authors additionally write: "thus it appears that the DMD cell-strains examined by Liechti-Gallati *et al.* behave in a manner consistent with nearly senescent fibroblasts," so they seem to have clearly understood what we meant discussing our findings. On the other hand, their explanation of our differences being caused by technical selectivity during treatment of the explant cultures is not plausible. It would imply that from the DMD strains we cultured mainly cells from the periphery, and from the controls mainly cells from the interior of the monolayer. This can certainly be excluded, because our handling of the cultures is always identical, irrespective of the cell types.

Furthermore, if we had worked with damaged cells, *all* strains should show a poor growth rate and high 5′-nucleotidase activities.

Our trypsinization method is well known and approved (we used Bacto-trypsin, which is much less aggressive than EDTA-trypsin, and Hank's solution as buffer). Even if trypsinization alone should be a direct or indirect cause of the slower growth rate of DMD fibroblasts compared to control cells, this would not indicate an artifact, but on the contrary be in favor of our and other investigators' conclusions (1, 2, 3, 5), that there is probably some defect in the plasma membrane of DMD fibroblasts.

The greater lag time in our kinetic studies can be explained by the possibility, that the inoculation density was not optimal. This does of course not alter the interpretation of our results, because the differences between DMD and control fibroblasts regarding growth kinetic and 5′-nucleotidase results remained identical in experiments with high inoculation density (4, Table 1). In the meantime our results proved to be reproduceable through many succeeding experiments up to approximately the 14th subculture. We noticed furthermore, that the pathologic findings in DMD fibroblasts are independent of the patients' ages (the youngest DMD boy is aged 14 months!). We were also able to identify correctly fibroblast strains as DMD or normal, which were sent to us codified from other laboratories, provided the culture conditions and the matching of controls for age were comparable to ours and the number of previous subcultures was not greater than twelve (we prefer subcultures no. three to eight). If Wertz and Roses are still doubtful about our findings, they are therefore also kindly invited to send to us codified fibroblast cultures from their DMD patients and matched controls under the same conditions.

We think that the authors' failure to reproduce our results may be due to technical differences between the two laboratories rather than serve as a suggestion, that our findings be the results of artifacts, the more as they are in accordance with the growth kinetic alterations described by Rodeman (5).

REFERENCES AND NOTES

1. Gelman B. B., Davis M. H., Morris R. E., and Gruenstein E.: Structural changes in lysosomes from cultured human fibroblasts in Duchenne's muscular dystrophy. J. Cell Biol., *88:* 329 (1981).
2. Jones G. E. and Witkowski J. A.: Analysis of skin fibroblast aggregation in Duchenne muscular dystrophy. J. Cell Sci., *48:* 291 (1981).
3. Jones G. E. and Witkowski J. A.: Reduced adhesiveness between skin fibroblasts from patients with Duchenne muscular dystrophy. J. Neurol. Sci., *43:* 465 (1979).
4. Liechti-Gallati S., Moser H., Siegrist H. P., Wiesmann U., and Herschkowitz N. N.: Abnormal growth kinetics and 5′-nucleotidase activities in cultured skin fibroblasts from patients with Duchenne muscular dystrophy. Pediatr. Res., *15:* 1411 (1981).
5. Rodemann H. P.: Die molekularen Mechanismen des pathophysiologischen Alterns: Untersuchungen an Hautfibroblasten von Patienten mit Duchenne Muskeldystrophie. Dissertation, Stuttgart-Hohenheim (1979).
6. Wiesmann U. N., Rossi E., and Herschkowitz N. N.: Acta Paediat. Scand., *61:* 296 (1972).
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