Serbian molecular biologists in Regensburg

Miodrag Guzvic

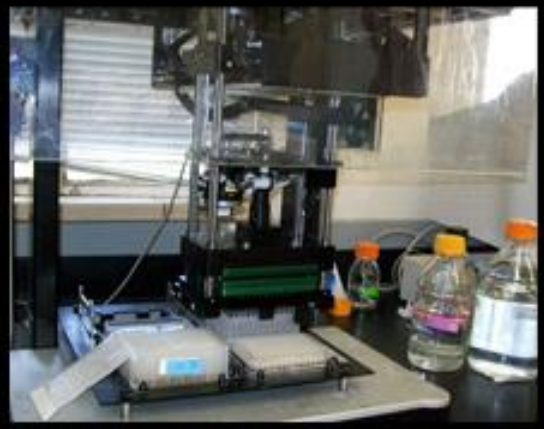
23.09.2019 Bamberg

Molecular Biologist

What my friends think I do

What my mom thinks I do

What society thinks I do



What my boss thinks I do

What I think I do

What I actually do

Why "molecular biologists"

- Molecular biology is increasingly becoming the part of almost all domains of classical biology
  - Still, most of research is related to human health
- Modern, dynamic, exciting, rewarding,...
- One of the key problems: it is very **expensive** field of science

conversion of 1 μmole of DPNH to DPN per min. by 10^{10} erythrocytes at 37° C. under our assay conditions. The results are summarized in Table 1. All the red blood cell enolase values from patients with hereditary spherocytosis were either normal or elevated. All the other glycolytic enzymes, as well as glucose-6-phosphate and 6-phosphogluconic dehydrogenases, were examined either singly or in multiple steps, and were found to be normal or increased in activity.

In order to determine whether enolase of the red cells in hereditary spherocytosis is more sensitive to fluoride than the enolase of normal red cells, sodium fluoride in different concentrations was added to the assay mixture and enolase activity was then measured. As shown in Fig. 1, under these conditions the enolase activity curve of red cells of hereditary spherocytosis was similar to that of the controls.

In conclusion, no glycolytic enzymatic deficiency was demonstrable in the erythrocytes of patients with hereditary spherocytosis. Specifically, the activity of enolase was normal or increased. There was no difference in the sensitivity to fluoride of the enolase activity of red cells of hereditary spherocytosis as compared with normal subjects under our assay conditions.

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RADIOBIOLOGY

Recovery of Ultra-Violet-irradiated *L* Strain Cells by means of Highly Polymerized Deoxyribonucleic Acid

It is known that ultra-violet irradiation may produce death of mammalian S_3 HeLa strain cells[1,2], strain *D*–98 and *D*–98C_6b (ref. 3) and *L* strain[4]. In our previous work we found that the rate of synthesis of nucleic acids in irradiated *L* strain cells is much slower than in normal cells[4]. A similar phenomenon has been observed in ultra-violet-irradiated bacteria[5,6]. It is probable that some of the most important radiation effects are closely related to the damage of structure and function in nucleic acid (NA) macromolecules, which may be responsible for the modification of the genetic make-up of the cells and for their death. Therefore, the problem of the repair of this damage is one of the most important in radiobiology. Thus, we have paid special attention to the problem of the recovery of irradiated organisms, investigating the macromolecular basis of the phenomena. For this purpose, irradiated cells were treated with highly polymerized isologous deoxyribonucleic acid (DNA), and results presented in this communication show that the irradiated cells survive better if they are treated after irradiation.

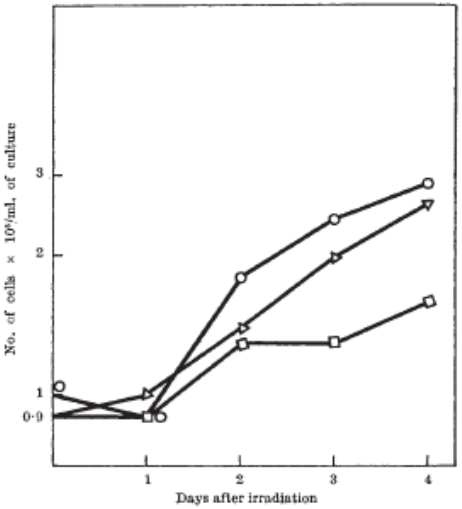

Fig. 1. Ultra-violet-irradiated *L* cells treated with 80 μgm. DNA/ml. of culture. ○, Control; △, irradiated + DNA; □, irradiated

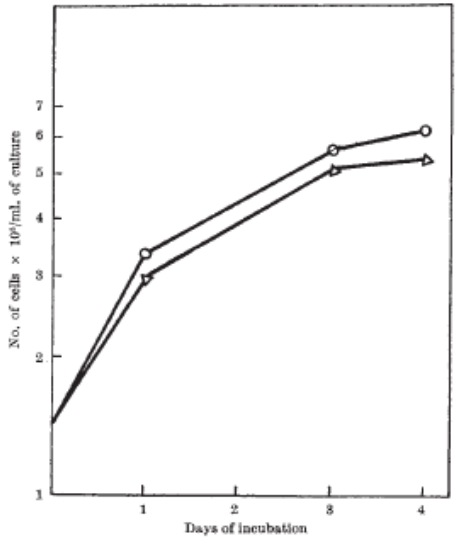

Fig. 2. Normal *L* cells treated with 40 μgm. DNA/ml. of culture. ○, Control; △, controls + DNA

For our experiments we have used *L* strain cells, which had been propagated in suspension as shown by Earle *et al.*[7]. Cells were cultivated in Eagle's medium supplemented with 20 per cent calf serum[8]. Cultures were obtained from L. Siminovitch of Ontario Cancer Institute (Toronto). As a source of radiation a Philips' ultra-violet lamp (30 W.) mounted at 105 cm. from the target plane was used. The radiant flux density at the target area was measured by the change of uridylic acid according to Shugar's technique[9]. The intensity was 700 ergs/min./mm.^{2}. Total dose of radiation was 2,800 ergs/mm.^{2}. DNA was extracted from normal *L* cells in logarithmic phase of growth by the technique of Zamenhof–Reiner[10]. Our DNA preparation contained some RNA.

Cells in the logarithmic phase of growth, suspended in saline *A*, were irradiated by ultra-violet light. The concentration of cells during irradiation was $0{\cdot}8 \times 10^6$ cells/ml. Just after radiation cells were resuspended in nutrient medium (10^5 cells/ml. of culture), containing highly polymerized DNA extracted from normal *L* cells in concentration of 20–80 μgm./ml. The samples for counting cells were taken every day.

From the results presented in Figs. 1 and 2, it is evident that highly polymerized DNA prepared from the same strain of cells aids recovery, that is, irradiated DNA-treated cells survive better the dose of radiation. As can be seen from the Fig. 1 in irradiated cultures treated with 80 μgm. DNA/ml. only 10 per cent of irradiated cells died in a 4-day interval following irradiation, whereas in non-treated cultures for the same time-interval about 50 per cent of irradiated cells lost the capacity to divide. This recovery effect, as can also be seen from Table 1, seems to depend upon the amount of DNA added to the irradiated cultures. We have examined this effect with 21, 50, 60 and 80 μgm. of DNA/ml. of culture.

Table 1. Recovery Effect of Various Amounts of DNA added to Irradiated Cultures

Amount of DNA added to the irradiated cultures (μgm.)	No. of cells surviving on the fourth day after irradiation relative to the irradiated non-treated cultures (control, 100 per cent)
21	123
50	134
60	147
80	155

These results show that a significant survival has been obtained with 60 and 80 μgm. of DNA/ml. of culture. The observed results may be of interest in correlation with a similar DNA recovery effect in irradiated rats[11], observed earlier in this laboratory.

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High-Resolution Autoradiography without Loss of Water-soluble Ions

The liquid-emulsion, stripping-film, and wet-mounting autoradiographic techniques developed by Bélanger and Leblond[1], Pelc[2], and Evans[3], respectively, are not directly applicable to tissue which contains water-soluble radioactive material. Variations to the last two of these techniques have been described in subsequent papers. Canny[4], Eisen[5], and Winteringham[6] described variations for mounting the stripping film without water coming into contact with the tissue, and Gallimore[7], Harris[8], and Kaminski[9] suggested different techniques for flattening and mounting paraffin sections directly on autoradiographic plates.

The purpose of this communication is to describe a technique devised in this laboratory that appears to provide a faster and less complex method for mounting paraffin sections directly on autoradiographic plates. By combining the procedures of flattening, mounting, and de-paraffinizing the sections, a new technique was devised to study the distribution of highly water-soluble sulphur-35 in barley roots.

After accumulating sulphur-35, the barley roots are quick-frozen in liquid nitrogen-cooled isopentane, and are cut into pieces 2–3 mm. long in a cryostat. They are then dehydrated by the freeze-dry method and infiltrated with paraffin under vacuum as suggested by Jensen[10]. After embedding in paraffin, sections are cut with a rotary microtome. A ribbon, containing the desired number of sections to be mounted on one autographic plate, is placed on top of a rubber stopper of convenient size.

The subsequent operations are all performed in a well-ventilated dark-room using a proper safe light. For large-scale preparations these manipulations are carried out in a ventilated glove box to prevent the toluene fumes from accumulating in the dark-room.

The paraffin ribbon that had been placed on the rubber stopper is transferred to the autoradiographic plate by inverting the stopper over it. The plate is next placed on a movable base to facilitate later manipulations. Two lead 'L's' of an embedding box are arranged on the plate to form an enclosure around the paraffin ribbon. The autoradiographic plates, used in this study, were prepared by wet-mounting pieces of Kodak '*AR*-10' stripping film, with the emulsion sides upward, on 'subbed' glass slides.

The arrangement described here is lowered into a container filled with enough toluene to cover the autoradiographic plate to a depth of 2–3 mm. The toluene flows slowly into the enclosure, dissolves the paraffin, and leaves the cleared tissue sections on the surface of the film. After about 3 min., the arrangement is removed from the toluene. The toluene that is trapped within the lead block enclosure is removed by gently pressing the straight edge of absorbent paper against the outside junction of the enclosure and the autoradiographic plate. Removing toluene in this manner permits the tissue sections to remain undisturbed on top of the film in the enclosure. The deparaffinizing is repeated with fresh toluene. The lead 'L's' are removed by sliding them away from the tissue sections and the autoradiographic plate is dried in a stream of air from a fan. The plates are then stored in a light-tight box in a refrigerator for exposure.

After the desired exposure-time has elapsed, the plates are removed and prepared for photographic development. First, the plates are dipped into water for 30 sec., then dried in a stream of air. This step helps to ensure that all the tissue sections stay in contact with the emulsion during the developing and fixing procedures. If water-soluble ions are lost from the tissue at this point, it is of no consequence since no further exposure of the film occurs. The plates are then placed for 5 min. in a coplin staining dish containing toluene to remove any trace of paraffin that might interfere with the development of the

Molecular biology in Serbia

- Serbia was one of the few countries in the world with dedicated molecular biology study direction in 1970s
- It was a top-notch study programme recognized worldwide
- While the study programme remains excellent, the main drawback are lab exercises and opportunities for practical work
  - ***Consequence: students have excellent and broad knowledge, but very little lab experience***

Personal experience

- Studied molecular biology in Belgrade
  - Diploma in 2002
- Researcher at Institute of Nuclear Sciences, Belgrade 2001 - 2006
- Research stays at Michigan State University, USA in 2004 and 2005
  - Magister of science 2005
- Research stay at University of Lübeck, Germany 2005/6

Serbs attract more Serbs

- Before my stay, the Lab in Lübeck hosted 2 molecular biologists from Serbia
- Good experience motivated the Lab head to bring more students from Serbia, and I was one of them
- After me, there were two Serbian molecular biologists visiting the Lab

B. Culjkovic –> A. Djarmati –> **M. Guzvic** –> A. Rakovic –> V. Dobricic –>...

Personal experience

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- Researcher at Institute of Nuclear Sciences, Belgrade 2001 - 2006
- Research stays at Michigan State University, USA in 2004 and 2005
  - Magister of science 2005
- Research stay at University of Lübeck, Germany 2005/6
- Start of PhD in Regensburg in 2006

Why Regensburg?

- I initially applied for position in Munich, but the whole Lab moved to Regensburg 6 months later

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- PhD (2006), followed by Postdoc (2011), followed by Group Leader (2018)



Serbs attract more Serbs

Milan Obradovic
PhD
(now Basel)

Serbs attract more Serbs

Milan Obradovic
PhD
(now Basel)

Ana Grujovic
PhD
(now Telexos)

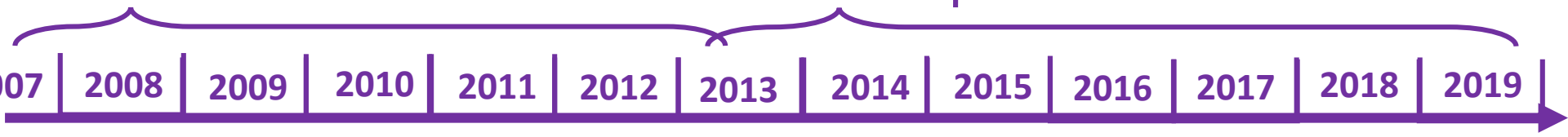
07 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019

Serbs attract more Serbs

Natasa Stojanovic
PostDoc
(PhD at TUM)

Milan Obradovic
PhD
(now Basel)

Ana Grujovic
PhD
(now Telexos)



Serbs attract more Serbs

Natasa Stojanovic
PostDoc
(PhD at TUM)

Vladan Milosevic, MD
PostDoc
(PhD in Italy)

Milan Obradovic
PhD
(now Basel)

Ana Grujovic
PhD
(now Telexos)

2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019

Others in Regensburg...

- There are/were few other Serbian molecular biologists in other Labs in Regensburg

Others in Regensburg...

- There are/were few other Serbian molecular biologists in other Labs in Regensburg


- ... and many many over the years in Munich



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Currently...

- Frequently contacted by students or researchers from Serbia
  - Students want to do internships
  - Researchers wish to collaborate on projects, get expertise, or train students
- Visits possible if they obtain own maintenance funding
- Research collaboration barely existing
  - Incompatible research interests
  - Difficulties in obtaining funding for such collaborations

Recent experience....

- Early summer 2019 attempted to apply for joint German-Serbian DAAD project
- Paperwork was very complex and time-consuming
  - Hardly any German researcher would use his time for this

We need

- Money that enables mobility and collaboration
  - Travel and maintenance costs
  - Basic costs for research projects
    - Seed money for obtaining grants from major funding agencies

Conclusions/Perspective

- Serbian molecular biologists are gladly accepted in German laboratories
  - Both short-term and long-term stays
- Many would return to Serbia
- Many would not leave at all if there would be more possibilities to "plug-in" into cutting-edge research